

University of Genoa

Doctorate in Sciences and Technologies of Chemistry and Materials

Curriculum: Chemical Sciences and Technology (STC)
XXXVIII cycle

Olive Mill Wastewater as a Source of Bio-Derived Active Ingredients: Process Development and Scale-Up for Agricultural and Industrial Applications

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Abstract

One of the main characteristics of the “Mediterranean diet” is the regular consumption of olive oil, which is renowned for its health benefits, primarily due to its high content of unsaturated fatty acids. However, in recent years, its minor components have also been the subject of extensive research. Polyphenols are particularly noteworthy due to their remarkable antioxidant, anti-inflammatory, antiatherogenic and antiproliferative biological activities. Nevertheless, due to their high hydrophilic structure, only a small proportion of polyphenols found in olive fruits end up in olive oil; the vast majority are present in by-products of oil production, particularly liquid waste. Therefore, the recovery of polyphenols from olive mill wastewaters (OMW) and their valorization could be a promising starting point for developing bio-pesticides or fertilisers and other high-value products. In this context, the present PhD thesis investigates an integrated and scalable strategy for the valorization of olive mill wastewater as a source of bio-derived active ingredients. The proposed approach combines an acidification pre-treatment with sulfuric acid to stabilize phenolic compounds and promote solid–liquid separation, thereby enhancing the removal of suspended solids and generating a clarified stream suitable for downstream processing. The supernatant was subsequently treated through an integrated cascade of membrane filtration processes (microfiltration, nanofiltration, diafiltration and reverse osmosis), achieving effective concentration and fractionation of soluble phenolic compounds while reducing organic load. Downstream purification was performed through adsorption on strong anionic resins and electrodialysis, desalting and refining of phenolic-rich fractions while preserving their antioxidant activity. Finally, to overcome the intrinsic hydrophilicity of OMW-derived phenolics and facilitate their incorporation into lipid-based formulations, an enzymatic lipophilization step was developed using immobilized lipases.

The most promising resulting products obtained from the different process streams were successfully formulated for potential applications both in agricultural and industrial fields. In particular, the sludge was tested as a feed supplement for black soldier fly (*Hermetia illucens*) larvae and together with the liquid fractions, after determining their cytotoxicity and phytotoxicity, were formulated for potential applications in fertilizers and biopesticides formulations. Moreover, preliminary investigations explored their incorporation into cosmetic products, such as antioxidant-enriched serums and gels. Preliminary techno-economic assessments of the main technologies investigated were presented as a basis for the future development of full life cycle assessment (LCA) and life cycle costing (LCC) analysis. Finally, based on the results obtained during this study, two main pathways were suggested for transferring the proposed technologies to real-scale applications.

Acronym	Definition
A	Ash
AEM	Anion Exchange Membrane
BER	Bioconversion Efficiency
BMED	Bipolar Membrane Electrodialysis
BOD	Biological Oxygen Demand
BSFL	Black Soldier Fly Larvae
CALB	Candida antarctica Lipase B
CAPEX	Capital Expenditure
CEM	Cation Exchange Membrane
CF	Concentration Factor
CFV	Cross-Flow Velocity
COD	Chemical Oxygen Demand
CRF	Capital Recovery Factor
DF	Diafiltration
DPPH	2,2-Diphenyl-1-picrylhydrazyl
DTG	Derivative Thermogravimetry
EC	Electrical Conductivity
ED	Electrodialysis
EDR	Electrodialysis Reversal
EDX	Energy-Dispersive X-ray Spectroscopy
EBCT	Empty Bed Contact Time
FC	Fixed Carbon
FE-SEM	Field Emission Scanning Electron Microscopy
FRR	Flux Recovery Ratio
FT-IR	Fourier Transform Infrared Spectroscopy
G	3,4-Dihydroxyphenylglycol
GAE	Gallic Acid Equivalents
GC-MS	Gas Chromatography–Mass Spectrometry
GI	Germination Index
GR	Growth Rate
H	Hydroxytyrosol
HaCaT	Human keratinocyte cell line
HEC	Hydroxyethyl Cellulose
HF	Hollow Fiber
HPLC	High-Performance Liquid Chromatography
LCA	Life Cycle Assessment
LCC	Life Cycle Costing
MC	Moisture Content
MF	Microfiltration
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MWCO	Molecular Weight Cut-Off
NF	Nanofiltration
NMR	Nuclear Magnetic Resonance
OMW	Olive Mill Wastewater
OPEX	Operating Expenditure
PES	Polyethersulfone
PP	Polypropylene
PVDF	Polyvinylidene Fluoride
RED	Reverse Electrodialysis
RO	Reverse Osmosis

RR	Substrate Reduction Rate
RSD	Relative Standard Deviation
SD	Standard Deviation
SED	Selective Electrodialysis
SGR	Specific Growth Rate
SR	Survival Rate
T	Tyrosol
TEA	Techno-economic Assessment
TGA	Thermogravimetric Analysis
TLC	Thin Layer Chromatography
TPC	Total Phenolic Content
TS	Total Solids
TSS	Total Suspended Solids
UF	Ultrafiltration
UV-Vis	Ultraviolet–Visible Spectroscopy
VCR	Volume Concentration Ratio
VM	Volatile Material
WRI	Weight Reduction Index

Symbol	Definition
α	Conversion
β	Heating Rate
μ	Viscosity
η	Dynamic Viscosity
A_m	Membrane Area
C	Concentration
E	Energy
E_a	Activation Energy
i	Annual Interest Rate
I	Electric Current
J	Flux
J_c	Critical Flux
m	Mass
n	Moles Number
P	Power
Q	Volumetric Flowrate
R	Rejection
R_{tot}	Total Filtration Resistance
R_m	Membrane Resistance
R_c	Cake Resistance
R_f	Fouling Resistance
t	Time
T	Temperature
TMP	Transmembrane Pressure
V	Voltage

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CHAPTER 1: GENERAL INTRODUCTION

1.1 Sustainability Transition in the European Agri-Food System

In recent years, the European agri-food system has entered a decisive sustainability transition. Increasing climate pressures, including heatwaves, altered precipitation and more frequent extreme events, are making production conditions more unstable and highlighting the limits of conventional, input intensive farming models [1,2]. At the same time, agriculture continues to exert a significant environmental impact. It contributes about 11% of total EU greenhouse gas emissions, mainly methane and nitrous oxide originating from livestock production and agricultural soils, while global food systems are estimated to account for roughly 26% of anthropogenic emissions [3,4].

In response to these challenges, the European Union has introduced a series of policy initiatives, the European Green Deal (COM(2019) 640 final) and the Farm to Fork Strategy, aimed at transforming the agri-food sector toward more sustainable models. Among them, the European Green Deal represents a comprehensive roadmap designed to achieve climate neutrality by 2050. The strategy promotes ambitious objectives including improved resource efficiency, circular material flows, reduction of pollution, and protection of biodiversity [5]. Within this framework, the Farm to Fork Strategy addresses sustainability across the entire food supply chain, from primary production to processing and consumption, and sets specific targets for 2030, such as reducing the use and risk of chemical pesticides by 50%, decreasing fertilizer use by 20%, halving antimicrobial sales for farmed animals, and expanding organic farming to 25% of agricultural land [6].

Achieving these goals requires not only incremental improvements in agricultural practices but also a broader transition toward sustainable and circular production systems. In this context, the circular bioeconomy has emerged as a key paradigm for the management of biological resources. It promotes the valorization of biomass, agro-industrial residues, and organic waste streams as feedstocks for the production of high-value products and bio-based materials [7]. This approach represents a shift from the traditional linear “take–make–dispose” model toward more circular production cycles, in which by-products and waste streams are reintegrated into new value chains [8]. Reintegrating these streams into productive cycles can simultaneously reduce environmental burdens, decrease reliance on synthetic chemicals, and enhance the economic resilience of rural territories.

In the European context, food systems currently generate a substantial amount of waste. Estimates from Eurostat indicate that the European Union produces over 58 million tonnes of food waste annually, corresponding to roughly 130 kg per capita and representing a significant reservoir of secondary biomass for valorization across the supply chain [9]. These streams represent a significant reservoir of secondary biomass that can potentially be converted into valuable resources through innovative processing technologies.

Among agro-industrial sectors, olive oil production represents a particularly relevant case. While olive oil is widely recognized for its nutritional and cultural importance, the extraction process

generates large volumes of by-products and liquid effluents, including olive mill wastewater (OMW). Traditionally regarded as a problematic waste due to its high organic load and phytotoxic properties, OMW has recently attracted increasing attention as a potential source of valuable bioactive compounds that can be recovered and valorized within circular bioeconomy frameworks.

1.2 Olive oil and its by-products

Olive oil, obtained from the mechanical extraction of *Olea europaea* L. fruits, is widely recognized as one of the cornerstone products of the Mediterranean diet [10,11]. Its production is a key sector for several European countries, especially Spain, Italy, and Greece. Spain has the largest area of olive cultivation (estimated at ca. 2.47 million ha), followed by Italy (ca. 1.16 million ha) and Greece (about 0.81 million ha) [10].

The nutritional and functional properties of olive oil have been extensively investigated, and its regular consumption has been associated with reduced incidence of cardiovascular diseases, metabolic disorders, and chronic inflammatory conditions [11,12]. Traditionally, the beneficial effects associated with the consumption of virgin olive oil were mainly attributed to its high content of mono- and polyunsaturated fatty acids. These fatty acids constitute the saponifiable fraction of olive oil and represent approximately 98–99% of its total composition. According to the International Olive Council, the fatty acid profile of olive oil is dominated by oleic acid (typically 55–83%), followed by linoleic acid (2.5–21%), depending on category and origin. Other relevant fatty acids include palmitic, stearic and linolenic acids [13].

However, it is now widely accepted that many of the health benefits associated with the regular consumption of virgin olive oil are actually related to the presence of minor constituents belonging to the unsaponifiable fraction [14,15]. Although this fraction accounts for only 1–2% of the oil's total weight, it comprises more than 230 distinct compounds [16]. These include liposoluble vitamins (especially tocopherols, that is vitamin E), carotenoids, chlorophylls, phytosterols, waxes and other bioactive molecules [17,18]. Among these minor components, phenolic compounds are particularly notable, contributing not only to the oil's characteristic bitter and pungent taste but also to its potent antioxidant and health-promoting properties [19]. Table 1.1 summarizes the main chemical components commonly found in olive oil.

Table 1.1: Main chemical components of olive oil.

Major components <i>(saponifiable fraction; 98-99%)</i>	Minor components <i>(unsaponifiable fraction; 1-2%)</i>
Oleic acid	Hydrocarbons (squalene)
Palmitic acid	Alcohols (aliphatic, terpenes)
Linoleic acid	Phytosterols, Tocopherols
Stearic acid	Pigments (chlorophylls, carotenoids)
Palmitoleic acid	Waxes
Linolenic acid	Phenols

Phenolic compounds in olive oil are structurally diverse and can be classified into several chemical classes (Figure 1.1) according to their core molecular structure [20–23]. These include:

- a) Phenolic acids: subdivided into hydroxybenzoic acid derivatives (e.g., vanillic, protocatechuic, gallic acids) and hydroxycinnamic acid derivatives (e.g., p-coumaric, caffeic, ferulic, sinapic acids).
- b) Phenolic alcohols: mainly hydroxytyrosol, tyrosol, 3,4-dihydroxyphenylglycol.
- c) Secoiridoids: compounds characterized by elenolic acid structure, often bound to phenolic compounds and/or to monosaccharides, as in oleuropein and ligstroside.
- d) Lignans: (+)-pinoresinol, (+)-1-acetoxypinoresinol.
- e) Flavonoids: luteolin, apigenin, quercetin, hesperidin.

These phenolic compounds are highly hydrophilic and, during olive oil extraction, predominantly partition into the aqueous phase. As a result, only a minor fraction of the total phenolics remains in the oil, while the majority is transferred into olive mill wastewater, which consists mainly of the vegetation water from the olive fruits and rinse water [24].

Olive oil can be obtained using three primary extraction systems: the traditional press, the three-phase decanter, and the two-phase centrifugation system. These methods differ not only in technology but also in their impacts on oil composition, by-product characteristics, and environmental performance [25]. In the traditional extraction system, olives are first crushed using millstones to obtain a paste, which is then subjected to mechanical pressing using stacks of fiber disks. This process separates the solid residue (olive skins, pulps and seeds) from the liquid fraction, which consists of a mixture of oil and vegetation water that is subsequently separated by decantation. Modern industrial plants typically employ centrifugal extraction systems, which allow the separation of phases based on density differences [26]. This system can operate in either a two or three phase, depending on the addition of water and the by-products generated. In the three-phase system, the addition of process water during centrifugation leads to the separation of three streams: olive oil, solid residue known as olive pomace, and a large volume of liquid effluent named olive mill wastewater. In contrast, the two-phase system reduces water addition and produces olive oil and a semi-solid by-product commonly referred to as wet pomace, which retains most of the vegetation water originally present in the olives [27,28]. Although the two-phase system significantly decreases wastewater production, considerable amounts of aqueous effluents are still generated during equipment washing and downstream processing. Furthermore, the seasonal nature of olive oil production leads to the generation of large volumes of wastewater within relatively short processing periods, which further complicates its management and treatment in olive-producing regions [29].

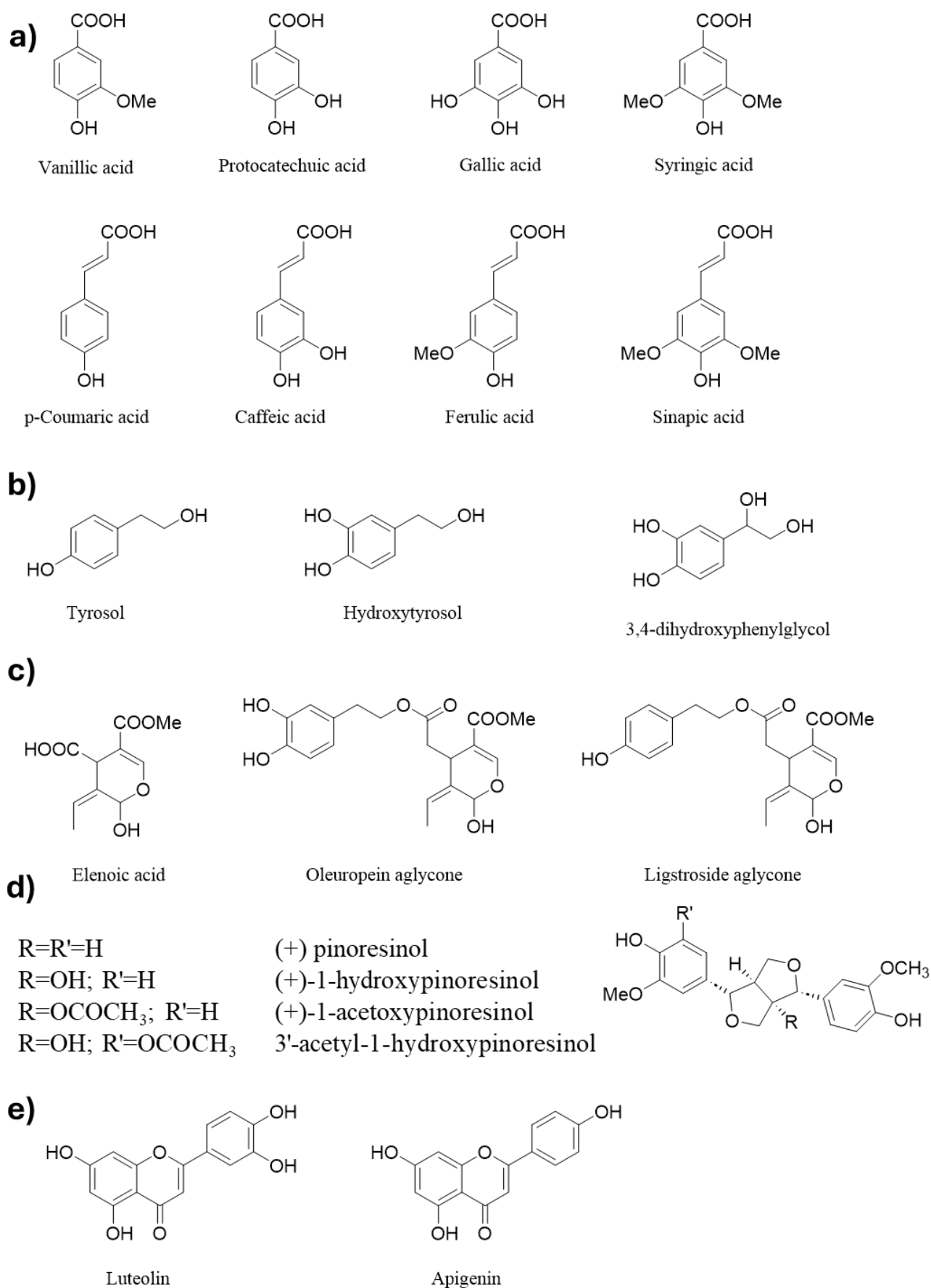


Figure 1.1: Examples of phenolic compounds identified in olive oil.

1.3 Olive Mill Wastewater: composition and environmental impact

Olive mill wastewater (OMW) is the main liquid effluent generated during the mechanical extraction of olive oil. It originates primarily from the vegetation water naturally present in olive fruits, together with the process water added during oil extraction and the washing water used during equipment cleaning. Depending on the extraction technology employed and the characteristics of the processed olives, OMW is typically produced in volumes ranging from approximately 0.5-1.5 m³ per ton of processed olives [30,31]. On a global scale, this corresponds to an estimated annual production ranging from 10 to 30 million m³/year of OMW [32,33].

OMW is a highly complex aqueous matrix whose physicochemical composition varies significantly depending on factors such as olive cultivar, fruit ripening stage, climatic conditions, agronomic practices, and extraction technology [10,29]. Despite this variability, some general compositional features can be identified. OMW typically contains 83-94% water, together with a wide variety of organic and inorganic constituents [34]. OMW is characterized by a very high organic load, reflected in elevated chemical oxygen demand (COD) values ranging from approximately 30 to 320 g/L, and biological oxygen demand (BOD) values between 35 and 132 g/L [35]. In addition, OMW contains suspended solids, colloidal particles, residual lipids, sugars, organic acids, and a variety of mineral salts, particularly potassium, calcium, magnesium, sodium, and nitrogen compounds [24,28].

One of the most distinctive features of OMW is the presence of phenolic compounds, typically occurring at concentrations up to 15 g/L, depending on processing conditions [36]. These compounds include simple phenols such as hydroxytyrosol, tyrosol, and 3,4-dihydroxyphenylglycol, as well as more complex phenolic derivatives originating from olive secoiridoids. While these molecules are responsible for the strong antioxidant activity associated with olive-derived products, they also contribute significantly to the toxicity of OMW. In particular, phenolic compounds exhibit antimicrobial activity that can inhibit microbial growth and interfere with biological degradation processes [37,38].

Due to the combination of high organic load, phenolic toxicity, acidic pH (typically between 3 and 6), and seasonal generation, olive mill wastewater is traditionally considered one of the most problematic agro-industrial effluents in Mediterranean countries [39]. At the same time, the high concentration of organic matter and bioactive compounds present in OMW has attracted increasing scientific interest in recent years, particularly with regard to the phenolic fraction, which represents a potentially valuable source of natural antioxidants and other bioactive molecules [40].

1.4 OMW Treatment and Valorization

Historically, research on olive mill wastewater has primarily focused on treatment technologies aimed at reducing pollutant load and mitigating its environmental impact. In Mediterranean countries, where olive oil production is highly seasonal, traditional management strategies have often relied on relatively simple disposal practices, such as storage in evaporation ponds or controlled land spreading

on agricultural soils. Evaporation ponds allow the natural reduction of wastewater volume through solar evaporation and the formation of semi-solid residues; however, they may also generate unpleasant odors, promote insect proliferation, and pose risks of soil and groundwater contamination if not properly managed [41]. Similarly, the agronomic application of OMW has been considered a potential strategy for recycling organic matter and nutrients. Nevertheless, its direct use is strongly limited by environmental regulations due to the high organic load, acidity, salinity, and the presence of phytotoxic phenolic compounds that can negatively affect soil microbial communities and plant growth [42,43].

In response to the environmental concerns associated with these traditional disposal practices, numerous studies have investigated different treatment technologies for olive mill wastewater. These approaches generally include physical, chemical, and biological processes, which are often applied individually or combined within multi-stage treatment systems.

Physical treatments are among the simplest approaches investigated for the preliminary clarification of olive mill wastewater. Processes such as sedimentation, centrifugation, and filtration can remove suspended solids but generally provide limited reduction of the organic load when applied alone. More effective results have been obtained by adding chemical reagents able to promote flocculation, aggregation, precipitation, or oxidation of organic compounds present in the wastewater. For example, Fleyfel et al. [44] reported significant reductions in COD, turbidity and phenolic compounds using a combined coagulation–flocculation and filtration process for the treatment of olive mill wastewater. In addition, advanced oxidation processes (AOPs) have attracted increasing attention due to their ability to degrade recalcitrant organic compounds. Among these technologies, Fenton and photo-Fenton reactions, ozonation, and electrochemical oxidation have demonstrated promising results for the degradation of phenolic compounds and the reduction of COD and BOD levels [45]. For example, Nieto et al. [46] reported COD removal efficiencies of up to 70% during photo-Fenton treatment of olive mill wastewater.

Biological treatments have also been extensively investigated as environmentally friendly options for reducing the organic load of OMW. Anaerobic digestion is often considered a promising approach because it allows the conversion of organic matter into biogas while simultaneously reducing COD levels. For example, Fezzani et al. [47] investigated the anaerobic co-digestion of olive mill wastewater and olive mill solid waste, demonstrating the significant conversions of organic compounds into methane, an economically valuable biogas that can be used for producing heat and electricity, under mesophilic condition. Aerobic biological treatments and composting processes have also been explored, particularly when OMW is co-treated with other organic wastes [48]. Nevertheless, the application of biological processes to raw OMW is often limited by the high concentration of phenolic compounds and the acidic nature of the effluent, which can inhibit microbial growth and reduce process stability. For this reason, biological treatments are frequently

preceded by physicochemical pretreatments aimed at reducing toxicity and improving biodegradability.

While these strategies can contribute to lowering the organic load and toxicity of the effluent, their effectiveness is often limited by the inhibitory activity of phenolic compounds and by the complex and variable composition of OMW. Moreover, many conventional treatment processes focus primarily on pollutant removal rather than on resource recovery, often resulting in the degradation or loss of valuable bioactive compounds present in the wastewater. In recent years, increasing attention has therefore shifted from simple waste treatment toward valorization strategies, in which olive mill wastewater is considered a potential source of valuable compounds rather than a waste requiring disposal. In particular, the recovery of phenolic compounds from OMW has attracted considerable interest due to their antioxidant activity and potential applications in food, pharmaceutical, cosmetic, and agricultural sectors.

Several technologies have been investigated for the recovery of phenolic compounds from olive mill wastewater. Among these, solvent extraction methods represent one of the earliest approaches proposed for phenolic recovery. Liquid–liquid extraction typically involves the acidification of the wastewater followed by the use of organic solvents such as ethyl acetate to selectively extract phenolic compounds including hydroxytyrosol and tyrosol [49]. Although this method can provide relatively high extraction efficiencies, its large-scale application is often limited by solvent consumption, environmental concerns, and the need for additional solvent recovery steps [27]. More recently, greener extraction strategies have also been explored for the recovery of phenolic compounds from OMW. For example, Hernández-Fernández et al. demonstrated that deep eutectic solvents can be effectively used to recover polyphenols from olive mill wastewater and olive leaves for biological applications [50].

Adsorption-based techniques have also been extensively investigated for phenolic recovery from OMW. Various porous materials, including activated carbon, polymeric resins, and natural biosorbents, have been tested for their ability to selectively capture phenolic compounds through hydrophobic interactions and π – π stacking mechanisms. For example, Agalias et al. [51] developed a process based on adsorption on polymeric resins that enabled the recovery of phenolic antioxidants from olive mill wastewater. While adsorption processes can produce phenolic-rich extracts with valuable biological properties, regeneration and desorption steps are generally required to recover the adsorbed compounds and restore the adsorbent material.

In addition to these approaches, pressure-driven membrane technologies have received increasing attention for the treatment and valorization of OMW. Membrane processes such as microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO) can be applied individually or in sequential configurations to clarify the wastewater, fractionate dissolved compounds, and concentrate phenolic-rich streams while simultaneously reducing the overall volume of effluents. For example, Bottino et al. [52] demonstrated that integrated membrane systems based on microfiltration

followed by reverse osmosis can effectively concentrate phenolic compounds from olive mill wastewater while producing a clarified permeate stream suitable for reuse. Compared with conventional solvent-based extraction methods, membrane separations operate under relatively mild conditions and avoid the use of organic solvents, making them particularly attractive from an environmental and operational perspective [53].

For these reasons, integrated separation strategies combining different technologies are increasingly investigated within a biorefinery framework, in which olive mill wastewater is considered a renewable feedstock for the recovery of bio-derived active ingredients rather than simply a waste requiring disposal [54–56].

1.5 Applications of OMW-derived products

Building upon this biorefinery perspective, recent research has demonstrated that OMW can support a broad spectrum of applications, extending far beyond the extraction of phenolic antioxidants. As a chemically rich and multifunctional feedstock, OMW enables the production of bioenergy carriers, functional materials, agronomic amendments, microbial metabolites, and high-value bioactive ingredients, positioning it as a versatile resource within modern circular-economy frameworks.

One of the most explored application domains concerns the use of OMW-derived products in agriculture, particularly as natural pesticides, plant protection agents, or soil amendments. The phenolic compounds contained in OMW exhibit antimicrobial and phytopathogen-inhibiting activity, making them promising candidates for sustainable crop protection. For example, Yangui et al. [57] demonstrated that hydroxytyrosol-rich extracts obtained from olive mill wastewater were able to inhibit the growth of *Pectobacterium carotovorum*, significantly reducing the development of soft rot in potato tubers during storage. These results highlight the potential of OMW-derived phenolics as natural agents for plant disease control. Additional studies have also reported the use of OMW extracts for biological control of plant pathogens and agricultural pests [58–62].

OMW has also been investigated as a substrate for microbial bioprocesses and bioenergy production due to its high organic load and significant carbon content. Crognale et al. [63] demonstrated that olive mill wastewater can be used as a fermentation substrate for the production of β -glucan by *Botryosphaeria rhodina*, showing that this effluent can act as a low-cost carbon source for microbial metabolite production. Similarly, OMW has been explored for the production of other microbial products and bioenergy carriers, including xanthan gum and biogas through anaerobic digestion [41,64].

Among the various valorization strategies, the recovery of phenolic compounds from OMW has attracted particular attention due to their strong antioxidant activity and potential industrial applications. Bernini et al. [65] demonstrated that hydroxytyrosol recovered from olive processing residues represents a highly valuable natural antioxidant with potential applications in food preservation and nutraceutical formulations. Numerous studies have also investigated

formulation-oriented approaches, including enzymatic lipophilization, to enhance the solubility, stability, and incorporation of OMW-derived phenolic compounds into lipid-based delivery systems, thereby broadening their applicability in food, cosmetic, and pharmaceutical formulations [66–68].

The strong antioxidant and anti-inflammatory properties of OMW-derived phenolic compounds have also stimulated growing interest in their use in cosmetic and biomedical formulations. Micheli et al. [69] highlighted the biological activities of hydroxytyrosol and oleuropein, including antioxidant, anti-inflammatory, and protective effects against oxidative stress-related disorders, suggesting their potential use as active ingredients in nutraceutical and dermocosmetic products. In this context, phenolic extracts derived from olive processing residues are increasingly investigated for applications in pharmaceuticals, functional foods, and skin-care formulations [28,70].

Beyond the recovery of individual molecules, OMW has also been explored as a precursor for the production of functional materials. For instance, thermochemical conversion of OMW can generate biochar or activated carbon materials with promising adsorption properties for environmental remediation [71]. In addition, phenolic-rich extracts obtained from olive processing by-products have been incorporated into functional materials and industrial products, including active packaging systems and advanced biomaterials [72]. More recently, growing attention has been devoted to the use of agro-industrial residues as substrates for insect bioconversion processes. In particular, organic wastes from the agri-food sector can be used as feed for larvae of species such as the black soldier fly (*Hermetia illucens*), enabling the production of insect biomass rich in proteins and lipids that can be further utilized in animal feed or other bio-based applications [73].

Taken together, these diverse applications position OMW as a renewable and versatile platform within modern biorefineries, capable of generating energy, materials, microbial metabolites, and high-value bioactive products. This expanded valorization landscape provides the conceptual foundation for the present research, which aims to explore integrated separation and refining strategies for the efficient recovery and application-oriented utilization of OMW-derived bioactive components.

From a technological perspective, the effective valorization of agro-industrial by-products such as OMW requires the implementation of integrated processing chains capable of progressively reducing matrix complexity while selectively isolating valuable compounds. This typically involves an initial pretreatment stage aimed at removing suspended solids and stabilizing the matrix, followed by a cascade of separation processes designed to fractionate and concentrate bioactive molecules. Once isolated and, when necessary, further purified, these compounds can be incorporated into industrial formulations and commercial products. Through such approaches, waste streams with negligible economic value can be transformed into sources of high-value bio-derived ingredients, contributing to the transition from waste management to resource-oriented biorefinery systems.

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CHAPTER 2: OBJECTIVES OF THE STUDY

2.1. Premises

This PhD project is developed within Spoke 6 – “Management models to promote sustainability and resilience of agricultural systems” of the AGRITECH National Research Centre, funded under Italy’s National Recovery and Resilience Plan (PNRR). AGRITECH promotes sustainable innovation in agri-food systems through coordinated research involving academia, industry and policy makers. Its activities are structured around five strategic pillars: resilience, aimed at enhancing sustainable productivity and climate adaptability; low impact, focused on reducing waste generation and environmental pressure; circularity, promoting circular economy strategies in agri-food chains; recovery, supporting the sustainable development of marginal areas; and traceability, improving safety and transparency in food supply chains, in synergy with the European Green Deal and the Farm-to-Fork strategy [1].

Within this framework, the present research contributes to the circularity and low-impact pillars by investigating strategies for the valorization of olive mill wastewater. In particular, this work explores integrated separation and refining approaches aimed at recovering bioactive compounds from OMW and converting them into value-added products for agricultural and industrial applications.

2.2. Research challenges

The valorization of olive mill wastewater is challenged by the intrinsic complexity of this matrix. Its composition is highly variable and depends on several factors such as olive cultivar, extraction technology and seasonal conditions, while large volumes are generated within a relatively short period during the olive oil extraction campaign [2–4].

In addition, the selective recovery of valuable bioactive compounds from OMW is further complicated by the high content of suspended solids and organic matter, which can promote fouling phenomena and significantly affect separation and fractionation processes [5,6]. Despite these challenges, the recovery of phenolic compounds remains of particular interest due to their biological activity. Among them, low-molecular-weight phenolic compounds represent some of the most valuable constituents of OMW due to their well-documented antioxidant and antimicrobial properties and their potential use in the development of bio-based products [7,8]. Finally, the high concentration of dissolved inorganic salts can interfere with purification processes and limit the direct valorization of OMW-derived fractions, requiring dedicated separation strategies.

2.3. Objective and thesis structure

Considering the challenges described above, the main objective of this research was to investigate strategies for the valorization of OMW through the recovery and fractionation of bioactive compounds, with particular focus on low-molecular-weight phenolic molecules, such as tyrosol, hydroxytyrosol and 3,4-dihydroxyphenylglycol (hereafter referred to as glycol). To achieve this goal,

different separation and refining strategies were explored to progressively reduce matrix complexity, recover valuable phenolic compounds and obtain fractions suitable for further valorization.

The procedures developed during this PhD project and discussed in the following chapters were progressively refined over the three-year research period. Initial exploratory experiments were conducted during the first year at laboratory scale, followed by process optimization and consolidation during the second and third years. During this period, collaborations with several academic and research institutions were also established, enabling the practical evaluation of the different streams generated within the proposed processing chain.

Chapter overview

Chapter 3 focuses on the pretreatment stage, consisting of an acidification step using concentrated sulfuric acid (98% H_2SO_4) followed by microfiltration using membranes with different materials and configurations. This combined approach aims to reduce suspended solids, residual oil processing materials (such as pulp, leaves and pomace), and part of the inorganic salts. At the same time, acidification contributes to stabilizing phenolic compounds, which remain protonated under acidic conditions and therefore less susceptible to oxidation or microbial degradation. The process also generates a secondary stream of interest, referred to as acidified sludge. One of the key objectives explored in this chapter is the comparison between different microfiltration configurations, including ceramic and polymeric membranes, as well as cross-flow and dead-end filtration modes, in order to evaluate whether lower-cost polymeric systems can approach the performance of more established ceramic membranes.

Chapter 4 describes the application of tighter membrane processes aimed at separating a phenolic-rich concentrate from a partially clarified aqueous stream. Nanofiltration was used to fractionate phenolic compounds according to their molecular weight, allowing the concentration of lower-molecular-weight phenolics with higher potential economic value. A diafiltration step was subsequently employed to recover smaller compounds that remained in the NF retentate. Finally, reverse osmosis was used to further concentrate both organic compounds and salts.

However, membrane processes alone are not sufficient to remove one of the main obstacles to the direct use of OMW-derived products: the high salt content.

For this reason, **Chapter 5** investigates the use of anion exchange resins (Amberlyst A26 in OH^- form) for the recovery of phenolic compounds from OMW nanofiltration permeate. Preliminary exploratory experiments were carried out to determine the optimal resin dosage and to investigate the influence of pH on adsorption phenomena. These studies led to the development of a packed-bed adsorption-desorption process capable of producing phenolic-rich fractions while partially separating them from acidic and inorganic species present in the feed.

Chapter 6 presents an innovative study conducted during the research stay at Lund University, focusing on electrodialysis (ED) as an alternative membrane process. Unlike pressure-driven

membrane systems, electrodialysis uses an electric field to separate positively and negatively charged ions across ion-exchange membranes. Although widely applied in fields such as brackish water desalination and food processing, this technology remains largely unexplored for OMW treatment. The experiments demonstrated that electrodialysis can significantly reduce the electrical conductivity of OMW while preserving most of the bioactive phenolic compounds.

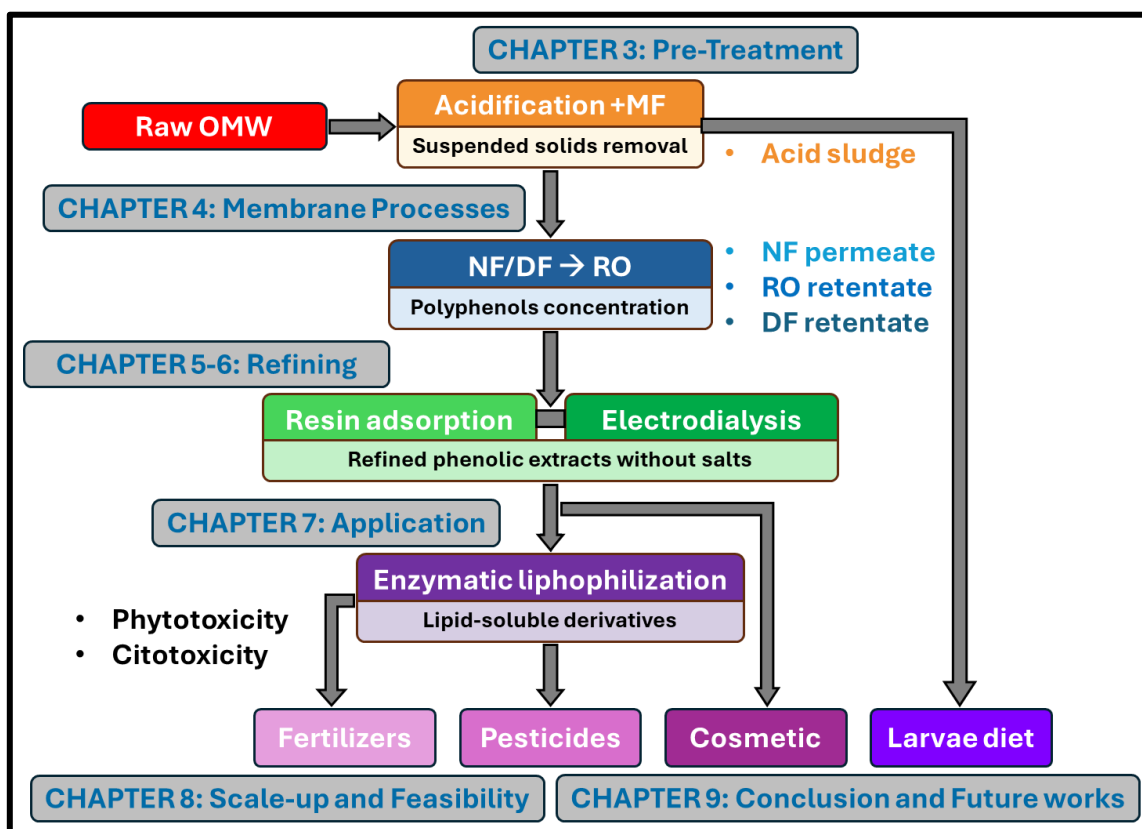
Chapter 7 explores several potential applications of the most promising fractions obtained throughout the process chain, including acidified sludge, NF permeate, and concentrates obtained from RO and DF stages. In addition, an enzymatic lipophilization reaction was investigated as a strategy to improve the formulation and distribution of phenolic compounds in commercial products. This reaction involves the attachment of long-chain esters to phenolic molecules, increasing their solubility in lipophilic matrices such as oils and fats. The biological properties of the obtained samples were also investigated through cytotoxicity and phytotoxicity assays, performed respectively on human keratinocyte cell line HaCaT and on cress seeds to evaluate germination index variations. Additional studies explored the use of acidified sludge as a supplement in diets for black soldier fly larvae, and the potential use of RO concentrates as active ingredients in cosmetic formulations, such as serums and hydrogels.

Chapter 8 presents a preliminary techno-economic assessment (TEA) aimed at evaluating the feasibility of the proposed process under different implementation scenarios. The first scenario considers the installation of simplified treatment systems directly at olive mills, prioritizing robustness and automation. The second scenario proposes a centralized processing facility where OMW from multiple mills could be treated and valorized through the integrated cascade process described in this work. Such an approach could enable the production of marketable bio-based products while transforming OMW from a problematic waste stream into a valuable secondary resource.

Finally, **Chapter 9** summarizes the main findings obtained during the three years of doctoral research and outlines potential directions for future work within the AGRITECH framework.

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CHAPTER 3: PRE-TREATMENT OF OMW BY ACIDIFICATION AND MICROFILTRATION

3.1. Introduction

Olive mill wastewater is a complex aqueous effluent generated during olive oil extraction, characterized by a highly heterogeneous physicochemical composition and a very high organic load. Depending on extraction technology, olive variety, and operating conditions, OMW typically contains large amounts of dissolved organic matter, suspended solids (TSS: 1-20 g/L), colloidal particles, lipids, inorganic salts, and phenolic compounds (0.5-15 g/L), contributing to its elevated COD (30-320 g/L) and significant environmental impact in Mediterranean olive-producing regions [1–4]. The organic components are mainly secoiridoids, phenolic acids, and phenolic compounds such as hydroxytyrosol, tyrosol. The latter represent a valuable fraction due to their strong antioxidant properties and potential applications in food, pharmaceutical, and cosmetic industries [5–12]. Despite this potential for valorization, the complex composition of OMW represents a major challenge for separation processes. In particular, the presence of suspended solids and colloidal material contributes to the turbidity of the wastewater and strongly affects membrane filtration, thereby necessitating effective pretreatment strategies.

Membrane-based processes have been widely investigated for OMW treatment and valorization due to their ability to operate under mild conditions while selectively separating suspended and dissolved fractions. Technologies such as microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO) have been proposed for wastewater clarification, phenolic compound recovery, and water reuse [7,13]. However, the application of membrane processes to raw OMW is strongly limited by membrane fouling, which arises from the accumulation of suspended solids, colloidal particles, and organic macromolecules on the membrane surface or within the pores.

Different fouling mechanisms may occur during OMW filtration, including pore blocking, adsorption of organic compounds, and the formation of cake layers composed of suspended particles and colloidal aggregates [14]. During concentration processes, the progressive accumulation of retained material may lead to the development of compressible cake layers, causing a rapid increase in hydraulic resistance and a decline in permeate flux [15]. For this reason, several operational concepts have been proposed to describe fouling-controlled filtration processes, including the critical flux, defined as the permeate flux below which fouling remains negligible over time, as well as the related concepts of threshold flux and sustainable flux, which better account for the gradual fouling observed in feeds such as OMW, where fouling-free operation is rarely achievable. In this framework, the threshold flux identifies the permeate flux above which fouling accelerates markedly, whereas the sustainable flux refers to the highest flux that can be maintained over long-term operation without inducing runaway fouling, thus ensuring stable filtration performance [16–18].

To mitigate fouling and improve filtration performance, various pretreatment strategies have been investigated for OMW prior to membrane filtration. These include sedimentation, centrifugation, coagulation–flocculation, and pH adjustment, which aim to reduce suspended solids and destabilize colloidal structures [19–21]. While these approaches can significantly improve feed filterability, they may also increase process complexity, generate additional sludge, or require chemical additives.

Recent studies emphasize that the treatment of OMW is often more effective when different technologies are combined within integrated treatment schemes, rather than relying on a single process [22,23]. Reviews on OMW treatment highlight that combined approaches involving physical, chemical, biological, and membrane processes can provide improved pollutant removal and facilitate resource recovery. Enaïme et al. [24] described a new combined process, comprising filtration of raw olive mill wastewater on two successive olive stone filters followed by a coagulation–flocculation. The results show a higher removal of total suspended solids and fatty matter from the raw OMW (about 82.5% and 73.8%, respectively) and a reduction of total phenolic compounds and chemical oxygen demand (COD) (11.3% and 23.2%, respectively). In another study Fleyfel et al. [25] presented an approach combining gravity filtration (gravel, sand, zeolite) and coagulation–flocculation achieving very high reductions in turbidity, fats, and TSS. Chidichimo et al. [26] proposed a combined treatment based on straw filtration followed by nanofiltration to clarify olive mill wastewater and recover valuable phenolic compounds. Straw filtration was used as a pretreatment step to remove suspended solids and reduce COD (about 70%), while the subsequent nanofiltration stage allowed an overall COD reduction up to 97.6% and high retention of polyphenols and antioxidant activity. Within these integrated strategies, microfiltration is frequently used as a clarification step, enabling the removal of suspended solids while allowing dissolved phenolic compounds to remain in the permeate stream for subsequent recovery through tighter membrane processes [27].

The performance of MF processes strongly depends on the membrane material and module configuration. Ceramic membranes are generally more resistant to fouling and allow the application of aggressive chemical cleaning procedures due to their high thermal and chemical stability, although they are typically associated with higher capital costs. Polymeric membranes, including hollow-fiber (HF) modules, are generally more cost-effective and widely used in water treatment applications, but they may require careful fouling control through hydrodynamic optimization and periodic cleaning operations [28,29]. In pressurized cross-flow systems, the feed stream flows tangentially along the membrane surface, generating shear stress that helps limit the accumulation of deposited material and mitigate cake layer formation. Conversely, hollow-fiber membranes can also be operated in submerged or dead-end configurations, where permeate is withdrawn by suction and fouling control relies on periodic backwashing and hydrodynamic scouring mechanisms. While these configurations allow operation at lower transmembrane pressures and reduced pumping energy, they are generally more sensitive to particle deposition and therefore require effective fouling management strategies [30].

Based on these considerations, the present work investigates an integrated pretreatment–membrane approach for OMW treatment. In the proposed strategy, raw OMW was first acidified with concentrated H_2SO_4 to pH 2, a step that promoted the aggregation and precipitation of suspended and colloidal material. The mixture was then allowed to settle, enabling the physical separation of an organic-rich solid material (sludge) from the clarified OMW (supernatant). The clarified fraction was then pumped into a separate tank and subsequently treated by microfiltration using three different configurations: (i) a ceramic multichannel membrane operated in cross-flow mode, (ii) a polymeric hollow-fiber module operated in cross-flow mode, and (iii) a submerged hollow-fiber system operated in dead-end suction mode. The objective was to characterize the sludge deposited at the bottom and to evaluate the filtration performance and fouling behavior of the three configurations when treating acidified OMW, as well as to assess their suitability as clarification steps prior to tighter membrane processes aimed at the recovery of phenolic compounds.

3.2. Materials and Methods

3.2.1. Materials

Olive mill wastewaters coming from three-phase system olive oil production were supplied by Frantoio Rocca Danilo (Tovo San Giacomo, Italy) and its composition is shown in Table 3.1.

Table 3.1: Composition of olive mill wastewater.

Parameter	Raw OMW
pH	4,5
Electrical conductivity	4777 $\mu\text{S}/\text{cm}$
Total suspended solids (TSS)	$7.0 \pm 0.6 \text{ g/L}$
Total solids (TS)	$18.7 \pm 0.6 \text{ g/L}$
Target polyphenols	$638.7 \pm 15.2 \text{ mg/L}$

3,4-dihydroxyphenylglycol, hydroxytyrosol, tyrosol were used as standard for HPLC calibrations. Acetonitrile and trifluoroacetic acid were used for HPLC mobile phase preparation. All the chemicals were purchased from Sigma Aldrich (Milan, Italy). Sulfuric acid (supplied by Merck KGaA, Darmstadt, Germany) were used to change the pH value of OMW. NaOH, NaOCl, HCl (Sigma Aldrich) were used for membrane cleaning and foulant removal. The characteristics of commercial membranes used in this work are summarized in Table 3.2.

Table 3.2: Membrane specifics.

Membrane	Material	Conformation	Cut-off	Area
Membralox EP19-40	Ceramic (α -Al ₂ O ₃)	3 units, each of 19 channels (4 mm Ø, 1020 mm lenght)	0.2 µm	3 x 0.24 m ²
Zenon ZW500	Polyvinylidene Fluoride (PVDF)	6 HF (1.2 mm OD, 200 mm length)	0.04 µm	0.0045 m ²
Pentair X-flow	Polyethersulfone (PES)	6 HF (1.5 mm OD, 240 mm length)	0.02 µm	0.0068 m ²
3M™ MF-PP	Polypropylene (PP)	5 HF (2.7 mm OD, 330 mm lenght)	0.2 µm	0.014 m ²
3M™ MF-PP	Polypropylene (PP)	20 HF (1.8 mm ID, 300 mm lenght)	0.2 µm	0.034 m ²

OD = Outside Diameter, ID = Inside Diameter, HF = hollow fiber

3.2.2. Methods

3.2.2.1. Acidification protocol

Raw olive mill wastewater (raw OMW), with an initial pH of 4.5, was collected from the olive mill and stored at room temperature until use. Before acidification, the sample was gently homogenized to ensure representative sampling. Acidification was then performed by gradual addition of 98% sulfuric acid until reaching pH 2. This pH value was selected based on literature reports indicating that acidification to pH 2 effectively promotes coagulation and settling of suspended and colloidal material, thus improving phase separation efficiency [19]. After acid addition, the acidified OMW was left to settle at room temperature for approximately 24 h to allow the suspended solids to sediment under gravity.

Following the settling period, the clarified supernatant, hereafter referred to as acidified and clarified OMW (OMW_pH2), was transferred to a separate tank using a peristaltic pump. To minimize the carry-over of particulate matter during the transfer, filter bags with a 200 µm opening (non-woven fabric) were employed. The settled fraction remaining at the bottom of the acidification vessel was collected and subsequently air-dried to obtain a semi-dry solid material, hereafter referred to as sludge. These three matrices, raw OMW, acidified and clarified OMW (OMW_pH2), and semi-dry sludge, represent the starting materials used in all subsequent physicochemical and analytical analyses.

3.2.2.2. Physicochemical analyses

pH and Electrical conductivity

The pH was measured using a Hanna pH209 pH meter, while electrical conductivity (EC) was determined using a Hanna EC215TM conductivity meter equipped with a Hanna HI76303TM probe.

Gravimetric analyses and FE-SEM/EDX elemental analysis

Gravimetric drying at increasing temperatures (105 °C, 180 °C, and 600 °C) was performed directly, without any prior evaporation step, to evaluate the distribution of water, volatile compounds, and mineral content in 20 mL of both raw and acidified olive mill wastewater (OMW_pH2), as well as in approximately 50 g of semi-dry sludge placed in porcelain crucibles. This procedure allowed the determination of total solids, corresponding to the dry weight at 105 °C, the volatile fraction (Equation 1), and the ash content (Equation 2). All measurements were carried out according to Standard Methods [31].

Volatile fraction:

$$(\text{Residue at } 105^{\circ}\text{C} - \text{Residue at } 600^{\circ}\text{C}) / \text{Residue at } 105^{\circ}\text{C} \times 100 \quad (1)$$

Ash content:

$$\text{Residue at } 600^{\circ}\text{C} / \text{Residue at } 105^{\circ}\text{C} \times 100 \quad (2)$$

A field emission scanning electron microscope (FE-SEM) (Zeiss Supra 40VP, Carl Zeiss, Germany) equipped with an energy-dispersive X-ray spectroscopy (EDX) detector was used to perform elemental analysis of the inorganic residue obtained after calcination at 600 °C. Analyses were conducted on raw OMW, OMW_pH2 and sludge.

Thermal analysesThermogravimetric analysis (TGA)

Thermal degradation of the samples under an inert atmosphere was performed using a thermal TGA 1 analyzer (Mettler Toledo, Milan, Italy). Thermogravimetric analysis (TGA) was carried out to estimate the higher heating value (HHV) of the sludge sample following a procedure based on ASTM E1131-20 [32]. Experiments were conducted on the solid part of the sludge obtained after acidification pre-treatment. Approximately 10 mg of solid samples pre-dried at 50 °C were placed in an alumina crucible for the analysis. In the first segment of the experiment, the sludge sample was heated at 10 °C /min in an inert environment (N₂ gas) from ambient temperature to 110 °C with a hold time of 5 min. Then, the sample was heated to 800 °C with the same heating rate and an isothermal period of 7 min at this temperature. After this, from 800 °C to 900°C, carrier gas was switched to O₂ with a dwelling time of 10 min to ensure complete combustion of the sample. N₂ and O₂ flow was maintained at 80 mL/min for the complete run. Prior to the sample run, blank experiments with an empty crucible were performed to obtain baseline correction. For accuracy of results, experiments were done in duplicate and reproducibility of the data was assured.

Proximate analysis

The proximate parameters were evaluated similar to ASTM Standard E1131-20 [32]: moisture content (MC) and volatile material (VM) were calculated in the nitrogen atmosphere; MC was measured as the weight loss between room temperature (starting temperature) and 110 °C; VM was considered to be the difference in mass at 110 °C and 800 °C, assuming that complete devolatilization took place before the combustion stage; fixed carbon (FC) was evaluated as the mass loss during the period between 800 °C and 900 °C when the N₂ carrier gas was substituted by O₂; Ash (A) was considered to be the residual mass at 800 °C in the oxidative atmosphere after combustion. These

proximate parameters were used to estimate the higher heating value (HHV) through Parikh's empirical correlation [33] (equation 3):

$$\text{HHV}(\text{MJ/kg}) = 0.03536\text{FC} + 0.1559\text{VM} - 0.0078\text{A} \quad (3)$$

where FC, VM, and A are the weight percentages on a dry basis yielding the HHV in MJ/kg.

Kinetic analysis (iso-conversional methods)

To investigate decomposition and devolatilization mechanisms during thermic programme, a kinetic study based on TGA data was carried out using model-free (iso-conversional) approaches. For each sample, thermogravimetric runs were carried out at several constant heating rates (typically 5-10-20-30 °C/min) generating a set of mass-loss curves (TG) required for iso-conversional analysis [34]. These methods support the assessment of energy potential and allow the determination of kinetic parameters without assuming a specific reaction mechanism.

In iso-conversional kinetics, the decomposition process is described as a function of the conversion degree (α), which represents the fractional mass loss relative to the total amount of material undergoing thermal degradation. Under the iso-conversional assumption, α is considered to depend only on temperature, while the temperature corresponding to a given conversion level (T_α) shifts with the applied heating rate β . This dependence $T_\alpha(\beta)$ provides the basis for determining the activation energy in iso-conversional methods [34].

The conversion was calculated using the following equation:

$$\alpha = \frac{m_0 - m_T}{m_0 - m_f} \quad (4)$$

where m_0 is the initial mass of the sample, m_T is the mass at a given temperature T , and m_f is the final mass after degradation in an inert environment.

Activation energy (E_a) was then obtained from the slope of the linearized equations derived from the Arrhenius equation, using three widely applied kinetic methods: Kissinger, Kissinger-Akahira-Sunose (KAS) Flynn-Wall-Ozawa (FWO) [35]. It should be noted that KAS and FWO are true iso-conversional methods, as they evaluate E_a at different α values, whereas Kissinger is not isoconversional, since it relies solely on the peak temperature T_m at each heating rate β .

$$\text{Kissinger} \quad \ln\left(\frac{\beta}{T_m^2}\right) = -\frac{E}{RT_m} + A \quad (5)$$

$$\text{FWO} \quad \ln(\beta) = -1.052 \frac{E}{RT_\alpha} + B \quad (6)$$

$$\text{KAS} \quad \ln\left(\frac{\beta}{T_\alpha^2}\right) = -\frac{E}{RT_\alpha} + C \quad (7)$$

Where: β = heating rate; T_p = temperature corresponding to the maximum degradation rate; E = activation energy; R = universal gas constant; T_α = temperature corresponding to a given conversion degree; A , B , and C = constants related to the pre-exponential factor.

Extraction and spectroscopic analysis

Soxhlet extraction

Soxhlet extraction was performed to determine the weight of water- and ethanol-soluble fractions of the acidified sludge. The extraction system consisted of a Soxhlet apparatus (30 mL extraction chamber) equipped with a Dimroth condenser (VWR, 533-2131) and coupled to a 250 mL round-bottom flask. Approximately 1 g of sludge, previously dried at 50 °C to constant weight, was placed in a cellulose thimble and inserted into the Soxhlet extractor. The flask was filled with 100 mL of Milli-Q water, and the system was heated using a hotplate (DIAB MS-H280-Pro) with temperature control. Extraction was carried out for 4 h under reflux conditions (~125 °C). After extraction, the liquid phase was collected, while the solid residue was dried at 50 °C to constant weight. The water-soluble fraction was determined gravimetrically from the mass difference before and after extraction. Subsequently, the dried residue was subjected to Soxhlet extraction using ethanol (95%) under similar conditions, with a heating temperature of approximately 105 °C.

FT-IR spectroscopy and GC-MS

Fourier Transform Infrared (FT-IR) spectroscopy analyses were performed to characterize both the dried sludge and the extracted liquids. Measurements were carried out using a Bruker Vertex 70 spectrophotometer equipped with an Attenuated Total Reflection (ATR) accessory. For each measurement, 128 scans were collected at a resolution of 2 cm⁻¹. Sludge samples were analysed after drying at 50 °C, while liquid extracts were dried at room temperature on aluminium foil prior to analysis. The ethanol-soluble fraction was further characterized by gas chromatography–mass spectrometry (GC–MS).

HPLC analysis

Target polyphenols quantification was carried out by high-performance liquid chromatography (HPLC) analysis. It was performed with a Shimadzu system (LC-10ATvp pump, SPD-10Avp detector, λ = 280 nm), equipped with a Synergi Hydro-RP column (150 × 3 mm, 4 μ m) and a pre-column filter. The mobile phase consisted of H₂O + 0.1% trifluoroacetic acid (A) and acetonitrile (B), with a 46 min gradient program. Injection volume: 5 μ L; flow rate: 0.7 mL min⁻¹; column temperature: 23–25 °C. Calibration curves for the target polyphenols and regression parameters are reported in the Supplementary Material.

3.2.2.3. Microfiltration setup and operating conditions

Microfiltration setup

Microfiltration trials were performed in batch mode using three different configurations: a cross-flow ceramic setup, a cross-flow polymeric hollow-fiber setup and a dead-end submerged hollow-fiber vacuum-suction setup. The three different plants are schematically showed in Figure 3.1.

Cross-flow ceramic setups. As shown in Figure 3.1 (a), OMW was fed by a centrifugal pump to the stainless-steel housing containing three parallel ceramic multichannel membranes (Membralox® EP19-40, Pall Corp., Port Washington, NY, USA). The system operated in outside-in cross-flow mode: the feed flowed through the channels, with the asymmetric filtration layer located on the inner

channel surface enabling selective permeation. The permeate passed radially through the porous support and was collected at the shell side.

Feed cross-flow velocity (CFV) was calculated from the ratio between the feed flow rate (measured by flow meter F) and the total cross-sectional area of the membrane channels. Operating pressure was monitored by two manometers (P1 and P2) installed before and after the module. The permeate flow rate was evaluated by measuring with a graduated tank the time necessary to produce a given permeate volume. Permeate flux was then calculated from the ratio between the permeate flow rate and the overall filtration surface area. The temperature measured by the thermometer, T, was kept constant by a cooling device immersed into the feed tank. A dedicated cleaning tank was used to circulate cleaning solutions (NaOH, NaOCl and HCl solutions) through the membrane module for the removal of organic fouling and inorganic scaling.

Cross-flow polymeric hollow-fiber setup. As shown in Figure 3.1 (b), OMW was fed by a centrifugal pump to two polymeric hollow-fiber modules connected in series. Each module contained a bundle of ten 3MTM MF-PP hollow fibers housed within a cylindrical shell. Filtration was operated in inside-out cross-flow mode, with the feed flowing through the fiber lumens and permeate collected on the shell side of the module. The retentate from the first module was directed to the second module before being returned to the feed tank.

During trials, additional hydraulic cleaning steps were applied to mitigate reversible lumen fouling. In these steps, valve V5 (feed-to-module valve) was closed to interrupt the forward cross-flow, generating a transient low-pressure differential on the permeate side that induced a permeate-driven backwash from the shell side to the lumen. The inverted TMP displaced detached foulants toward the lumen. Valve V5 was then reopened to resume normal cross-flow operation.

Dead-end submerged hollow-fiber vacuum-suction setup. As shown in Figure 3.1 (c), a polymeric hollow fibers module (3MTM MF-PP¹, Zenon ZW500, Pentair X-Flow) was fully immersed in a feed tank and connected at both ends to a permeate line. Permeate was withdrawn through the lumens by (i) a vacuum gauge regulating negative pressure and (ii) a peristaltic pump generating controlled suction.

This configuration resulted in dead-end inside-out vacuum-driven filtration, where the feed surrounding the fibers was drawn across the external membrane wall into the lumens. The permeate driving force (transmembrane pressure, TMP) was established by the combination of hydrostatic head in the tank and negative pressure applied on the permeate side.

¹ 3MTM MF-PP fiber are hydrophobic membranes – for this reason was necessary to wet them first with an alcoholic solution (ethanol) and then with water to make them lipophilic and therefore suitable to be used for OMW filtration.

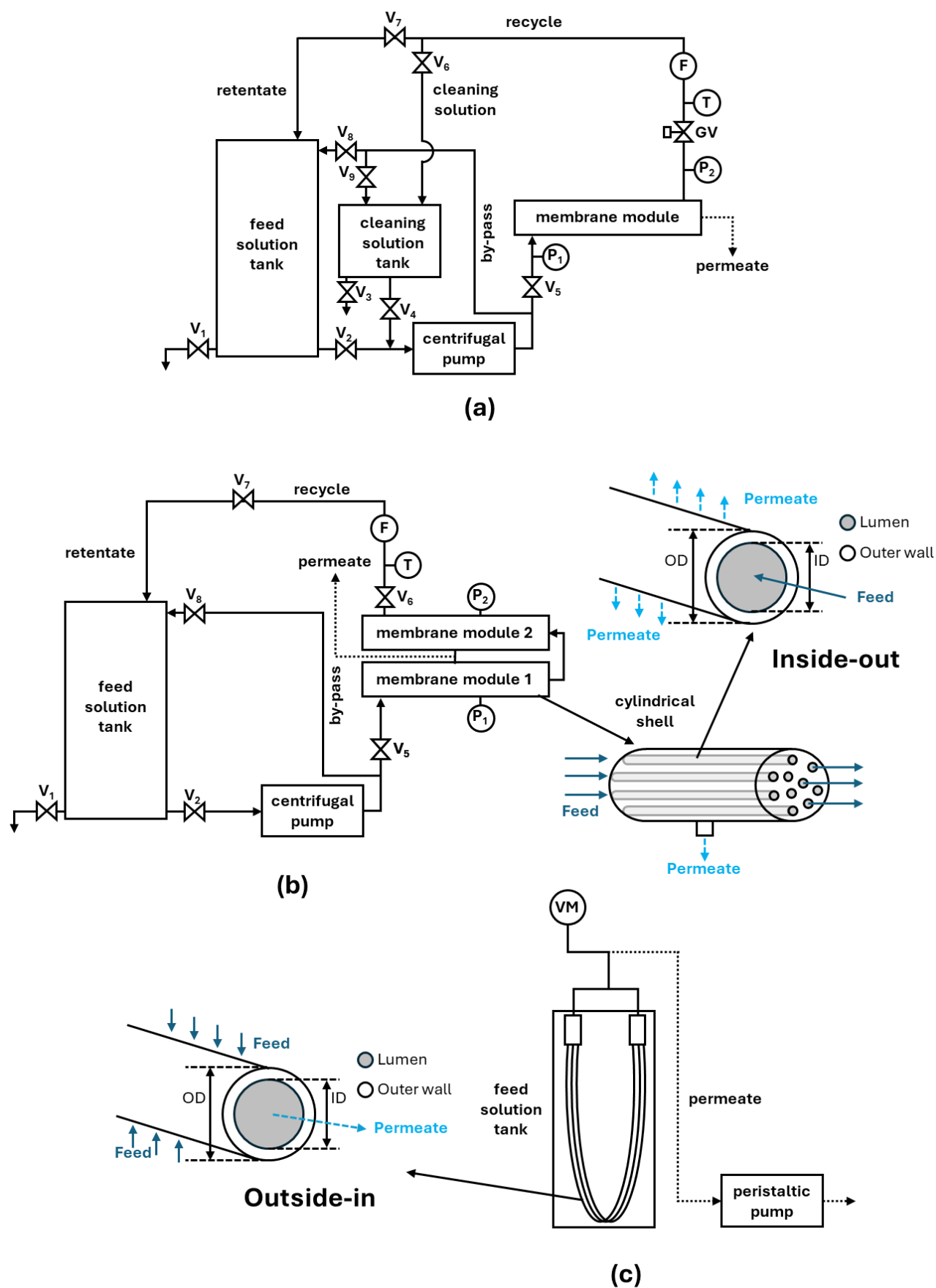


Figure 3.1: Schematic of the plants used for MF test with (a) Membralox EP19-40: V1-9 ball valves; GV gate valve; P1,2 manometers, T thermometer; F flowmeter. (b) V1-8 ball valves, P1,2 manometers, T thermometer; F flowmeter. (c) Hollow fiber: VM vacuum meter.

Operating conditions

Table 3.3 shows the operating conditions adopted for each configuration and the filtration surface for each module (ceramic cross-flow, polymeric cross-flow and polymeric dead-end).

Table 3.3: Operating condition and modules filtration surface.

Parameter	Ceramic cross-flow	Polymeric cross-flow	Polymeric dead-end
Temperature	25 °C	25 °C	25°C
Transmembrane Pressure	2.5 bar	0.9 bar	0.2-0.4 bar (via vacuum)
Filtration surface	0.72 m ²	0.034 m ²	0.0045-0.014 m ²
Feed flow rate	9000 L/h	100 L/h	/
Cross-flow velocity (CFV)	3.5 m/s	0.55 m/s	/

During the experiments, two main operating modes were adopted with the three filtration setups:

- Recirculation mode, where both permeate and retentate streams were continuously recycle to the feed tank, maintaining constant feed volume and solute concentration throughout the experiment. It was used to monitor flux stability over time.
- Concentration mode, where retentate was recycled to the feed tank while permeate was withdrawn from the system, resulting in a progressive decrease in feed volume and an increase in solute concentration over time. It was used for mimicking continuous operation.

Membrane selectivity was evaluated in terms of rejection of the solutes that are retained inside the membrane and calculated according to the following equation:

$$R(\%) = \frac{\sigma_{Feed} - \sigma_{Permeate}}{\sigma_{Feed}} \times 100 \quad (8)$$

where R (%) is the percentage rejection, and σ represents the variable examined. For example, sigma can be the concentration of polyphenols (from HPLC measurement) or the electrical conductivity.

The ratio between the initial volume of the feed and that of the concentrate (equation 8) defines the volume concentration ratio (VCR).

$$VCR = \frac{V_{feed}}{V_{retentate}} \quad (9)$$

After each filtration experiment, the membranes were chemically cleaned using alkaline solutions containing NaOH and/or NaOCl at concentrations ranging from 0.1 to 1 wt% (approximately 1–10 g/L), depending on the membrane type and the severity of fouling. Cleaning was performed at temperatures between 25 and 30 °C for polymeric membranes and up to 60 °C for ceramic membranes, with contact times ranging from 30 to 60 min. When alkaline cleaning alone was insufficient to restore membrane performance, an additional acid cleaning step was carried out using HCl solutions at concentrations between 0.1 and 1 wt% (approximately 1–10 g/L). Thorough rinsing

with deionized water was performed between alkaline and acidic cleaning steps to avoid neutralization reactions and ensure effective membrane regeneration. The effectiveness of the cleaning protocol was evaluated by measuring the pure water flux before (J_{W0}) and after filtration (J_{WF}) and flux recovery ratio (FRR) was defined as:

$$FRR = (J_{WF} / J_{W0}) \times 100 \quad (10)$$

3.2.2.4. Critical flux evaluation

To investigate membrane fouling behaviour and determine the critical flux (J_c), filtration experiments were carried out through both short-term and long-term trials.

The critical flux was determined in short-term experiments using a flux-step method [30]. The method was applied by progressively increasing the suction setpoint (adjusting peristaltic pump/vacuum level) to impose stepwise increases in permeate flux (J). At each step, TMP was continuously recorded until stabilization or until a monotonic increase was observed. For each flux step, two TMP values were considered: (i) the initial TMP, corresponding to the rapid increase in filtration resistance immediately after the flux increment, and (ii) the final TMP, corresponding to the TMP measured at the end of the step. From these values, two fouling-related parameters were derived: the average TMP and the rate of TMP increase ($dTMP/dt$). The critical flux was defined as the permeate flux at which $dTMP/dt \geq 0.5$ mbar/min [36].

The total filtration resistance (R_{tot}) was estimated using the resistance in series model:

$$R_{tot} = R_m + R_c + R_f \quad (11)$$

where R_m is the intrinsic membrane resistance (obtained from pure water permeability measurements, performed prior to filtration experiments), R_c is the cake resistance and R_f is the fouling resistance due to irreversible adsorption and pore plugging. Under pure water conditions, no critical flux is observed, and R_m can be determined from the slope of the linear relationship between permeate flux and transmembrane pressure.

R_m and R_{tot} were calculated according to Darcy's law:

$$R_{tot} = TMP / \mu \cdot J \quad (12)$$

where μ is the fluid viscosity that was assumed equal to that of water at the operating temperature.

After identifying the critical point during the ramp-up sequence, the protocol was repeated in reverse (ramp-down) by progressively decreasing the permeate flux in order to evaluate possible hysteresis effects between increasing and decreasing flux steps.

Long-term trials were carried out to evaluate the fouling behavior of the submerged hollow-fiber modules during extended operation at constant permeate flux. In these experiments, the permeate was continuously withdrawn from the feed tank, and the filtration was therefore performed in continuous mode. A dual-channel peristaltic pump was used to operate two hollow-fiber modules in parallel, allowing the direct comparison of their filtration performance over time. The evolution of TMP was

continuously monitored during the experiments. Filtration tests were conducted under three operating conditions relative to the critical flux determined during short-term trials: (i) below the critical flux, (ii) close to the critical flux, and (iii) above the critical flux.

To assess fouling reversibility, periodic back-flush operations were performed by reversing the permeate flow direction for a fixed time interval. The effect of back-flushing was evaluated by comparing the TMP evolution before and after the cleaning step. At the end of each experiment, membrane permeability was assessed through pure water flux measurements to evaluate the extent of irreversible fouling and the effectiveness of the already mentioned cleaning protocol.

3.3. Results and Discussion

3.3.1. Acidification

The effect of acidification on phase separation is illustrated in Figure 3.2, which shows the evolution of OMW in Imhoff settling cones following the addition of sulfuric acid.

The addition of sulfuric acid immediately promoted flocculation and precipitation of suspended solids and colloidal material generating a settled layer at the bottom and leaving a relatively clearer acid OMW (supernatant) at the top. A substantial part of the separation was already evident within 1 h after acid addition. After 24 h, the settled layer was estimated to occupy approximately 10% of the initial OMW volume. This treatment resulted in an increase in electrical conductivity of the OMW due to the addition of sulfate ions. To validate the observations obtained at bench scale, the procedure was scaled up to approximately 1000 L of raw OMW, which were acidified with about 1.5 L of 98% H_2SO_4 (2.8 kg) until reaching pH 2. After the settling step, approximately 920 L of acidified and clarified OMW (OMW_pH2) were recovered as the supernatant, while the settled fraction amounted to approximately 80 L. Following air-drying, this settled material yielded about 30 kg of semi-dry matter.

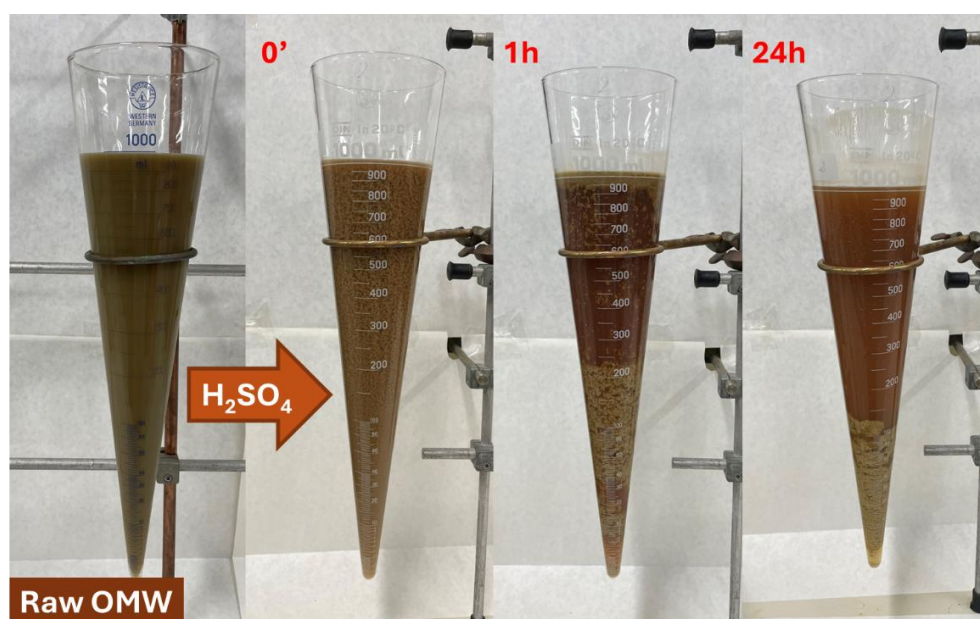


Figure 3.2: Imhoff settling cone with OMW and its evolution after H_2SO_4 addition.

Overall, the acidification pre-treatment produced two distinct fractions from the raw OMW: (i) an acidified and clarified liquid phase (OMW_pH2), corresponding to the supernatant subsequently fed to microfiltration, and (ii) a semi-solid fraction obtained after supernatant removal, consisting of the material settled at the bottom and then air-dried to yield a semi-dry sludge. These three samples, raw OMW, acidified and clarified OMW, and semi-dry sludge, constitute the starting materials characterized and processed in the following sections.

3.3.1.1. Gravimetric and FE-SEM/EDX analysis

The three samples were subjected directly, without any prior evaporation step, to a thermal analysis sequence typically applied to aqueous matrices. The residue at 105 °C represented the total solids (TS), including both organic and inorganic matter. The weight loss between 105 °C and 600 °C corresponded to the volatile fraction, while the residue remaining at 600 °C represented the inorganic ash content. To better interpret the transformations occurring during heating, intermediate mass-loss steps were also considered. The weight loss between 105 °C and 180 °C was mainly associated with weakly bound or physically trapped water (e.g., hydration or crystallization water) together with light volatile organic compounds. The loss between 180 °C and 600 °C reflected the thermal degradation of the remaining organic matrix, generally referred to as volatile solids (VS).

The results of the gravimetric analysis for raw OMW and OMW_pH2 liquids and solid sludge are summarized in Table 3.4. For each determination, 20 mL of liquid were weighed into porcelain crucibles for raw OMW and OMW_pH2, whereas approximately 50 g (semi-wet basis) of the sludge were used.

Table 3.4: Gravimetric analysis results (TS, VS, ash) for OMW, OMW_pH2 and sludge.

Parameter	OMW	OMW_pH2	Sludge
pH	4.5	2	/
Electrical conductivity (μS/cm)	4777	8330	/
Total solids (% w/w)	1.9	1.4	27.5
Volatile solids (% w/w)	1.5	0.9	27.0
Ash Content (% w/w)	0.4	0.5	0.5
VS (% TS)	79	66	98
Ash (% TS)	21	34	2
Loss 105–180 °C (% TS)	30	11	12
Loss 180–600 °C (% TS)	50	55	86

% w/w normalized to initial wet mass; % TS normalized to total solids (residue at 105 °C). Stepwise losses (105–180 °C; 180–600 °C) calculated by subtraction. Each value refers to analyses performed on 20 mL (liquids) or ~50 g (sludge).

Raw OMW is a liquid matrix with a relatively low solids content per unit mass, as typically observed for this type of effluent [37]. Approximately 79% of the total solids decomposed between 180 and 600 °C, indicating a predominantly organic nature, while 21% remained as ash, confirming the presence of dissolved salts and mineral components. The acidified OMW showed a lower total solid content but a similar ash percentage, suggesting that acidification mainly removed organic and

colloidal matter while preserving the ionic load. At the same time, the relative inorganic fraction in the liquid increased (higher ash/TS ratio) due both to the removal of organic colloids and to the introduction of sulfate ions during acidification. Most of the organic fraction, following acidification with H_2SO_4 , settled as sludge at the bottom of the storage tank, consisting of approximately 98% organic matter and 2% ash, thereby facilitating clarification of the supernatant.

The sludge therefore represents a dense and concentrated organic matrix with a very low ash content, whereas the resulting supernatant exhibited lower total solids (diluted phase) and a higher relative inorganic fraction, consistent with sulfate addition and the removal of organic colloids. Overall, these results indicate that acidification acts as an effective pretreatment for reducing organic fouling potential.

FE-SEM/EDX analysis

To estimate the elemental composition of the inorganic ash in the three samples, SEM–EDX analyses were performed on the residues obtained after calcination at 600 °C. The most abundant elements are reported as relative percentages in Figure 3.3.

For clarity of presentation, oxygen (O), although detected and considered in the overall mass closure, is not shown in the bar chart. Carbon (C) is also not shown, as its signal is largely influenced by the carbon support/contamination and is not quantitative for these matrices.

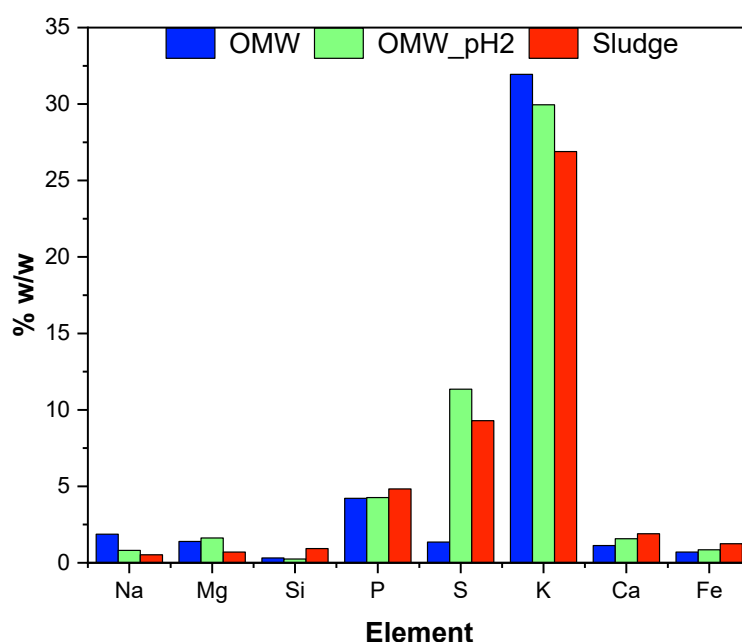


Figure 3.3: EM–EDX elemental composition of raw OMW, OMW_pH2 and sludge. Oxygen (O) was detected but omitted from the plot for clarity. Carbon (C) was not reported owing to substrate/contamination contributions and its non-quantitative character for these samples.

As also observed by C. Paredes et al. [38], K was the most abundant element in all three matrices. Its relative percentage was lower in the settled fraction, plausibly due to the high solubility of potassium salts, which tend to remain in the liquid phase. Acidification also led to a clear increase in the sulfur signal in both OMW_pH2 and the sludge, consistent with the introduction of sulfate ions via H_2SO_4 .

These EDX results provide a preliminary picture of the inorganic elements present in the three samples.

The elemental composition obtained by EDX analysis, combined with the ash content determined from the residue at 600 °C, was used to estimate the molar amount of each element present in 1 m³ of raw OMW (feed basis) and in the derived fractions. On this basis, the most probable inorganic salts were reconstructed through a stepwise stoichiometric allocation, sequentially assigning elements in the order sulfates → phosphates → oxides. The resulting compounds are reported in Table 3.5 as equivalent salts/oxides, with masses expressed as kg per 1 m³ of raw OMW and percentages reported as weight percent of total ash (W% of ash) within each stream. The unassigned percentage primarily corresponds to oxygen included in the stoichiometric mass closure (oxygen is not shown in the EDX bar chart for clarity).

Table 3.5: Reconstructed inorganic compounds for raw OMW, OMW_pH2 and sludge, obtained by combining SEM–EDX elemental abundances with gravimetric ash at 600 °C.

Equivalent salts/oxides	OMW		OMW_pH2		Sludge	
	Mass (kg/m ³)*	W% ash**	Mass (kg/m ³)*	W% ash**	Mass (kg/m ³)*	W% ash**
K ₂ O	0.85	22	/	/	/	/
K ₃ PO ₄	0.85	22	0.43	10	0.022	14
Mg ₃ (PO ₄) ₂	0.23	6	0.26	6	0.0038	3
Na ₃ PO ₄	/	/	0.085	2	0.0019	1
CaSO ₄	0.19	5	0.24	5	0.0097	7
K ₂ SO ₄	0.12	3	2.42	55	0.063	42
Fe ₂ O ₃	0.039	1	0.053	1	0.0027	2
SiO ₂	0.026	1	0.023	1	0.0030	2
Reconstructed salts	2.31	60	3.51	80	0.11	71
Unassigned***	1.54	40	0.9	20	0.4	29
Total ash	3.85	100	4.41	100	0.15	100

* Mass values expressed as kg per 1m³ of raw OMW (acidification of 1 m³ yielded 0.92 m³ of OMW_pH2 + 30 kg of dry sludge)

** Weight % of the stream's total ash per compound; *** Unassigned oxygen in mass closure

The obtained values represent a theoretical reconstruction consistent with the elemental quantities measured by SEM-EDX, but they do not constitute a direct determination of the mineralogical species present. This does not represent a thermodynamic speciation model, as potential equilibria influenced by pH, solubility, ionic activities, kinetic factors, or temperature were not considered.

The mass balance performed on the ash fraction revealed a significant redistribution of mineral species following acidification. In particular, the increase in ash/TS in the acidified OMW (from 21% to 34%) indicates a relative enrichment of the inorganic component, mainly attributable to sulfate introduction through the addition of H₂SO₄ (mainly K₂SO₄ and CaSO₄). In the sludge, the low ash fraction (2% of TS) suggests that settling primarily concentrated volatile solids, with selective co-precipitation of some mineral species (especially calcium phosphates/sulfates). The residual unassigned fraction is attributable to oxygen accounted for in the stoichiometric closure and to phases not readily expressible as simple salts (e.g., complex oxides, amorphous phases), with a possible

contribution from refractory carbon, which was not quantified in this analysis, as previously stated. XRD analysis would allow identification of the crystalline phases effectively present, while ICP-OES or ICP-MS analysis after total digestion would enable quantitative metal determination and cross-validation of the EDX results. For example, Miranda et al. [39] report high percentages of K_2O and P_2O_5 in concentrated olive mill wastewater (COMWW) using X-ray fluorescence spectrometer analysis.

3.3.1.2. Extraction test

Soxhlet extraction revealed a high extractable fraction in the acidified sludge. Approximately 25 wt% of the dry mass was solubilized in water, while an additional 50 wt% was extracted using ethanol, resulting in a total extractable fraction of about 75 wt%. These results indicate that the sludge contains a large proportion of solvent-extractable organic matter, including both low-molecular-weight compounds and potentially also extractable macromolecular or colloidal components. The remaining fraction (25 wt%) is likely composed of more recalcitrant organic matter, including condensed aromatic or lignin-like structures, which are known to occur in olive mill wastewater as part of its hydrophobic-neutral high-molecular-weight fraction [40].

The aqueous extract exhibited a yellow–ochre coloration, while the ethanol extract showed an intense green color, qualitatively indicating a compositional differentiation between the extracted fractions. In particular, water appears to preferentially solubilize polar oxidized compounds, such as low-molecular-weight organic acids and hydrophilic phenolic species, whereas ethanol extracts less polar species such as pigments and lipid-like molecules.

FT-IR analysis was performed on the raw acidified sludge and on the solid and liquid fractions obtained after sequential Soxhlet extraction with water and ethanol, in order to investigate the chemical differences among the extracted fractions. Band assignments were performed based on standard FT-IR interpretation of organic and biomass-derived materials reported in the literature [41,42]. It should be noted that the spectra were not normalized; therefore, variations in band intensity are discussed qualitatively.

In Figure 3.4 (a), corresponding to water extraction, the raw sludge (black curve) exhibits a broad band in the region $3600\text{--}3200\text{ cm}^{-1}$, attributed to O–H stretching vibrations associated with hydroxyl groups in phenolic compounds and residual moisture. Bands at approximately 2920 and 2850 cm^{-1} correspond to aliphatic C–H stretching, while the signal around $1700\text{--}1730\text{ cm}^{-1}$ is assigned to carbonyl groups ($C=O$), typical of organic acids and esters. After extraction, the solid residue (blue curve) shows a qualitative attenuation of bands associated with hydroxyl and carbonyl groups, indicating the removal of oxygenated compounds. The spectrum of the aqueous extract (cyan curve) confirms this behavior, showing pronounced bands in the O–H, C–O, and carboxylate regions.

In particular, the bands observed at ~ 1570 and $\sim 1400\text{ cm}^{-1}$ are attributed to asymmetric and symmetric stretching vibrations of carboxylate groups (COO^-), suggesting that organic acids are predominantly present in their deprotonated or salt form in the aqueous phase. Additionally, the band at $\sim 1090\text{ cm}^{-1}$

is assigned to C–O stretching vibrations, consistent with the presence of oxygenated compounds such as alcohols, phenols, and carbohydrate-like structures.

In Figure 3.4 (b), the starting material for ethanol extraction (hereafter referred to as sludge') corresponds to the solid residue obtained after water extraction (blue curve). Compared to sludge', the solid residue after ethanol extraction (dark-green curve) shows a reduction of signals in the aliphatic ($2920\text{--}2850\text{ cm}^{-1}$) and aromatic ($1600\text{--}1500\text{ cm}^{-1}$) regions, indicating the removal of semi-polar organic compounds. The ethanol extract exhibits characteristic bands in both the aliphatic and carbonyl regions. In particular, a dominant band at $\sim 1710\text{ cm}^{-1}$ is attributed to C=O stretching vibrations of carboxylic acids, while a weaker band at $\sim 1740\text{ cm}^{-1}$ indicates the presence of ester functionalities, suggesting a mixture of free fatty acids and esterified lipid-like compounds. Bands at ~ 1460 and $\sim 1410\text{ cm}^{-1}$ are associated with CH_2/CH_3 deformation vibrations, supporting the presence of long aliphatic chains. Additionally, a band at $\sim 940\text{ cm}^{-1}$ can be attributed to out-of-plane =C–H bending vibrations, consistent with unsaturated structures such as fatty acids.

The comparison between the two extraction steps highlights a clear and progressive fractionation of the sludge components. Water extraction preferentially removes polar oxygenated compounds, whereas ethanol targets aromatic and less polar organic species. This behavior is consistent with Soxhlet results, which showed that approximately 25 wt% and 50 wt% of the sludge are extracted by water and ethanol, respectively, confirming the predominance of solvent-extractable organic matter. Similar fractionation behavior has been reported for olive mill wastewater and related agro-industrial residues, where the use of solvents with different polarity enables the selective recovery of phenolic and lipid fractions [43,44]. In particular, solvent-based extraction approaches have been widely applied to olive-derived materials for the effectiveness recovery of phenolic and lipophilic compounds, including through Soxhlet extraction techniques [45]. The chemical features observed in the FT-IR spectra, including hydroxyl, carbonyl, and aromatic functional groups, are consistent with the known composition of olive mill wastewater, which is characterized by a complex mixture of phenolic and oxygenated compounds [41].

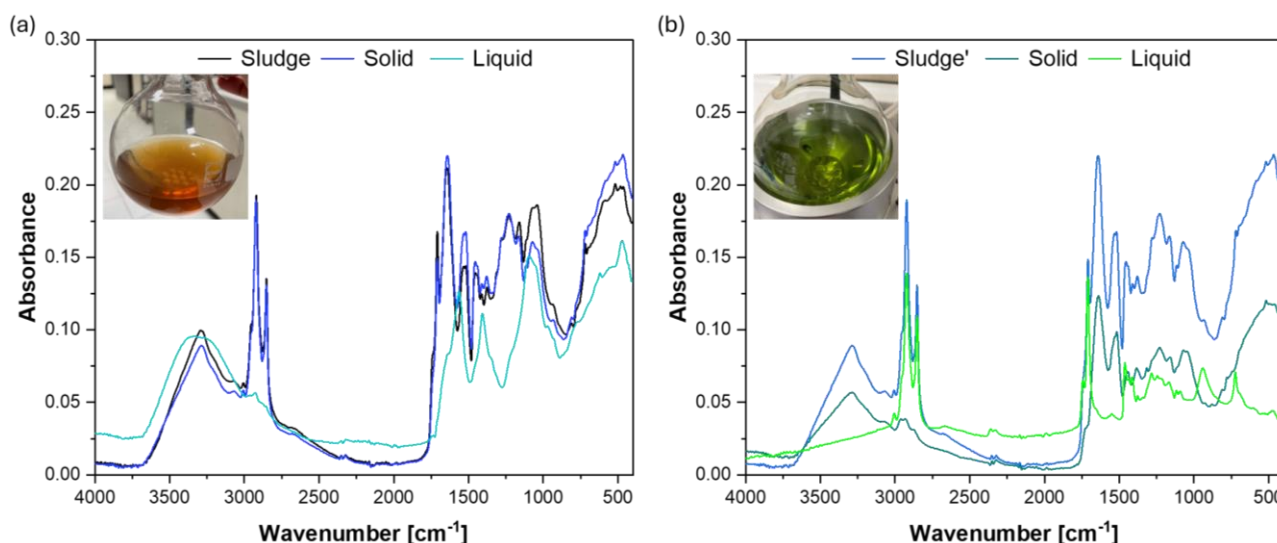


Figure 3.4: FT-IR spectra of sludge dried at 50°C and products after Soxhlet extraction using (a) water and (b) ethanol.

GC–MS qualitative analysis of the ethanol extract revealed the presence of fatty acids, with oleic acid identified among the main volatile components, confirming the extraction of lipid-like compounds by ethanol. This result is consistent with findings by Zghari et al. [41] study on olive mill wastewater composition. Conversely, HPLC analysis did not detect significant concentrations of the target phenolic compounds (3,4-dihydroxyphenylglycol, hydroxytyrosol, and tyrosol), suggesting that these compounds were not retained in the sludge and likely remained in the supernatant during the acidification step. This result is consistent with Leouifoudi et al. work [45], which reports significantly lower polyphenol content in olive cake (0.95 mg/g) compared to olive mill wastewater, where phenolic compounds are mainly concentrated in the liquid phase.

3.3.1.3. Thermogravimetric analysis

Estimation of higher heating value (HHV)

TGA was used for the mass loss experiments to estimate the HHV of the sludge sample and understand its behaviour in terms of energy potential. The results of this analysis can give better insight into the usage of sludge for energy and the resource recovery phase. Moisture content (MC), volatile material (VM), fixed carbon (FC), and ash (A) were calculated from TGA curves as outlined in Section 3.2.2.2, and an example of this can be seen in Figure 3.5, showing the (a) TG curve of the sludge sample previously dried at 50°C and (b) its derivative (DTG) which represents the rate of mass loss.

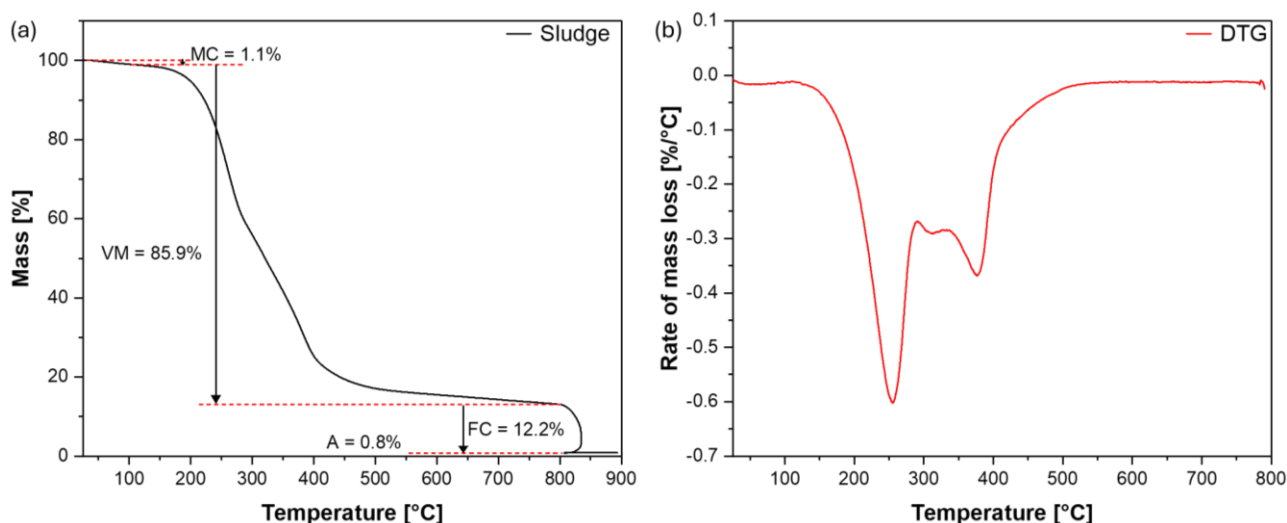


Figure 3.5: (a) TG and (b) DTG curves of the dried sludge obtained during thermogravimetric analysis at a heating rate of 10 °C/min.

For TG curve, it can be observed that there was an initial mass loss attributed to water volatilization, expressed as MC. A major weight loss occurs in the temperature range of approximately 200–500 °C, corresponding to the decomposition of the main volatile constituents of the sludge, in agreement with the high volatile matter content (VM = 85.9 wt%). After reaching 800 °C under inert atmosphere, the system was switched to an oxidizing environment (O₂), allowing complete combustion of the remaining carbonaceous residue. At this stage, fixed carbon (FC) and ash (A) were determined. The FC was calculated as the difference between the initial mass and the sum of MC, VM, and ash

fractions. The obtained proximate composition (high VM and low ash content) indicates that the sludge behaves as a highly organic and volatile-rich material rather than a mineral-rich residue.

The DTG curves (Figure 3.5 b) exhibit a main degradation region between approximately 200 and 500 °C, with multiple overlapping peaks, indicating a complex multi-step decomposition process. In particular, it can be identified a main peak centered at approximately 250 °C, followed by a shoulder at around 310–320 °C and a broader secondary peak at approximately 370–380 °C. In lignocellulosic materials, similar temperature ranges are often attributed to the degradation of hemicellulose, cellulose, and lignin [46,47]. However, such assignments are not directly applicable in the present case. Although lignocellulosic components cannot be excluded, their contribution to the thermal behavior is not dominant. Indeed, Soxhlet revealed that approximately 75 wt% of the sludge is solvent-extractable, while FT-IR analysis indicated a predominance of oxygenated, aromatic, and lipid-like compounds. These results suggest that the sludge consists mainly of a complex mixture of low- and medium-molecular-weight organic species rather than structural lignocellulosic polymers, which are well known to be insoluble in ethanol. Therefore, the DTG features are more appropriately interpreted as follows: the main peak (~250 °C) is associated with the decomposition of thermally labile compounds, including low-molecular-weight oxygenated species; the intermediate shoulder (~315 °C) reflects overlapping degradation of compounds with intermediate stability; and the higher-temperature peak (~370–380 °C) is attributed to more recalcitrant structures, likely involving condensed aromatic species and char precursors. This multi-step and partially overlapping behavior is typical of heterogeneous organic matrices and is consistent with previous studies on olive mill residues and organic-rich wastes [48,49].

HHV was estimated from thermogravimetric analysis using an empirical equation from the literature. The resulting HHV was 17.7 MJ/kg, typical of biomass and vegetal residues rather than conventional mineral-rich sludges. It is the VM that mainly contributed to the heating value of sludge (85.9%). From the literature, olive oil by-products with VM 70-80 % had a HHV of 20.7-26.3 MJ/kg (using a Parr 1351 Adiabatic Bomb) [39]. Other studies [49,50] report HHV ranged from 18 to 21 MJ/kg for OMW samples and from 17 to 24 MJ/kg for solid agricultural biomasses, confirming the potential of the sludge as a bio-based fuel.

Kinetic Models

The thermal degradation kinetics of the sludge were investigated using iso-conversional methods based on TG-DTG data obtained at four heating rates (5, 10, 20, and 30 °C/min). This approach allows the determination of the apparent activation energy (E_a) without assuming a specific reaction mechanism. Three methods were applied, namely Kissinger, Flynn–Wall–Ozawa (FWO), and Kissinger–Akahira–Sunose (KAS). The activation energy was calculated over a conversion range (α) from 0.1 to 0.8, with a step size of 0.1. Figure 3.6 shows the TG–DTG curves at four different heating rates together with the linear fits obtained for the three iso-conversional methods. The determination of the straight-line slopes from Figure 3.6 allows the calculation of the activation energy, as summarized in Table 3.6.

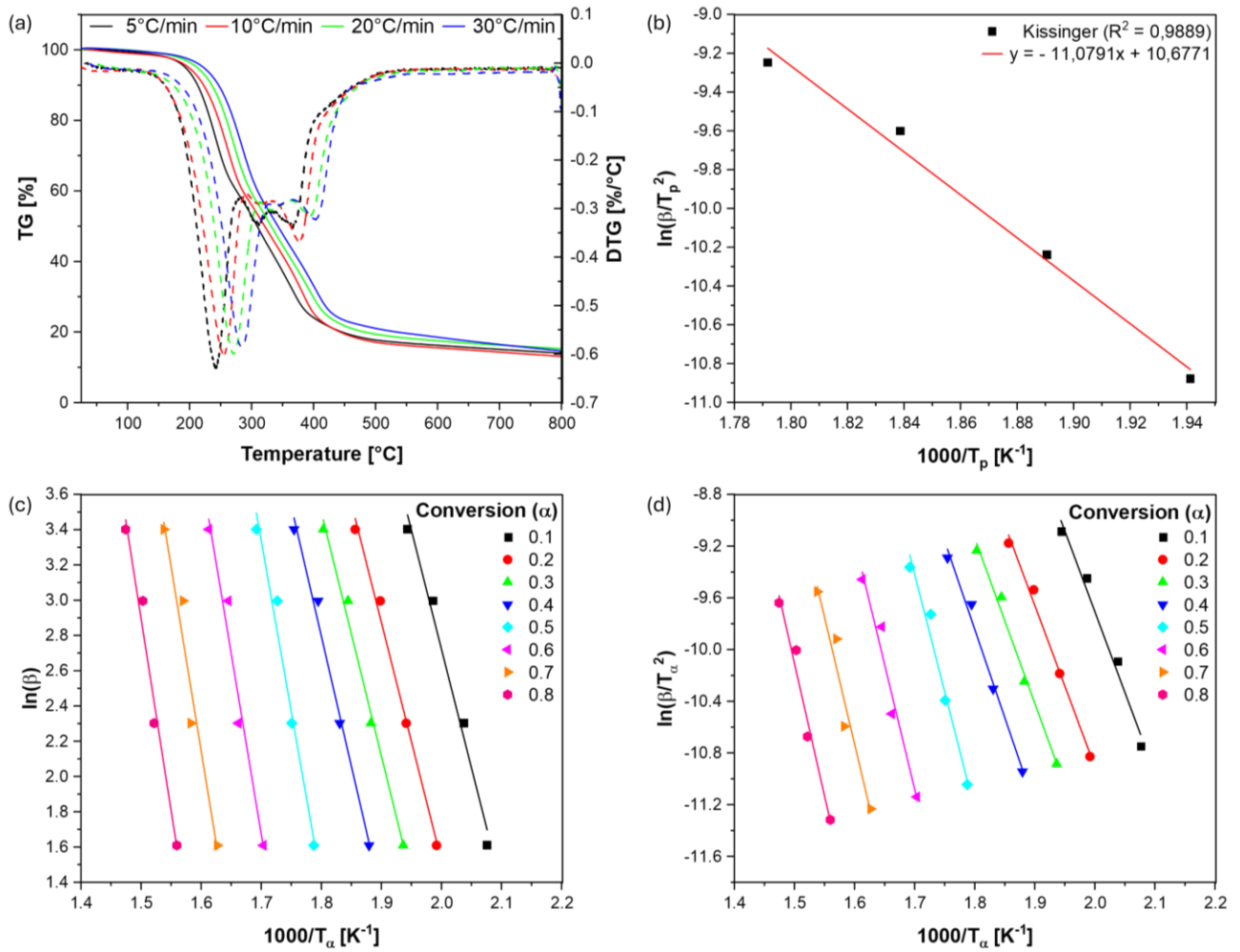


Figure 3.6: (a) TG-DTG curves of dried sludge at 5, 10, 20, 30 °C/min heating rate and graphical representation of (b) Kissinger, (c) FWO, (d) KAS method.

Kissinger analysis yielded a global average activation energy of 92 kJ/mol for the main degradation process, considering only the main DTG peak in the temperature range 240–280 °C.

Table 3.6: Activation energies E_a and coefficient of determination R^2 versus conversion rate α for dried sludge.

α	<i>FWO</i>		<i>KAS</i>	
	E_a (kJ/mol)	R^2	E_a (kJ/mol)	R^2
0.1	106.5	0.985	103.8	0.982
0.2	106.9	0.992	103.8	0.991
0.3	109.4	0.990	106.2	0.988
0.4	116.5	0.989	113.5	0.987
0.5	153.1	0.976	151.5	0.973
0.6	161.9	0.964	160.3	0.960
0.7	165.0	0.951	163.1	0.945
0.8	172.2	0.976	170.2	0.973

The results show that the apparent activation energy increases with the degree of conversion. In particular, E_a values obtained from FWO and KAS methods range from approximately 106–110 kJ/mol at low conversion ($\alpha = 0.1$ – 0.3) to about 165–172 kJ/mol at higher conversion levels ($\alpha = 0.6$ –

0.8), with high correlation coefficients ($R^2 > 0.97$), confirming the reliability of the analysis. In the intermediate conversion range ($\alpha = 0.4$ – 0.6), E_a shows a marked increase (115–160 kJ/mol), corresponding to the main devolatilization stage observed in the DTG curves. This behavior reflects the progressive transition from the degradation of thermally labile compounds to more stable and recalcitrant organic fractions (e.g., condensed aromatic structures or lipid-derived residues). Overall, the continuous increase in E_a indicates a multi-step degradation process involving overlapping reactions, in agreement with the DTG profile and the heterogeneous nature of the sludge. Although lignocellulosic components cannot be excluded, the present sludge does not exhibit the typical kinetic behavior of structured lignocellulosic biomass, where distinct kinetic regions can be associated with hemicellulose, cellulose, and lignin. Instead, it shows a continuous distribution of activation energies, consistent with Soxhlet and FT-IR results indicating a predominance of extractable oxygenated, aromatic, and lipid-like compounds. Similar trends have been reported for olive mill residues and other organic-rich wastes [48,49], where the increase in activation energy with conversion reflects the progressive decomposition of complex organic mixtures rather than discrete structural components.

These results, combined with the relatively high HHV value, highlight the potential of the sludge as a reactive biomass for thermochemical valorization processes such as pyrolysis, torrefaction, or energy recovery. The obtained parameters, consistent with those of olive-derived residues and agricultural biomasses ($\text{HHV} \approx 15$ – 20 MJ/kg), support the classification of the sludge as a promising bio-based fuel, with potential for reducing disposal and supporting circular bioeconomy strategies in the olive oil sector.

3.3.1.4. Polyphenols quantification

The distribution and quantification of target polyphenols were evaluated by HPLC analysis for raw OMW and acidified OMW, and the results are reported in Table 3.7.

Table 3.7: Concentration of target polyphenols [mg/L] in raw OMW and OMW_pH2.

	Glycol [mg/L]	Hydroxytyrosol [mg/L]	Tyrosol [mg/L]	Total [mg/L]
Raw OMW	263.4 ± 13.2	276.1 ± 6.9	99.2 ± 3.0	638.7 ± 15.2
OMW_pH2	255.6 ± 12.8	303.2 ± 7.6	107.4 ± 3.2	666.2 ± 15.3

The data show that acidification affects the phenolic composition in a compound-dependent manner. In particular, hydroxytyrosol and tyrosol concentrations increase significantly after acidification (from 276.1 to 303.2 mg/L and from 99.2 to 107.4 mg/L, respectively), while 3,4-dihydroxyphenylglycol decreases from 263.4 to 255.6 mg/L. As a result, the total concentration of the selected phenols shows a slight overall increase. This behavior is consistent with literature findings [51], which report that acidification can both enhance and reduce specific phenolic compounds depending on their chemical stability. In particular, the increase in hydroxytyrosol and tyrosol may be attributed to the hydrolysis of bound or glycosylated forms secoiridoid aglycons [52,53], leading to the release of free phenols. Conversely, the decrease in 3,4-dihydroxyphenylglycol

suggests possible degradation or transformation into other phenolic derivatives under acidic conditions.

No detectable concentrations of the target phenolic compounds were found in the sludge obtained after acidification, indicating that these compounds were not retained in the solid phase and were preferentially partitioned in the liquid fraction. This observation is consistent with the previous study introduced before of Leouifoudi et. al. [45] on olive cake, where is reported that phenolic compounds are mainly associated with the liquid phase of olive mill wastewater, while only limited amounts are found in the solid residues. This selective redistribution of phenolic compounds is particularly relevant for downstream recovery processes, where specific high-value phenols can be targeted.

3.3.2. Microfiltration

3.3.2.1. Ceramic Membrane: Benchmark Performance

The ceramic membrane operated under pressurized crossflow conditions provided the first benchmark for MF performance both on raw and acidified OMW. The evolution of permeate flux as a function of the volume concentration ratio (VCR) is reported in Figure 3.7. Filtration was performed in concentration mode from the beginning of the experiment, without a preliminary recirculation step. In addition, any overnight stops are indicated by an asterisk.

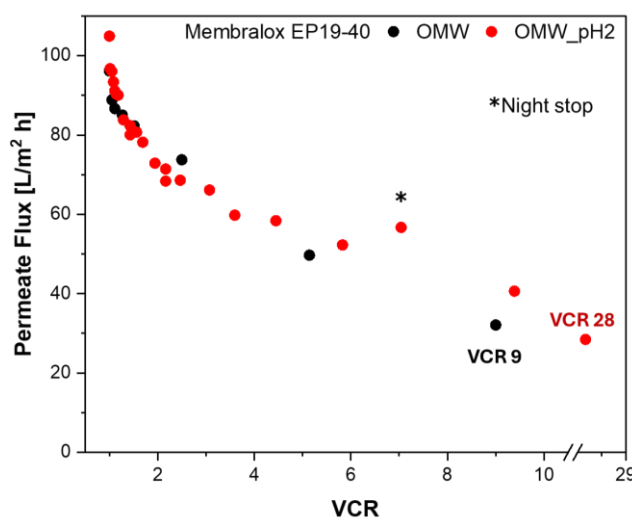


Figure 3.7: Evolution of permeate flux during concentration of raw and acidified OMW using a ceramic membrane module (Membralox).

For both raw and acidified OMW, permeate flux decreases with increasing VCR due to the combined effects of fouling and progressive concentration of retained species [54]. Interestingly, the two feeds exhibit comparable permeate fluxes up to VCR of 5, indicating that under moderate concentration conditions the impact of acidification on filtration performance is limited. In this regime, fouling is mainly governed by initial pore blocking and the early formation of a deposit layer, which appear to occur similarly in both systems [26,55].

Beyond this threshold, however, the filtration behavior diverges in different ways. Raw OMW shows a marked decline in flux, likely due to its high initial suspended solids content (7.0 g/L), which

promotes the formation of a dense and highly compressible cake layer [29]. As VCR increases, the progressive accumulation of solids resulted in cake thickening and compression, coupled with an increase in retentate viscosity. These effects result in a rapid rise in hydraulic resistance, as expected for microfiltration systems [56,57], ultimately limiting the process to VCR 9.

In contrast, OMW_pH2 maintained higher permeability at increasing VCR values. The lower initial suspended solids content (1.4 g/L), resulting from acidification, mitigated cake layer buildup and limited its compressibility [19,26]. As a result, the system can be operated up to VCR of 28, significantly extending the achievable concentration range. Notably, the permeate flux measured at VCR of 28 for OMW_pH2 (28.5 L/m²h), even after prolonged operation, was only slightly lower than that measured for raw OMW at VCR of 9 (32 L/m²h).

Overall, these results demonstrate that acidification has a limited effect on filtration performance at low-to-moderate concentration levels, but becomes crucial at higher VCR values, where it enables deeper concentration of the feed and prevents premature process limitation.

In both cases, the high concentration levels achieved required a chemical cleaning step at the end of the filtration cycle. This was particularly evident for raw OMW, where the retentate reached high suspended solids concentrations of approximately 40 g/L, resulting in significant membrane fouling and requiring a more intensive alkaline cleaning procedure performed at 60 °C for 1h, followed by acid cleaning with a 1 wt% HCl solution. The flux recovery ratio was found to be very close to 100%, indicating the effectiveness of the cleaning procedure and the complete restoration of membrane permeability. A similar behavior was observed for OMW_pH2. Although acidification significantly reduced the suspended solids content of the feed, the high concentration levels reached during filtration (VCR up to 28) still resulted in fouling of the ceramic module. After MF, the pure water flux dropped to approximately 25% of the initial value; nevertheless, complete permeability recovery (FRR ~100%) was achieved using a cleaning protocol similar to that adopted for raw OMW.

The composition of feed, permeate, and retentate streams was analysed in terms of electrical conductivity (Table 3.8) and target polyphenols distribution (Figure 3.8) for both raw and acidified OMW.

Table 3.8: Electrical conductivity (EC) of raw and acidified OMW and their retention (%).

Electrical Conductivity [$\mu\text{S}/\text{cm}$]	Raw OMW	R(%)	OMW_pH2	R(%)
Feed	4777	12	8330	13
Permeate	4200		7250	
Retentate	5030		8370	

Electrical conductivity showed only minor variations between feed and permeate in both cases, confirming that dissolved ions pass through the membrane pores. The acidified sample exhibited higher EC values, which can be attributed to the enhanced solubilization of inorganic species under acidic conditions.

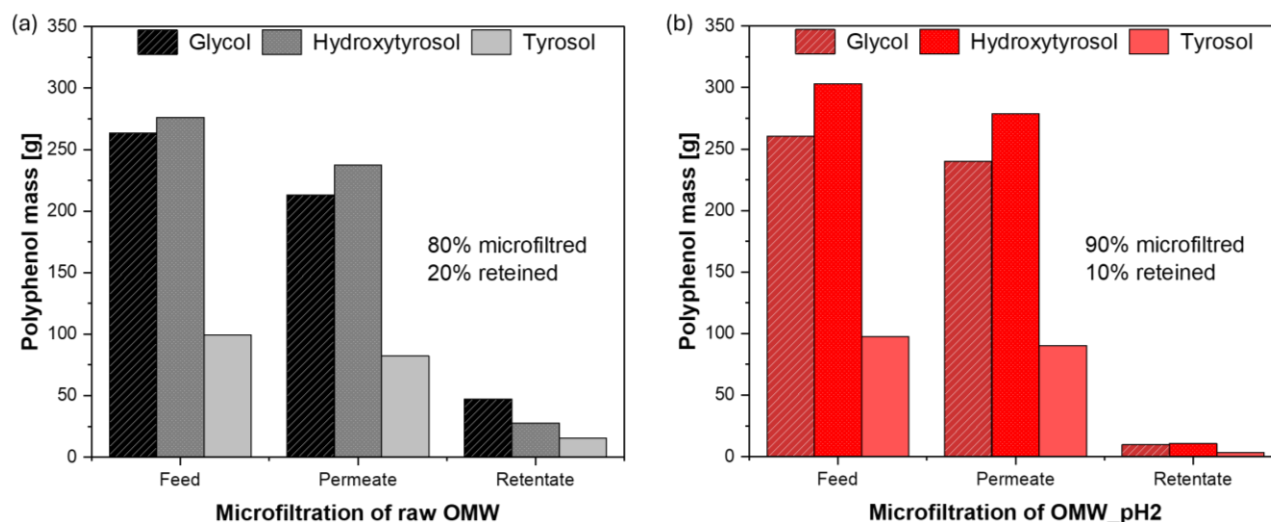


Figure 3.8: Mass balance of three target polyphenols for c) NF and d) DF process with their distribution between the permeate and retentate stream, calculated relative to the initial mass in the feed

Polyphenol distribution exhibited a distinct behavior compared to suspended solids. For both raw OMW and OMW_pH2, the majority of target phenolic compounds were detected in the permeate, indicating their predominant association in the dissolved phase.

A higher amount (g) of polyphenols was observed in the permeate obtained from OMW_pH2 compared with raw OMW. This reflects not only their higher initial concentration in the acidified feed, as discussed in Section 3.3.1.4, but also a greater fraction of compounds passing through the membrane (90% for OMW_pH2 vs 80% for raw OMW). This difference can be attributed to the different partitioning of phenolic compounds between dissolved and particulate phases. In raw OMW, a fraction of phenolics is associated with suspended solids and colloidal structures and is therefore partially retained during microfiltration. A similar behavior was reported by García-Castelló et al. [13], who observed that approximately 78% of polyphenols were recovered in the permeate after microfiltration of OMW. In contrast, acidification promotes the release of phenolic compounds into the dissolved phase, through the destabilization of colloidal interactions and the hydrolysis of more complex structures into simpler molecules such as hydroxytyrosol and tyrosol [53]. As a result, a larger proportion of phenolics is present in a freely soluble form and can pass through the membrane.

These results confirm that microfiltration does not provide effective separation of low-molecular-weight phenolic compounds but rather preserves them in the permeate stream. Consequently, MF acts as a clarification step while maintaining valuable bioactive compounds in solution, making the permeate suitable for subsequent recovery through tighter membrane processes such as nanofiltration or reverse osmosis. The MF retentate, enriched in suspended solids and colloidal material, can be combined with the sludge fraction produced during acidification and directed to conventional solid waste valorization routes such as composting or anaerobic digestion, as commonly reported for OMW treatment processes.

3.3.2.2. Pressurized Double-Module Polymeric System

A cross-flow concentration test of OMW_pH2 was performed using a double polymeric hollow-fiber module (2x10 fibers) and compared with the ceramic Membralox system. Due to the different operating conditions (TMP: 0.9 vs 2.5 bar; membrane filtration surface: 0.034 vs 0.72 m²; feed flow rate: 100 vs 9000 L/h), permeate fluxes were normalized to their initial values (J/J_0) to allow a comparison of the relative fouling behavior. The initial permeate flux was significantly higher for the ceramic membrane ($J_0 = 105 \text{ L/m}^2 \text{ h}$) than for the polymeric module ($J_0 = 42 \text{ L/m}^2 \text{ h}$), reflecting the higher TMP and cross-flow velocity.

Figure 3.9 shows the evolution of normalized permeate flux as a function of VCR. Despite the differences in operating conditions, the normalized flux profiles exhibited comparable initial decline for both systems, followed by a more stable behavior for the hollow-fiber module. In particular, at VCR ~5-6, the ceramic membrane reached $J/J_0 = 0.5$ (VCR 5.8), whereas the polymeric system maintained a higher relative permeability ($J/J_0 = 0.6$ at VCR 6.5). This suggests that, under the investigated conditions, the polymeric configuration experienced a less pronounced permeability decline during concentration, even when taking into account the differences in cross-flow velocity, module geometries, and TMP.

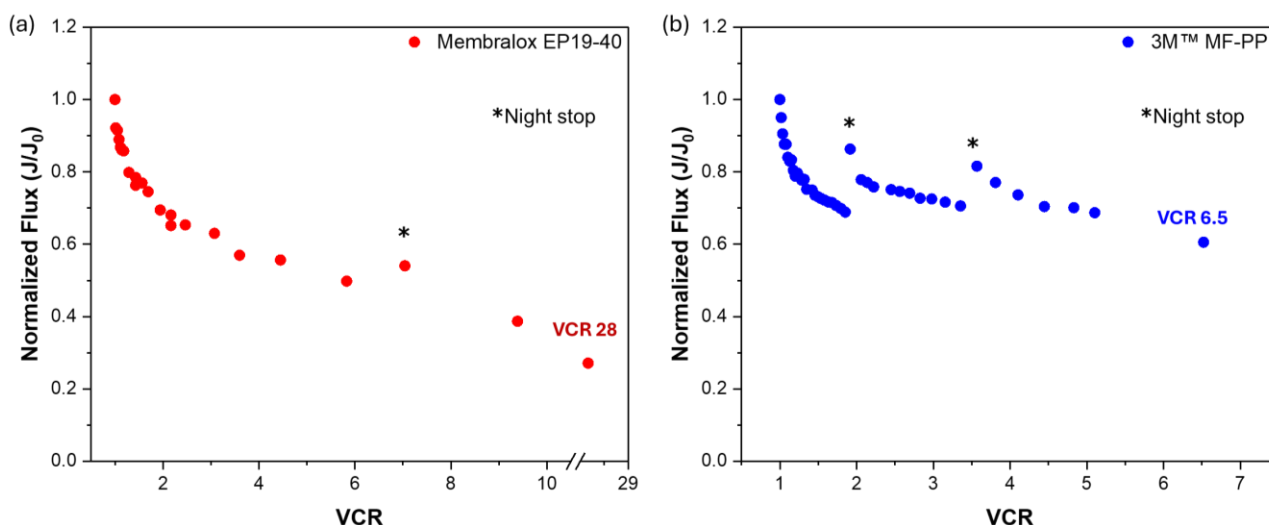


Figure 3.9: Normalized permeate flux profiles of OMW_pH2 over VCR with both ceramic (Membralox EP19-40) and polymeric module (3MTM MF-PP).

The temporary increases in flux observed during the test corresponded to system restarts after overnight interruptions, as well as to the application of periodic backflush operations in the hollow-fiber module, which were performed when a significant decline in flux was observed. These cleaning steps partially restored membrane permeability, indicating that a fraction of the fouling was reversible. This behavior is consistent with literature reporting that polymeric hollow-fiber membranes can benefit from periodic backwashing, which effectively removes loosely attached foulants and mitigates reversible organic fouling during wastewater treatment [58,59]. In contrast, ceramic membranes typically show higher fouling resistance and allow more aggressive cleaning strategies. The different fouling behavior can also be related to the module configuration. The ceramic

membrane operated in an outside-in cross-flow mode, whereas in the hollow-fiber module the feed flowed inside the fiber lumen and permeate was collected on the shell side. This geometry, combined with lower TMP and hydrodynamic stress, may have limited cake layer compaction and favored the removal of loosely attached foulants during back-flush cycles [60].

Despite the favorable normalized flux trend, the two systems differed significantly in terms of process performance. The ceramic membrane enabled concentration up to VCR 28 and treated approximately 480 L in 3.2 h, whereas the polymeric module reached VCR 6.5 and processed only 12 L in 13 h. These differences were mainly due to the much larger membrane area and higher operating pressures of the ceramic system. After the cross-flow concentration tests, the fouling behavior of the polymeric hollow-fiber module was further evaluated through water flux measurements before and after cleaning. Following the filtration run up to VCR 6.5, the pure water flux decreased to approximately 50% of its initial value, indicating the formation of a fouling layer during operation. However, a mild alkaline cleaning step using NaOH 0.1 wt% was sufficient to completely restore the original water permeability. The full recovery of the initial flux suggests that fouling was predominantly reversible and mainly associated with the deposition of loosely attached organic and colloidal matter on the membrane surface. This behavior is consistent with the treatment of pre-acidified OMW, where the reduction of suspended solids limits severe cake compaction and facilitates the removal of foulants during chemical cleaning.

Overall, these results indicate that, when treating pre-acidified OMW, polymeric hollow-fiber membranes can exhibit manageable fouling behavior and benefit from back-flush-assisted operation. However, ceramic membranes remain more suitable for high-throughput applications and deep concentration, while polymeric systems may represent a viable option for lower-scale or energy-limited processes. These findings suggest that appropriate pre-treatment strategies may help to reduce the performance gap between polymeric and ceramic membranes, with potential implications for process cost optimization.

3.3.2.3. Critical Flux Evaluation of Polymeric Membranes

Given the interesting fouling behavior observed during the cross-flow filtration of OMW_{pH2} using the double polymeric hollow-fiber module, additional tests were carried out to evaluate an alternative configuration based on submerged dead-end filtration. In this approach, commercial hollow fibers were assembled into custom modules and operated under vacuum, directly immersed in acidified OMW, with the aim of mimicking membrane bioreactor (MBR)-like conditions.

Short-term trials

Figure 3.10 (a-c) shows the flux stepping method used for the determination of critical flux (J_c) of OMW_{pH2} using hollow-fiber modules covering the MF to tight UF range (0.2, 0.04 and 0.02 μm). For each flux step, TMP initially exhibited a rapid increase due to the instantaneous rise in hydraulic resistance, followed either by stabilization or by a gradual increase associated with fouling

development. The critical flux was identified from the evolution of the TMP slope ($dTMP/dt$). When the TMP increase rate exceeded 0.5 mbar/min, the corresponding permeate flux was considered as the critical flux J_C . In all tested modules, the transition between stable and fouling-dominated regimes appeared gradual rather than sharp, indicating progressive fouling development. Figure 3.10 (d) shows the flux vs. TMP behavior, during the ascending and descending run, of the three membrane modules. The hysteresis semi-loop observed during the flux stepping test provided further insight into the nature of fouling. After reaching fluxes above J_C , the TMP did not return to its initial values upon flux reduction, indicating the presence of residual fouling. This behavior reflected the coexistence of reversible and irreversible fouling mechanisms, where loosely attached material could be partially removed, while a fraction of foulants remained strongly associated with the membrane surface [17,61].

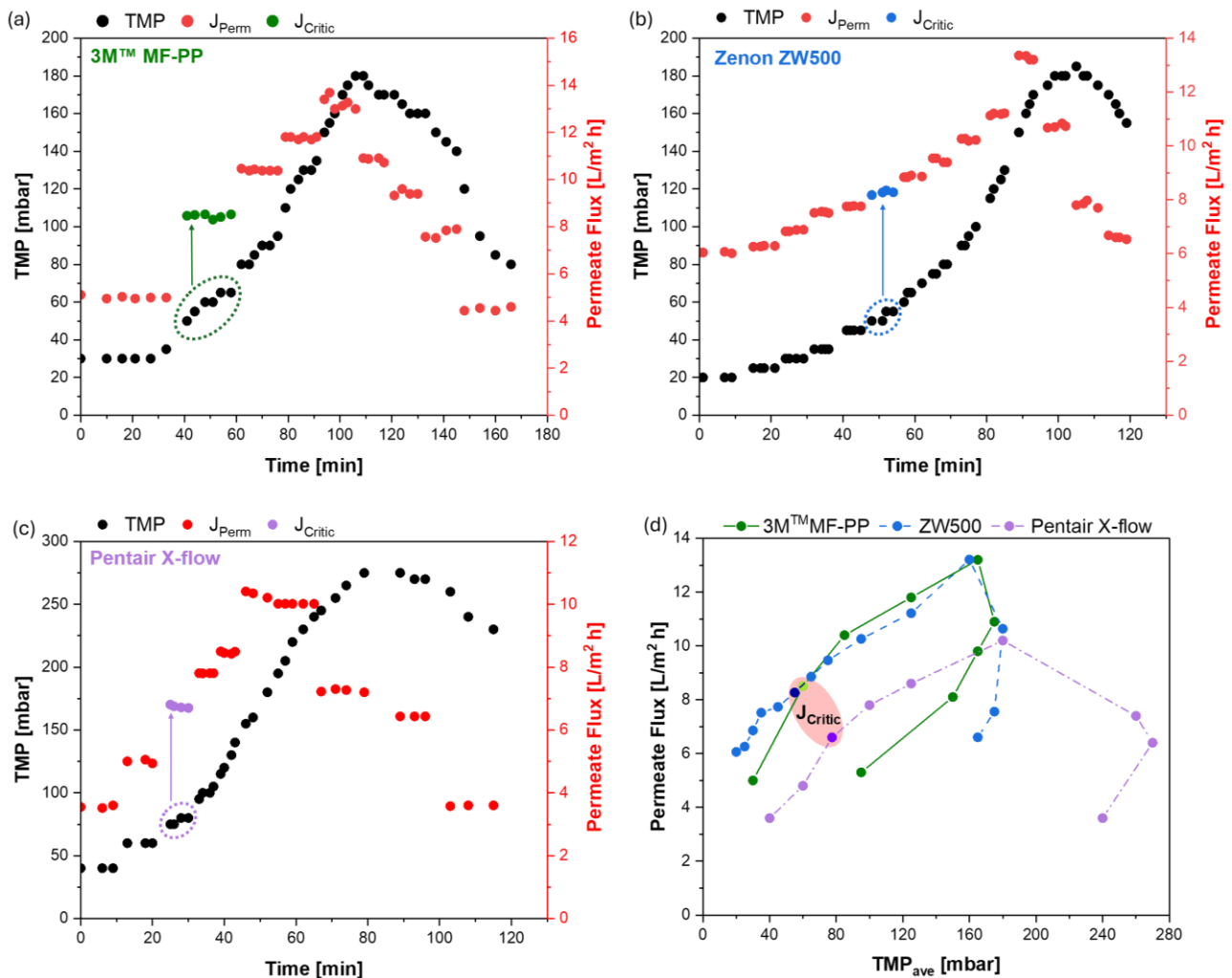


Figure 3.10: Flux stepping method used for the determination of critical flux (J_C) of OMW_pH2 with (a) 3M™ MF-PP, (b) Zenon ZW50 (c) Pentair X-flow hollow fiber modules and (d) relative hysteresis loop.

Based on the TMP and permeate flux values measured during the flux-stepping experiments, the total resistance (R_{tot}) was obtained from Darcy's law. The intrinsic membrane resistance (R_m) was derived from pure water permeability measurements performed prior to filtration tests. The additional resistance associated with cake formation and fouling ($R_c + R_f$) was estimated by difference ($R_{tot} -$

R_m). The critical point values (TMP and J) for the three types of membranes are reported in Table 3.9, along with the values of various resistances.

Table 3.9: TMP, critical flux and calculated filtration resistances for the hollow-fiber membranes during flux-stepping tests.

Membrane	TMP _{Critic} [mbar]	J _{Critic} [L/m ² h]	R _{tot} [10 ¹² m ⁻¹]	R _m [10 ¹² m ⁻¹]	R _c + R _f [10 ¹² m ⁻¹]
3M TM MF-PP	57.5	8.5	2.74	0.19	2.55
Zenon ZW500	55	8.3	2.68	0.48	2.20
Pentair X-flow	77.5	6.6	4.76	1.38	3.38

The results showed that the contribution of fouling-related resistance ($R_c + R_f$) represents the dominant fraction of the total resistance under the tested conditions, confirming that membrane performance during submerged filtration was largely governed by the development of fouling layers rather than by the intrinsic membrane permeability.

The MF-PP (0.2 μm) and the ZW500 (0.04 μm) membrane exhibited similar J_c and R_{tot} values, suggesting that the onset of critical flux was not strictly controlled by membrane pore size. Instead, the gradual transition to a fouling-controlled regime appeared to be driven by the formation of a surface deposit layer and by module-specific hydrodynamic conditions [17]. This behavior is consistent with the treatment of acidified OMW, where the reduction of suspended solids limits rapid cake formation while colloidal and dissolved organic matter still contributes to the progressive development of a fouling layer [18]. In contrast, the tighter X-flow (0.02 μm) membrane showed higher resistance and lower J_c , suggesting an earlier transition to a fouling-dominated regime, likely due to its higher sensitivity to colloidal and organic matter accumulation.

Water flux recovery measurements further support this interpretation. The 3MTM MF-PP module showed a limited recovery of 20% after OMW filtration, which increased to about 50% after alkaline cleaning, indicating the presence of strongly adsorbed organic foulants. The Pentair X-flow module exhibited similarly incomplete recovery (40%), suggesting a higher sensitivity to persistent fouling. In contrast, the ZW500 module showed an apparent increase in water permeability after filtration and cleaning, which may be attributed to incomplete initial wetting or progressive air removal from the pores, leading to an underestimation of the initial flux and higher permeability after operation and cleaning. Overall, these results confirm that, although acidification reduces severe particulate fouling, the system remains affected by organic and colloidal fouling, which is only partially reversible and becomes more pronounced when operating above the critical flux.

Long-term trials

Figure 3.11 reports the temporal evolution of TMP during constant-flux operation for the 3MTM MF-PP (0.2 μm) and Zenon ZW500 (0.04 μm) hollow-fiber modules at fluxes imposed below, close to, and above the critical flux previously determined by the flux-stepping method. The tests were performed in parallel using a dual-channel peristaltic pump, with the permeate continuously withdrawn from the system, thus progressively concentrating the bulk solution.

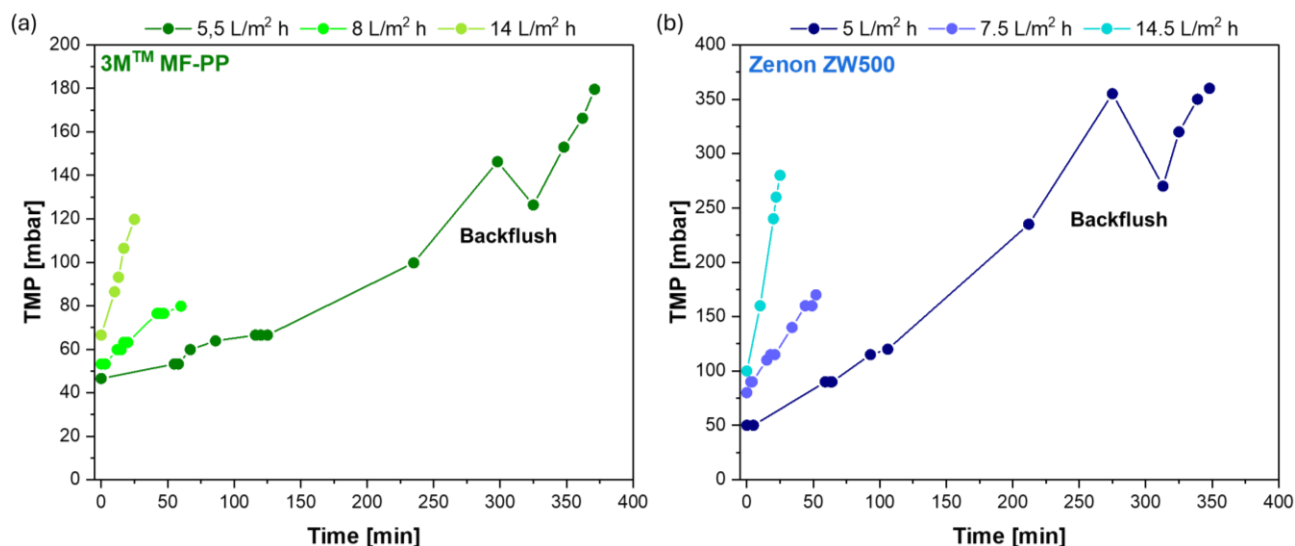


Figure 3.11: Behaviour of a) 3M™ MF-PP and b) Zenon ZW500 modules at fluxes below, close to, and above the critical fluxes.

Under subcritical conditions, both membranes exhibited a relatively moderate increase of TMP over time, although the rate of fouling was noticeably different. Starting from similar initial TMP values (~ 50 mbar), the MF-PP membrane showed a slower increase, reaching approximately 66.5 mbar after 2 h and about 100 mbar after 3.5 h of operation. In contrast, the tighter ZW500 membrane exhibited a much faster increase, reaching about 120 mbar after 2 h and approximately 245 mbar after 3.5 h. After reaching high TMP values, a back-flush step was applied by reversing the permeate flow for approximately 20 minutes. This procedure partially restored membrane permeability, indicating that part of the fouling layer was reversible. However, the subsequent increase of TMP during continued operation suggests that a fraction of the fouling remained irreversible, likely associated with adsorption of organic matter or compression of the deposited layer [62].

When operating near the critical flux, the difference between the two membranes became more pronounced. After one hour of filtration, TMP increased from 50 to about 80 mbar for the PP membrane, while it reached approximately 170 mbar for the ZW500 module. Under supercritical conditions, both membranes exhibited a rapid TMP increase; however, the ZW500 membrane reached significantly higher pressures (280 mbar) within only 25 minutes compared with 120 mbar for the MF-PP membrane.

These results indicated that, although the two membranes exhibited comparable critical flux values in the short-term flux stepping tests, their long-term fouling kinetics differed substantially. The tighter membrane appeared more sensitive to the accumulation and compaction of colloidal and dissolved organic matter, leading to a faster transition toward a fouling-dominated regime. Similar behavior has been widely reported in constant-flux membrane filtration studies [63,64], where the increase of TMP over time reflects the progressive growth and compaction of the fouling layer under sustained permeation conditions. In addition, the difference between short-term critical flux determination and long-term filtration performance is consistent with previous studies showing that flux-step methods provide useful comparative information but cannot fully predict the long-term TMP evolution when

the feed composition progressively changes during operation. In particular, when filtration is performed in concentration mode, the increasing accumulation of colloidal and organic matter can progressively shift the system toward more severe fouling conditions, even when operating close to the critical flux [30].

These observations highlight that critical flux values obtained from short-term experiments should be interpreted cautiously when applied to prolonged filtration processes. Recent work has revisited the classical concept of critical flux, highlighting that real filtration systems often exhibit gradual fouling even below the nominal critical flux, particularly when complex feeds are treated. In this framework, the concept of threshold or sustainable flux has been proposed as a more practical descriptor of membrane performance under realistic operating conditions, where fouling evolves progressively with time and concentration of foulants [65,66].

Overall, the present study provides a preliminary evaluation of submerged hollow-fiber filtration applied to acidified OMW. While the results demonstrate the feasibility of the approach, further work should focus on optimizing module configuration, increasing membrane surface area, and refining the operating strategy in order to better control fouling dynamics and evaluate long-term sustainable flux conditions. This configuration, well known for its lower energy requirements and simpler hydraulic setup, can significantly reduce operating costs and offers the advantage of minimizing membrane fouling when operated below the critical flux threshold.

3.4. Conclusions

The present work demonstrated the effectiveness of acidification as a key pre-treatment step for olive mill wastewater, enabling the separation of a solid sludge phase and a clarified liquid fraction suitable for membrane processing. The acidified sludge was extensively characterized through SEM–EDX, TGA and solvent extraction techniques. SEM–EDX analysis revealed the presence of inorganic species mainly associated with oxides and sulfates of alkaline elements, particularly K and Ca. Sequential Soxhlet extraction showed that approximately 25 wt% of the sludge was water-soluble, while an additional 50 wt% was extractable in ethanol. FT-IR analysis suggested that the aqueous extract mainly contained oxygenated polar compounds, whereas the ethanol fraction was enriched in lipid-like and aromatic species. GC–MS analysis further identified oleic acid among the most abundant volatile components of the ethanol extract. No relevant concentrations of target polyphenols were detected in the water extract by HPLC measurement.

Thermogravimetric analysis allowed the determination of proximate composition and the estimation of the higher heating value (HHV=17.7 MJ/kg), as well as the evaluation of kinetic parameters of thermal degradation. The obtained results indicate that the acidified sludge exhibited characteristics typical of organic-rich biomass, with high volatile content and significant energetic potential. These findings suggest that the recovered sludge may represent a promising feedstock for thermochemical valorization processes, while additional potential applications will be discussed in the following Chapter 7.

The acidified supernatant was instead employed to investigate microfiltration as a clarification step using different membrane materials and configurations, including ceramic and polymeric membranes operated under cross-flow and submerged hollow-fiber conditions. Acidification significantly reduced the suspended solids concentration (from 7 to 1.4 g/L), enabling stable microfiltration and allowing the ceramic membrane system to reach high volume concentration ratios (VCR up to 28). At the same time, the permeate stream retained approximately 90% of the phenolic compounds initially present in the feed. The increase in the concentration of target phenolics such as tyrosol and hydroxytyrosol in the acidified feed was consistent with acid-catalyzed hydrolysis reactions affecting glycosylated or secoiridoid phenolic precursors.

A comparative analysis between cross-flow ceramic and polymeric HF membranes operating with OMW_{pH2} showed that, although the polymeric double-module operated at lower hydraulic throughput due to the adopted laboratory configuration, it was still able to concentrate the feed while maintaining relatively stable permeate fluxes. This result highlights the potential applicability of polymeric hollow-fiber membranes for OMW clarification. However, submerged dead-end filtration experiments revealed stronger differences between membranes: tighter hollow-fiber modules exhibited faster fouling kinetics despite showing similar critical flux values during short-term flux-stepping tests. These observations confirm that membrane performance cannot be fully assessed through critical-flux measurements alone and should be evaluated through long-term filtration tests under realistic operating conditions. In this perspective, recent studies have emphasized the importance of alternative concepts such as threshold or sustainable flux when dealing with complex feeds where fouling develops progressively over time.

Overall, the coupling of acidification and microfiltration proved to be an effective strategy for OMW treatment, allowing efficient removal of suspended solids while maintaining dissolved organic compounds, including valuable phenolic species, in the permeate stream. In addition to improving process stability, the possibility of employing polymeric membranes instead of ceramic ones may significantly reduce capital expenditure costs (CAPEX), making the process more economically attractive for large-scale applications. Furthermore, the recovery of phenolic compounds in the permeate highlights the suitability of MF as a pre-treatment step prior to tighter membrane processes such as nanofiltration (NF) and reverse osmosis (RO), where further concentration and valorization of bioactive compounds can be achieved.

Future work should therefore focus on optimizing both cross-flow polymeric and submerged membrane configurations, increasing membrane surface area, and refining hydrodynamic control strategies in order to better manage fouling dynamics and identify sustainable operating conditions for scale-up applications.

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CHAPTER 4: INTEGRATED MEMBRANE PROCESSES FOR POLYPHENOL FRACTIONATION AND RECOVERY

4.1. Introduction

Olive mill wastewater, generated during olive oil production, represents not only an environmental challenge but also a potential source of valuable bioactive compounds, particularly phenolic molecules such as hydroxytyrosol, tyrosol, and related derivatives. These compounds are widely recognized for their antioxidant properties and potential applications in food, pharmaceutical, and cosmetic sectors [1–5]. For this reason, considerable research efforts have been directed toward the development of sustainable technologies capable of recovering these compounds while simultaneously reducing the environmental impact associated with OMW disposal.

Among the technologies proposed for OMW treatment and valorization, pressure-driven membrane processes have received increasing attention due to their ability to operate under relatively mild conditions while enabling selective separation of solutes based membrane selectivity and on molecular size [6–9]. In particular, microfiltration is commonly used as a clarification step to remove suspended solids, while ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO) membranes have been widely investigated as sequential processes for the purification and concentration of phenolic compounds from OMW [10–14].

Ultrafiltration is typically applied as an intermediate purification step to remove colloidal material and macromolecular organic compounds while allowing low-molecular-weight phenolics to remain in the permeate stream. Turano et al. [8] investigated an integrated centrifugation–ultrafiltration system for olive mill wastewater treatment and reported a significant reduction of waste pollution, with removals of about 80% for ashes and suspended solids and up to 90% for COD. Similarly, El-Abbassi et al. [15] demonstrated that UF membranes can be effectively used as a pretreatment step for olive processing wastewaters under acidic conditions, improving both phenolic recovery from the permeate and the performance of subsequent membrane processes. Other studies have also confirmed that UF pretreatment contributes to reducing membrane fouling and improving the efficiency of downstream nanofiltration operations [6,11].

Nanofiltration has been extensively studied for the separation and concentration of phenolic compounds from OMW due to its molecular weight cut-off typically ranging between 200 and 400 Da, which is comparable to the molecular weight of several phenolic molecules present in these effluents. Coskun et al. [16] evaluated the performance of several NF membranes for the treatment of olive mill wastewater and reported significant retention of phenolic compounds together with moderate permeate fluxes depending on membrane characteristics. Similarly, Ochando-Pulido et al. [17] investigated nanofiltration processes for OMW treatment and reported that NF membranes can

effectively reduce the organic load while concentrating antioxidant compounds in the retentate stream. Other authors have also confirmed the suitability of NF membranes for polyphenol recovery from agro-industrial wastewaters [13,18–20].

In order to improve separation efficiency and process performance, several integrated membrane strategies have been proposed. Bazzarelli et al. [21] investigated membrane-based approaches for the treatment and valorization of olive mill wastewater, highlighting that combining different membrane operations can enhance separation efficiency and facilitate the fractionation of valuable compounds. García-Castello et al. [12] also evaluated integrated membrane processes for the recovery of polyphenols from olive mill wastewater and emphasized the advantages of combining different membrane operations in order to achieve both pollutant removal and compound recovery.

Reverse osmosis has been widely applied as a final concentration step for OMW treatment. Due to their dense selective layer, RO membranes exhibit very high rejection of organic solutes and inorganic salts, enabling the concentration of dissolved species in a significantly reduced volume while producing a permeate stream that can potentially be reused within the process [16,22–24]. Integrated membrane systems combining MF, UF, or NF with RO have therefore been proposed for both pollutant removal and resource recovery from olive mill wastewater.

Despite the advantages of membrane technologies, fouling remains one of the main limitations in OMW filtration. The high concentration of organic matter, colloids, and macromolecules promotes concentration polarization, cake layer formation, and adsorption of organic compounds on the membrane surface or within membrane pores [25–27]. Ochando-Pulido et al. [22] described the progressive development of concentration polarization and cake layer formation during nanofiltration of OMW streams, which leads to a gradual decline in permeate flux. Similar fouling mechanisms have been observed in several studies investigating membrane filtration of agro-industrial wastewaters [28–30]. To mitigate these limitations, appropriate pretreatment steps and sequential membrane operations have been proposed. For instance, Bottino et al. [31] investigated treatment schemes based on microfiltration followed by reverse osmosis, demonstrating that adequate pretreatment significantly improves the performance of spiral-wound RO membranes and enables the concentration of organic compounds while producing a permeate stream potentially suitable for reuse.

In addition to pressure-driven membrane operations, diafiltration has been proposed as a complementary technique to enhance the recovery of diffusible solutes. During diafiltration, fresh solvent is added to the retentate while permeate is continuously withdrawn, promoting the removal of permeable compounds across the membrane while retaining less permeable macromolecular species [32]. The feasibility of this approach for olive mill wastewater treatment was demonstrated by Gkoutsidis et al. [33], who showed that diafiltration can effectively enhance the removal of diffusible compounds from OMW streams.

Most studies applying nanofiltration to OMW have focused on maximizing the rejection of phenolic compounds and concentrating them in the retentate stream. However, the rejection of phenolics

strongly depends on their molecular weight and physicochemical properties, and low-molecular-weight compounds such as hydroxytyrosol and tyrosol may partially permeate through relatively loose nanofiltration membranes. This behavior can be exploited to design separation strategies based on selective fractionation rather than simple concentration.

Based on these considerations, the present work investigates an integrated membrane approach for the fractionation and concentration of phenolic compounds from acidified and microfiltered olive mill wastewater. Several flat-sheet nanofiltration membranes were first evaluated to identify the most suitable membrane for selective polyphenol transmission. A relatively loose nanofiltration membrane was then applied in a spiral-wound configuration to process larger volumes of feed and promote the transfer of low-molecular-weight phenolics into the permeate. Subsequently, diafiltration was used to enhance the recovery of diffusible compounds from the retentate, while reverse osmosis was finally employed to concentrate the phenolic-rich stream. This NF–DF–RO sequence was therefore explored as a strategy to combine early-stage fractionation with efficient downstream concentration while mitigating the impact of organic fouling.

4.2. Materials and Methods

4.2.1. Materials

Olive mill wastewaters coming from three-phase system olive oil production were supplied by Frantoio Rocca Danilo (Tovo San Giacomo, Italy). The raw effluent was subsequently pretreated by acidification, followed by a microfiltration stage. The MF process was carried out to eliminate the major organic matter. It was performed with Membralox EP19-40 membrane, under the operating conditions of 2.5 bar of TMP and a cross-flow velocity (CFV) of 3.5 m/s (see Chapter 3). 3,4-dihydroxyphenylglycol, hydroxytyrosol, tyrosol were used as standard for HPLC calibrations. Acetonitrile and trifluoroacetic acid were used for HPLC mobile phase preparation. All the chemicals were purchased from Sigma Aldrich (Milan, Italy). NaOH, NaOCl, HCl (Sigma Aldrich) were used for membrane cleaning and foulant removal. Five different flat-sheet membranes and one spiral-wound membrane were tested for NF process. The spiral-wound module (NP010) was also tested for diafiltration tests. A spiral-wound module was tested for reverse osmosis trial. The main characteristics of the membranes investigated are reported in Table 4.1.

Table 4.1: Membrane specifics.

Model	Manufacturer	Material	Type	Cut-off	Salt Rejection	
NF90	Dow-Filmtech	Polyamide TFC	Flat-sheet	150-200 Da	MgSO ₄ NaCl	99.0% 90.0%
NFX	Synder Filtration	Polyamide TFC	Flat-sheet	150-300 Da	MgSO ₄ NaCl	99.0% 40.0%
NF270	Dow-Filmtech	Polyamide TFC	Flat-sheet	200-400 Da	MgSO ₄ NaCl	97.0% 40-60%
TS40	Mann+Hummel	Polypiperazine	Flat-sheet	200-300 Da	MgSO ₄ NaCl	>98.5% 40-60%
NP010	Mann+Hummel	Polyethersufone	Flat-sheet	~1000 Da	Na ₂ SO ₄ NaCl	35.0% 10.0%
NP010	Mann+Hummel	Polyethersufone	Spiral-wound			
BW30	DuPont	Polyamide TFC	Spiral-wound	~100 Da	MgSO ₄ NaCl	>99.5% 99.0%

TFC: Thin-film Composite

4.2.2. Methods

4.2.2.1. Physicochemical analyses

The pH was measured using a Hanna pH209 pH meter, while electrical conductivity (EC) was determined using a Hanna EC215TM conductivity meter equipped with a Hanna HI76303TM probe. Target polyphenols quantification was carried out by high-performance liquid chromatography (HPLC) analysis. It was performed with a Shimadzu system (LC-10ATvp pump, SPD-10Avp detector, $\lambda = 280$ nm), equipped with a Synergi Hydro-RP column (150 × 3 mm, 4 μ m) and a pre-column filter. The mobile phase consisted of H₂O + 0.1% trifluoroacetic acid (A) and acetonitrile (B), with a 46 min gradient program. Injection volume: 5 μ L; flow rate: 0.7 mL min⁻¹; column temperature: 23–25 °C. Calibration curves for the target polyphenols and regression parameters are reported in the Supplementary Material.

4.2.2.2. NF/DF/RO Set-up and Operation

NF/DF/RO trials were performed in batch mode using a pressurized cross-flow set-up, schematically shown in Figure 4.1. It was used for experiments carried out with both flat-sheet and spiral-wound membranes. As illustrated in Figure 4.1, microfiltered OMW_{pH2} was fed to the membrane module by a piston pump at a feed flow rate, Q, measured by a flow meter and regulated through a variable frequency drive (inverter). The operating pressure was controlled with a pressure control valve (globe valve) and monitored by a manometer (P) located after the membrane module. A system of ball valves allowed the selection of either of the two membrane modules. The permeate flow rate was determined by measuring the time required to collect a known permeate volume in a graduated tank. The permeate flux was then calculated as the ratio between the permeate flow rate and the total effective membrane surface area. The temperature, monitored by a thermometer (T), was maintained constant at 25 °C by

means of a cooling device immersed in the feed tank. A dedicated cleaning tank was used to circulate cleaning solutions (NaOH, NaOCl and HCl solutions) through the membrane module for the removal of organic fouling and inorganic scaling.

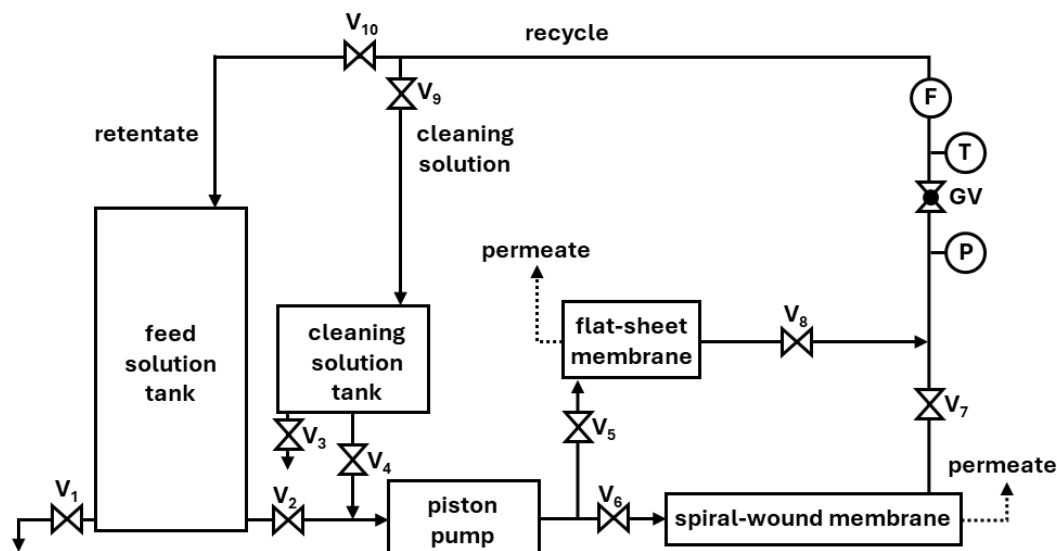


Figure 4.1: Schematic of the plants used for NF/DF and RO tests with flat-sheet and spiral wound membranes: V1-10 ball valves; GV globe valve; P manometer; T thermometer; F flowmeter.

The flat-sheet membranes were used for preliminary test. These tests were carried out keeping the feed concentration constant, and continuously recycling both permeate and retentate streams to the feed tank. Concentration tests were performed with spiral wound module recycling the retentate to the feed tank while the clean permeate was continuously withdrawn to be used for future application. Table 4.2 reported the operating conditions adopted for each membrane process.

Table 4.2: Operating condition.

Parameter	Flat-sheet membrane (NF)	Spiral-wound membrane (NF/DF)	Spiral-wound membrane (RO)
Temperature	25 °C	25°C	25 °C
Transmembrane Pressure (TMP)	20-30 bar	30 bar	20 bar
Filtration surface	0.0066 m ²	6.3 m ²	7.3 m ²
Feed flow rate	500 L/h	1500 L/h	1000 L/h
Feed velocity	0.77 m/s	1.1 m/s	1.0 m/s

Three representative samples were collected for each test: initial feed, medium permeate and final retentate. Membrane selectivity was evaluated in terms of rejection of the solutes that are retained inside the membrane and calculated according to the following equation:

$$R(\%) = \frac{\sigma_{Feed} - \sigma_{Permeate}}{\sigma_{Feed}} \times 100 \quad (1)$$

where R (%) is the percentage rejection, and σ represents the variable examined. For example, sigma can be the concentration of polyphenols (determined by HPLC analysis) or the electrical conductivity.

the distribution of target polyphenols between permeate and retentate streams was evaluated through mass balance calculations and expressed relative to the initial mass in the feed:

$$PPs \text{ distribution } (\%) = \frac{mass_{\text{Permeate or Retentate}}}{mass_{\text{Feed}}} \times 100 \quad (2)$$

The ratio between the initial volume of the feed and that of the retentate (equation 3) determined the volume concentration ratio (VCR), which describes the degree of volumetric concentration achieved during the process. The corresponding concentration-based parameter is the concentration factor (CF), defined as the ratio between the solute concentration in a given stream and that in the initial feed (equation 4).

$$VCR = \frac{V_{\text{feed}}}{V_{\text{retentate}}} \quad (3)$$

$$CF = \frac{C_{\text{feed}}}{C_{\text{retentate}}} \quad (4)$$

After each filtration experiment, membrane cleaning was performed according to the procedure described in Chapter 3, consisting of alkaline cleaning (NaOH and/or NaOCl) followed, when required, by acid cleaning with HCl. The effectiveness of the cleaning protocol was evaluated by measuring the pure water flux before (J_{W0}) and after filtration (J_{WF}) and flux recovery ratio (FRR) was defined as:

$$FRR = (J_{WF} / J_{W0}) \times 100 \quad (5)$$

4.3. Results and Discussion

4.3.1. Nanofiltration and diafiltration

Figure 4.2 presents the performance of five flat-sheet polymeric nanofiltration membranes tested in recirculation mode using acidified, microfiltered OMW. For each membrane, stable permeate fluxes ($\pm$ variance, based on the results obtained by Jezowska et al. [34]) are plotted as data points, while rejection (R%), calculated according to Equation 1, for electrical conductivity and concentrations of three target polyphenols (HPLC-determined) are shown as bar charts. Panels a and b show results obtained at 20 bar and 30 bar, respectively.

The permeate flux values reported for all five nanofiltration membranes represent the plateau region of operation, meaning they were measured after the initial decline associated with early-stage fouling had stabilised. This provides a clearer indication of membrane permeability and applied pressure dependence when filtering microfiltered OMW_{pH2}.

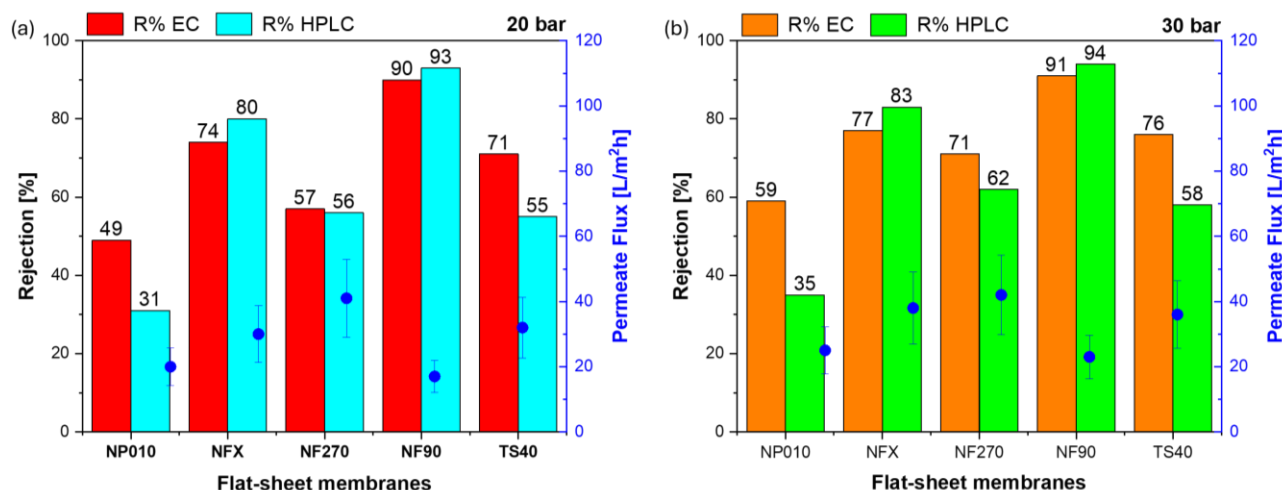


Figure 4.2: Comparison of salt rejection, target polyphenol rejection, and stable permeate flux for the investigated flat-sheet membranes (NP010, NFX, NF270, NF90, and TS40). (a) Results obtained at 20 bar; (b) results obtained at 30 bar.

At 20 bar, NF270, TS40, and NFX membranes exhibited the highest fluxes, reaching 41, 32, and 30 L/m²h, respectively. These values reflect their relatively larger pore size and higher intrinsic water permeability, as discussed in the work of Ochando-Pulido et al. [35]. NP010, which is looser but less permeable, exhibits a moderate flux of 20 L/m²h, while NF90, the densest membrane, shows the lowest flux (17 L/m²h). These permeate fluxes are higher than those reported in some flat-sheet nanofiltration studies on OMW and similar matrices, where values around 10–12 L/m²h [36] at comparable pressures have been observed, likely due to differences in operating mode and feed pretreatment. When the pressure was increased to 30 bar, all membranes show an increase in productivity. This was also demonstrated by Bottino et al. [31] who conducted a similar study on OMWs and showed that flow increases with applied pressure, particularly in the initial phase before the effects of concentration and fouling predominate.

Retention performance shows distinct membrane-specific behaviours for both ionic species (EC removal) and phenolic compounds. Salt rejection increases with membrane tightness, ranging from 49–59% for NP010 up to 90–91% for NF90, with intermediate values for NFX, TS40, and NF270. Polyphenol rejection follows a similar order but with generally higher values, particularly for NF90 (93–94%) and NFX (80–83%). The intermediate membranes (NF270 and TS40) provide moderate phenolic rejection, while NP010 shows the lowest retention due to its higher molecular weight cut-off (MWCO). The observed differences in flux and rejection among the tested membranes are consistent with the well-established trade-off between permeability and selectivity in nanofiltration processes, where membranes with larger pore size exhibit higher permeate fluxes but lower rejection, while tighter membranes provide higher selectivity at the expense of permeability [37,38]. However, membrane MWCO alone cannot fully explain the observed retention behavior. The experiments were conducted at pH ≈ 2, where the phenolic hydroxyl groups of polyphenols, as well as most carboxylic functionalities present in the matrix, are expected to remain predominantly protonated. Consequently, although the membrane surface is likely positively charged under these conditions due to the operating pH being below the isoelectric point of most NF membranes, electrostatic interactions and

Donnan exclusion effects are expected to play a limited role in solute separation. In addition to steric hindrance, the chemical nature of the membrane material may therefore contribute to the observed rejection patterns through affinity-related phenomena such as hydrophobic interactions, hydrogen bonding and, in the case of aromatic polyamide membranes, possible π – π interactions with aromatic phenolic compounds [39].

Among the membranes, NP010 was chosen to scale-up the process on a pilot plant since it exhibits the lowest rejection of target polyphenols which present high antioxidant activity. For this reason, a spiral-wound module was used to filter larger volumes of microfiltered OMW_pH2. Figure 4.3 (a) shows the flows as a function of VCR, operating at 30 bar and with a CFV of approximately 1.1 m/s. The feed was concentrated significantly (VCR $\sim$ 7). In the first part, the flow rate through the membrane was reduced sharply to a VCR of 2. In the second part, the flux decline was slower until reaching a steady state condition. Several factors cause flux decline during the filtration process, including concentration polarization, gel layer formation on the membrane surface and clogging of the membrane pores by compounds in the OMW [36]. These factors may contribute to additional resistance on the feed side of the membrane, thereby reducing the effective driving force for permeation. At the beginning of filtration, the dominant factor is typically concentration polarization, which leads to a rapid initial flux drop. Subsequently, cake deposition develops on the membrane surface, producing a second, more gradual decline phase [11]. In this case as well, R% of EC and polyphenols was evaluated, resulting in 48% and 35%, respectively. In particular, during this stage of NF, NP010 allows almost all of the tyrosol to pass into the permeate (R = 12%), while retaining higher quantities of glycol and hydroxytyrosol (47.5–43.5%). This behavior may be related to its lower MW and to weaker interactions with the membrane material compared to hydroxytyrosol and glycol, which contain a greater number of hydroxyl groups.

Since a significant portion of the polyphenols remained in the NF retentate, deionized and water obtained from previous reverse osmosis processes was added in three additions to wash the remaining target polyphenols into the permeate (diafiltration process). Figure 4.3 (b) shows the permeate flux as a function of VCR. The fluxes are very low, and it can be attributed to the fact that the membrane had already been in operation for several hours and was likely partially fouled or clogged. EC and polyphenols R% were found to be 75% and 50%, respectively. Also in this case, tyrosol shows the highest permeation into the permeate (26%), compared to the other two polyphenols, which are retained at around 60%. Despite the flux decline and partial fouling of the membrane, the diafiltration process allowed a further recovery of the remaining target polyphenols from the NF retentate.

Table 4.3 reports the electrical conductivity values and the corresponding R% as well as the concentrations of the three target polyphenols and their respective R% in the feed, permeate, and retentate streams for both membrane processes. Figure 3 c and d display the mass balances of the polyphenols of feed, permeate and retentate and their distribution between the permeate and retentate stream.

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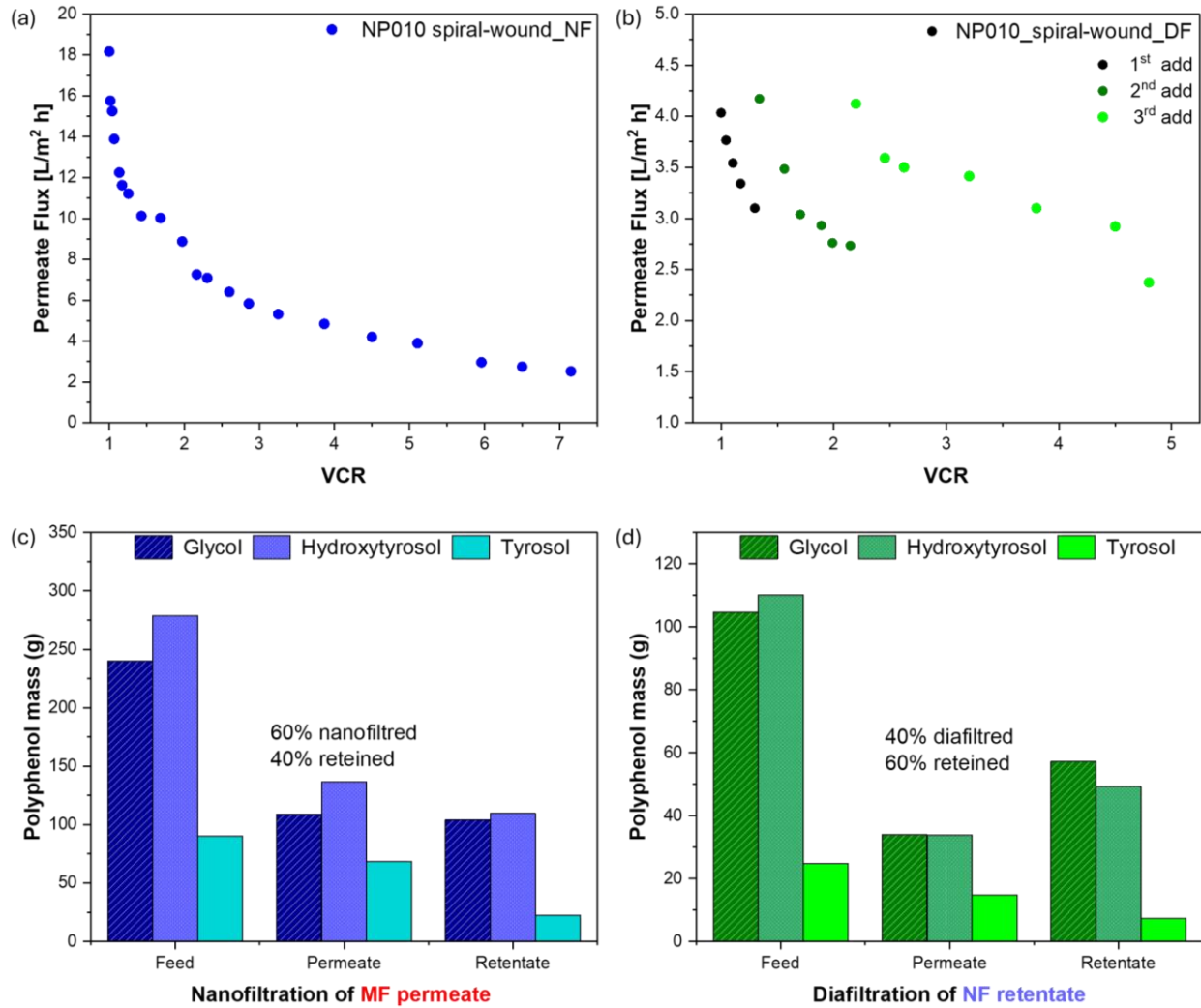


Figure 4.3: Permeate flux trends over VCR for a) nanofiltration of MF permeate and b) diafiltration of NF retentate, both operated in concentration mode using a spiral-wound NP010 membrane. Mass balance of target polyphenols for c) NF and d) DF process with their distribution between the permeate and retentate stream, calculated relative to the initial mass in the feed.

Table 4.3: Electrical conductivity (EC) and target polyphenol concentrations in the feed, permeate, and retentate streams, together with the corresponding membrane rejection (R%).

		NP010_NF			NP010_DF		
EC (μS/cm)	Feed	9230			13050		
	Permeate	4770			3250		
	Retentate	24500			20700		
R% EC		48			75		
Volume (L) 1m ³ of OMW_pH2	Feed	964			461		
	Permeate	834			372		
	Retentate	130			92		
Target polyphenols concentration (mg/L)		G	H	T	G	H	T
	Feed	249,1	289,3	93,8	227,0*	238,9*	53,8*
	Permeate	130,8	163,6	82,4	91,5	91,1	39,9
	Retentate	813,5	857,2	175,1	620,5	535,0	80,4
R% HPLC		35			50		

*concentration of NF retentate diluted with H₂O/RO permeate

Overall, the results obtained for NF and subsequent diafiltration of microfiltered OMW at pH 2 are consistent with trends widely reported for pressure-driven membrane processes applied to olive mill wastewater and other phenolic-rich agro-industrial streams. The marked initial flux decline followed by a slower decrease at increasing VCR reflects the combined effects of concentration polarization, osmotic pressure build-up and progressive cake layer formation, which increase mass transfer resistance and reduce the effective driving force for permeation. Similar behavior has been extensively described for NF and RO systems treating OMW and comparable high-organic-load matrices [40,41].

Significantly, the use of a loose NF membrane enabled a different separation strategy compared to conventional approaches. While most literature studies focus on retaining and concentrating polyphenols in the NF retentate [42], it has been widely reported that the rejection of phenolic compounds in nanofiltration strongly depends on their molecular weight and structure, with low-molecular-weight species showing partial transmission through the membrane [43]. In this context, the present work deliberately exploits this behavior by using a loose NF membrane (NP010) to promote the transmission of target polyphenols into the permeate (60% pass through the membrane). The observed behavior is also consistent with the strongly acidic operating conditions adopted in this study, under which the target phenolics remain predominantly neutral. As a result, steric effects and solute–membrane affinity interactions are expected to dominate the separation process, favoring the transmission of low-molecular-weight phenolics through the loose NF membrane.

Furthermore, the diafiltration step allowed an increased recovery of target polyphenols from the NF retentate. This behavior is consistent with the fundamental principles of diafiltration, where the addition of fresh solvent reduces the concentration of diffusible solutes in the retentate and promotes their transfer across the membrane, while less permeable macromolecular species remain retained [44,45].

From a process perspective, the combined NF–DF approach enables a controlled fractionation of the feed stream, resulting in the recovery of antioxidant low-molecular-weight polyphenols in the permeate, while concentrating higher-molecular-weight organic matter in the final retentate. This confirms the potential of membrane-based processes not only as separation technologies but also as tools for selective valorization of agro-industrial by-products.

The final concentrate resulting from the combined NF and DF streams was a dark-brown water rich in high-molecular-weight organic compounds, which have been only partially characterized. As a starting point, the liquid was extracted using solvents with different polarities, namely iso-butanol and ethyl acetate. After concentrating and drying the organic phases, it was observed that iso-butanol tended to extract a larger amount of species (brown-colored extract), whereas ethyl acetate appeared to be more selective and therefore extracted fewer compounds (orange-colored extract). Differences in extraction yield and selectivity with solvents of different polarity are consistent with literature on

polyphenol fractionation using successive solvents and on measured partition coefficients of phenolics in biphasic systems (water/ethyl acetate vs water/butanol-type alcohols) [46]. Both extracts were analyzed by HPLC to determine the concentration of target polyphenols. In particular, starting from two separate solutions of the extracts (1.2 mg/mL of extract dissolved in ACN:H₂O + 0.1% TFA, 1:1), a higher amount of target polyphenols was recovered from the ethyl acetate extract (G: 15.9 mg/L, H: 148.1 mg/L, T: 29.2 mg/L) compared to the iso-butanol extract (G: nd, H: 87.4 mg/L, T: 4.8 mg/L). These results are consistent with literature reports on solvent polarity and solute–solvent partitioning behavior [47], and align with the findings of Galanakis et al., who identified ethyl acetate (AcOEt) as a suitable solvent for the recovery of tyrosol and hydroxytyrosol [48]. At pH 2, polyphenols are in their neutral form (phenolic groups not dissociated), which increases their tendency to partition into an organic phase. Ethyl acetate has intermediate polarity, low miscibility with water, and a good ability to solvate aromatic compounds bearing –OH groups; therefore, it promotes a clearer distribution between the aqueous and organic phases, increasing the partition coefficient of phenols. Iso-butanol, on the other hand, is more polar, partially miscible with water, and capable of forming strong hydrogen bonds with water [49]. As a result, phase separation is less distinct, part of the solvent remains in the aqueous phase, and phenols are not extracted as efficiently into the organic phase. Moreover, a higher amount of non-target polar compounds may be co-extracted. Further investigation is therefore needed to quantify the possible presence of polysaccharides such as pectin, lipids, tannins, flavonoids and other higher-molecular-weight macromolecular complexes in the extracts [50].

4.3.2. Reverse osmosis

Figure 4.4 shows the permeate flux as a function of VCR, obtained by concentrating in reduced liquid volumes the permeate from NF (panel a) and the permeate from DF (panel b) using a BW30 spiral-wound RO membrane. The higher initial flux and relatively steeper decline with VCR reflect the progressive concentration polarization and mainly osmotic pressure effects, despite starting in both cases from a relatively “clean” feed. Similar trends have been reported in pressure-driven membrane treatments of OMW, where the increase in osmotic pressure and solute accumulation adjacent to the membrane significantly reduces flux at higher concentration factors [31]. As in the case of the spiral-wound NF and DF membranes, Table 4.4 reports the results obtained from measurements of electrical conductivity and polyphenol concentrations, along with their corresponding rejection values. The mass balances of the target polyphenols and their distribution between permeate and retentate streams are shown in Figures 4.4 (c) and 4.4 (d).

CHAPTER 4: INTEGRATED MEMBRANE PROCESSES FOR POLYPHENOL FRACTIONATION AND RECOVERY

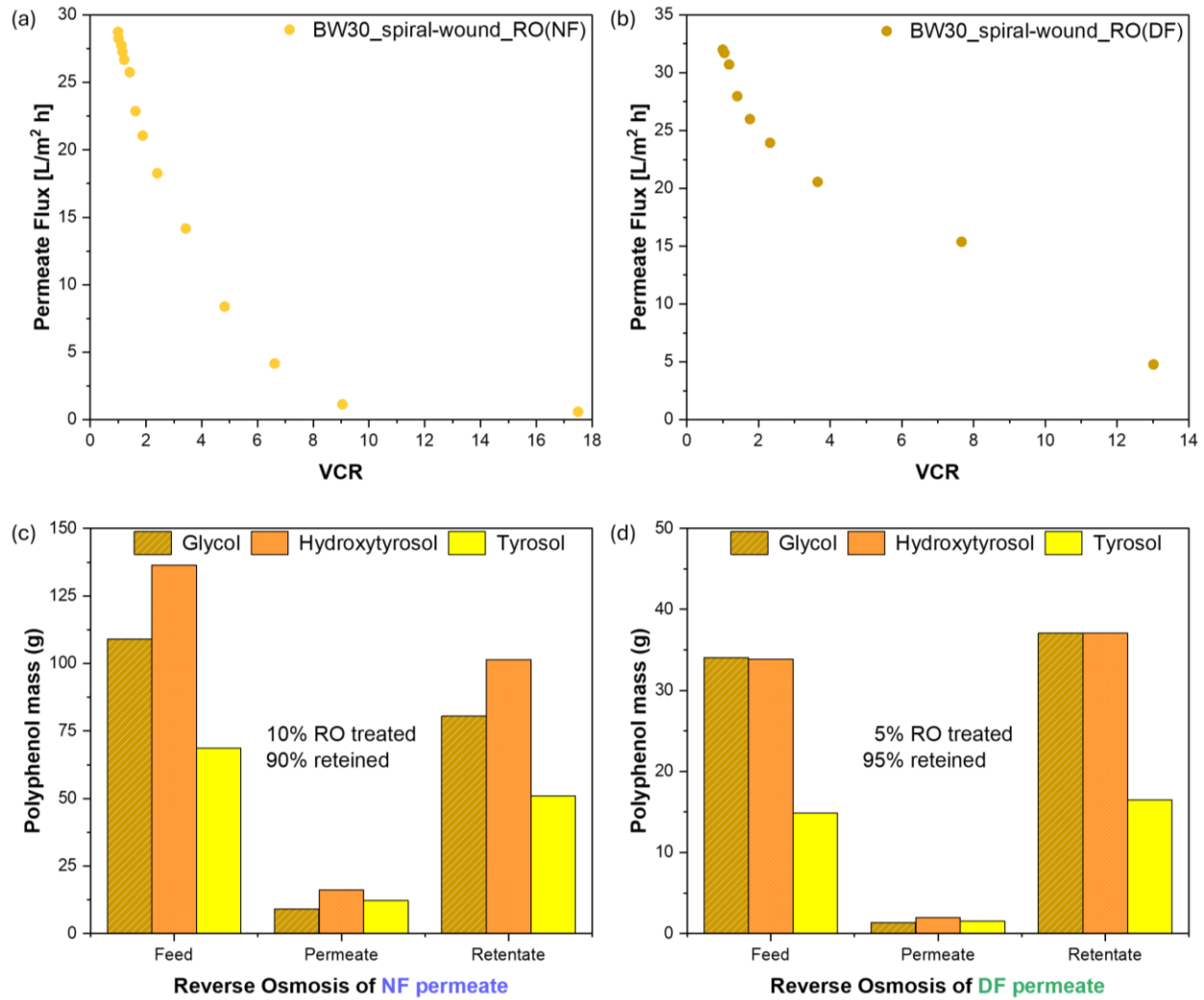


Figure 4.4: Permeate flux trends over VCR for a reverse osmosis process of a) NF permeate and b) DF permeate, both operated in concentration mode using a spiral-wound BW30 membrane. Mass balance of target polyphenols for c) RO(NF) and d) RO(DF) process with their distribution between the permeate and retentate stream, calculated relative to the initial mass in the feed.

Table 4.4: Electrical conductivity (EC) and target polyphenol concentrations in the feed, permeate, and retentate streams, together with the corresponding membrane rejection (R%).

		BW30_RO(NF)			BW30_RO(DF)		
EC (μS/cm)	Feed	4770			3250		
	Permeate	939			356		
	Retentate	38600			30200		
R% EC		80			89		
Volume (L) 1m ³ of OMW_pH2	Feed	834			372		
	Permeate	790			342		
	Retentate	48			29		
Target polyphenols concentration (mg/L)		G	H	T	G	H	T
	Feed	130,8	163,6	82,4	91,5	91,1	39,9
	Permeate	11,2	19,9	15,0	4,0	5,8	4,5
	Retentate	1694,4	2133,0	1070,6	1297,2	1296,9	578,0
R% HPLC		87			93		

Thanks to the upstream pretreatment and fractionation steps (MF–NF–DF), the RO stage could be operated up to $VCR = 17$, which is notably higher than values commonly reported for RO concentration of OMW streams, where flux is typically severely limited at lower VCR due to osmotic pressure build-up, concentration polarization, organic fouling and inorganic scavenging [31]. This highlights the key role of pretreatment in reducing fouling propensity and enabling higher concentration factors in RO, even as the effective driving force progressively decreases with increasing feed concentration and polarization effects. The relatively low salt rejection ($\sim 80\%$) observed in this study, compared with the typical values reported for RO membranes ($>95\%$), can likely be attributed to the high VCR values achieved. At elevated concentration factors, the increase in osmotic pressure and solute accumulation near the membrane surface enhances diffusive transport of salts across the membrane, leading to a reduction in apparent rejection. A similar behavior was observed for the target polyphenols: although they were effectively concentrated in the retentate, a partial passage into the permeate was also detected.

From an application perspective, the quality of the RO permeate is a key parameter for process valorization. The relatively low electrical conductivity achieved (939 and $356 \mu\text{S}/\text{cm}$ for NF and DF permeates, respectively, as RO feed), particularly in the case of the DF permeate, suggests that this stream could be reused as process water or recycled within the system, for instance in diafiltration steps. Similar internal reuse strategies have been widely proposed in integrated membrane processes for olive mill wastewater, where permeate streams are valorized rather than discharged [51]. Additional potential applications include its use for membrane cleaning operations, feed dilution, or other internal process needs. Furthermore, RO permeate streams could be investigated for integration with biological processes. In particular, residual organic carbon present in treated effluents can contribute to biological denitrification, reducing the need for external carbon sources as reported in Collivignarelli et al. work [52]. Moreover, given its relatively low salinity, the permeate may also be considered for agricultural reuse, although its suitability would require further assessment of residual organic compounds and potential phytotoxicity.

On the other hand, the RO retentate represents one of the most relevant output streams of the process, as it contains the target polyphenols concentrated in a significantly reduced volume. The final retentates reached 4.90 g/L and 3.17 g/L of total target phenolics in the RO(NF) and RO(DF) streams, respectively, confirming the strong concentration capability of the RO step. At the same time, the concentrates still exhibit relatively high electrical conductivity due to the accumulation of dissolved salts during the concentration process ($EC \sim 35 \text{ mS}/\text{cm}$). Unlike conventional MF–RO or UF–RO configurations, where a wide range of organic compounds is co-concentrated, the upstream NF and diafiltration steps enable a selective pre-fractionation of the feed. In particular, the use of a loose NF membrane allows preferential transmission of target polyphenols into the permeate, while higher-molecular-weight organic matter is largely retained. The subsequent diafiltration step further enhances the recovery of diffusible phenolics, leading to a feed stream for RO that is already enriched in target compounds and partially purified.

A summary of the main process streams and their corresponding concentration of target phenolics is reported in Table 4.5, highlighting the progressive enrichment achieved along the integrated MF–NF–DF–RO process.

Table 4.5: Distribution and enrichment of the three target phenolics (3,4-dihydroxyphenylglycol, hydroxytyrosol and tyrosol) in the main process streams obtained during the integrated MF–NF–DF–RO treatment of olive mill wastewater.

Stream	Total target polyphenols (mg/L)	Concentration factor (CF)
Raw OMW	638.7 ± 15.2	1.0
Sludge	n.d.	/
MF retentate	692.6 ± 16.5	1.1
NF retentate	1845.8 ± 46.3	2.9
DF retentate	1235.9 ± 33.9	1.9
RO(NF) retentate	4898.0 ± 105.1	7.7
RO(DF) retentate	3172.1 ± 74.6	5.0

Starting from raw OMW containing 0.64 g/L of total target phenolics, the process progressively enriched these compounds, reaching up to 4.90 g/L in the final RO(NF) retentate, corresponding to concentration factor of approximately 7.7. Intermediate streams, such as the NF retentate and the RO(DF) retentate, also showed a significant enrichment, confirming the effectiveness of the sequential membrane operations in progressively concentrating the phenolic compounds.

The concentrated stream rich in polyphenol obtained in this work can therefore be considered a suitable intermediate for further purification steps (e.g., salt removal), enabling its subsequent use in the formulation of high-value products for food, cosmetic, or agricultural applications.

4.4. Conclusions

The preliminary screening of flat-sheet nanofiltration membranes provided a clear framework for selecting the most suitable membrane for process scale-up. The results highlighted a trade-off between permeability and selectivity, with tighter membranes such as NF90 achieving high rejection of both salts and polyphenols, while looser membranes such as NP010 allowed significantly higher transmission of low-molecular-weight phenolic compounds. This behavior is consistent with the well-established dependence of nanofiltration performance on membrane permeability, MWCO, and membrane material properties. In addition to steric effects, specific solute–membrane interactions may also contribute to the observed retention patterns, particularly under the strongly acidic conditions adopted in this work. Although membranes such as NF270, TS40, and NFX exhibited higher permeate fluxes (above 45 L/m²h at 30 bar), their rejection of target polyphenols remained significant, limiting their suitability for selective target polyphenols recovery. Conversely, NP010 showed the lowest polyphenol rejection, confirming its potential as a fractionation tool rather than a concentration membrane and enabling the preferential transmission of low-molecular-weight phenolics into the permeate stream. This observation supports the concept that membrane selection

should be guided not only by permeability or retention performance alone, but by the desired separation pathway of target compounds.

Based on these results, NP010 was selected for further investigation in a spiral-wound configuration to process larger volumes. The subsequent experiments confirmed that, under increasing VCR conditions, the system exhibited the typical two-stage flux decline behavior associated with pressure-driven membrane processes, with an initial sharp decrease followed by a more gradual decline. This trend reflects the combined effects of concentration polarization, osmotic pressure build-up, and progressive cake layer formation. The application of diafiltration further enhanced this selective recovery by promoting the removal of valuable phenolic compounds from the retentate. As a consequence, the final retentate became progressively enriched in higher-molecular-weight organic compounds.

A subsequent reverse osmosis step enabled operation on a stream that was already partially purified, improving the overall efficiency of the concentration process. Starting from raw OMW containing 0.64 g/L of total target phenolics, the integrated membrane cascade progressively enriched these compounds up to 4.90 g/L in the RO(NF) retentate, corresponding to a concentration factor of approximately 7.7.

Overall, the proposed NF–DF–RO configuration represents a shift from conventional membrane strategies for OMW treatment, where nanofiltration is generally applied to maximize polyphenol retention in the retentate. In contrast, this work demonstrates that loose nanofiltration membranes can be used as selective fractionation tools to promote the transmission of low-molecular-weight phenolics into the permeate, enabling their subsequent recovery and concentration through diafiltration and reverse osmosis. Although the final concentrates still exhibit relatively high electrical conductivity, they represent a phenolic-rich intermediate stream that can be further purified (e.g., by salt removal) for the production of value-added products.

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CHAPTER 5: REFINING OF OMW BY ANION EXCHANGE RESIN

5.1. Introduction

As already stated in the general introduction, phenolic compounds present in OMW are known for various biological activities, including their potential applications as pesticides [1]. However, the concentrated solutions obtained through the methods described in the previous chapters contain a significant amount of inorganic salts. High salinity can negatively affect potential applications such as irrigation or soil amendment, as OMW is characterized by elevated salt content and can alter soil chemical and biological properties, leading to reduced seed germination, plant growth inhibition, and modifications in microbial activity [2]. Excessive ionic strength may induce osmotic stress and impair plant development, while also affecting soil structure and hydraulic properties [3]. These effects are especially critical when OMW is directly applied in agriculture or used for foliar treatments, where salt accumulation may damage plant tissues and reduce crop yield. Conversely, under controlled conditions and appropriate dilution or pretreatment, OMW-derived streams with reduced salinity can be valorized as irrigation water, soil amendments, or even as sources of bioactive compounds for agricultural or industrial applications [4,5].

Several technologies have been proposed for the treatment and valorization of OMW, including chemical oxidation, coagulation–flocculation, solvent extraction, membrane processes, and adsorption [6–13]. Among these, membrane-based processes have gained particular attention due to their ability to efficiently fractionate complex effluents without the addition of chemicals. In particular, nanofiltration has been widely reported as an effective technique for separating low-molecular-weight compounds, allowing the production of streams enriched in phenolic compounds while reducing the concentration of salts and other inorganic species. Nevertheless, membrane processes alone are often insufficient to achieve the desired selectivity and purity for specific reuse or valorization pathways. As a result, additional downstream purification steps are typically required to further concentrate or selectively recover target compounds from nanofiltration permeates [14,15].

In this context, adsorption processes have been widely investigated as an effective and versatile approach for the separation of organic micropollutants from aqueous solutions. Adsorption onto polymeric resins, in particular, has emerged as a promising option for the recovery of aromatic compounds, due to their tunable surface properties, ease of regeneration, and relatively low operational costs [16,17]. Polymeric resins used for this purpose can be broadly classified into two main categories: non-ionic adsorbent resins and ion exchange resins [17,18]. Non-ionic resins, typically based on styrene–divinylbenzene matrices, interact with phenolic compounds mainly through hydrophobic interactions and π – π stacking, as widely reported both for model phenolic systems and real agro-industrial effluents [16,19,20]. In contrast, ion exchange resins rely on

electrostatic interactions and can retain ionized species, with their performance strongly influenced by pH and solution composition [8,17,21].

While non-ionic resins are generally preferred for selective phenolic recovery, ion exchange resins, especially in hydroxide form, offer the potential advantage of simultaneously remove acidic anions and promote the retention of aromatic compounds [8,21]. This aspect becomes particularly relevant when treating an already processed stream. In this context, the use of nanofiltration as a pre-treatment step can modify the chemical composition of the feed stream and generate permeates or retentates with different phenolic and inorganic profiles, potentially affecting resin performance [15,22]. Therefore, the application of a strong-base anion exchange resin in hydroxide form may promote the neutralization and partial removal of acidic inorganic and organic species, while simultaneously enabling the recovery of phenolic compounds through adsorption–desorption processes. This integrated approach could contribute to the purification and valorization of OMW-derived streams, although the balance between adsorption mechanisms, ion-exchange phenomena, competition effects, and regeneration efficiency remains a critical aspect to be investigated [21,23,24].

In this framework, the present chapter investigates the use of a strong-base anion exchange resin (Amberlyst® A26, OH⁻ form) for the treatment of nanofiltration permeate derived from OMW. The study focuses on the reduction of residual acidity and ionic species, together with the adsorption–desorption behavior of phenolic compounds. Batch and column experiments were performed to evaluate the influence of operating parameters, including resin dosage and pH, as well as the recovery of target compounds. In addition, potentiometric titration was carried out to investigate the acid–base behavior of the nanofiltration permeate upon NaOH addition, providing insight into the ionization of organic species and its possible impact on resin performance. Finally, a preliminary techno-economic assessment was carried out to evaluate the feasibility and sustainability of the proposed approach.

5.2. Materials and Methods

5.2.1. Materials

Olive mill wastewaters coming from three-phase system olive oil production were supplied by Frantoio Rocca Danilo (Tovo San Giacomo, Italy). The raw effluent was subsequently pretreated by acidification, followed by a coupled microfiltration and nanofiltration stage. The NF permeate become the feed of the subsequent trials. 3,4-dihydroxyphenylglycol, hydroxytyrosol, tyrosol were used as standard for HPLC calibrations. Acetonitrile and trifluoroacetic acid were used for HPLC mobile phase preparation. All the chemicals were purchased from Sigma Aldrich (Milan, Italy). Gallic acid (99% w/w, Sigma-Aldrich) was used as an analytical standard to prepare the calibration curve for the spectrophotometric determination of UV-absorbing compounds. Concentrations of the analyzed samples were expressed as gallic acid equivalents (GAE). NaOH 1 M (Sigma-Aldrich) was used both for OMW pH adjusting and resin's regeneration. Amberlyst A26® strong base anion exchange resin (OH⁻ form, purchased from Sigma Aldrich) was used for ion-exchange experiments,

which physical and chemical characteristics are summarized in Table 5.1. Water, methanol and acetic acid were used as solvents for the desorption stage.

Table 5.1: Amberlyst A26[®] physical and chemical characteristics.

Resin features	Amberlyst A26 [®]
Type of IE resin	Strong-base anionic
Matric	Styrene-divinylbenzene (macroreticular)
Functional group	Quaternary ammonium
Ion form as shipped	OH ⁻
Adsorption capacity (eq/L)	0.8
Density (g/L)	656.8
Operative pH range	0-14

5.2.2. Methods

5.2.2.1. Physicochemical analyses

The pH was measured using a Hanna pH209 pH meter, while electrical conductivity (EC) was determined using a Hanna EC215TM conductivity meter equipped with a Hanna HI76303TM probe. The concentration of UV-absorbing compounds was estimated from absorbance measurements at 280 nm and expressed as gallic acid equivalents (GAE), using a Spectroquant[®] Prove spectrophotometer (Merck, Darmstadt, Germany). Calibration curves with gallic acid (5–100 mg L⁻¹) yielded R² values of 0.999.

According to literature, hydroxytyrosol shows a maximum absorbance at approximately 280 nm, whereas tyrosol exhibits a maximum at around 276 nm [25]. Therefore, measurements at 280 nm allow effective monitoring of phenolic compounds commonly present in OMW. This approach was preferred over the Folin–Ciocalteu method due to its faster analysis time and suitability for processing a large number of samples. Target polyphenols quantification was carried out by high-performance liquid chromatography (HPLC) analysis. It was performed with a Shimadzu system (LC-10ATvp pump, SPD-10Avp detector, $\lambda = 280$ nm), equipped with a Synergi Hydro-RP column (150 × 3 mm, 4 μ m) and a pre-column filter. The mobile phase consisted of H₂O + 0.1% trifluoroacetic acid (A) and acetonitrile (B), with a 46 min gradient program. Injection volume: 5 μ L; flow rate: 0.7 mL min⁻¹; column temperature: 23–25 °C. Calibration curves are reported in the Supplementary Material.

Potentiometric Titration

A potentiometric titration was performed to characterize the acid–base behavior of the NF permeate and to determine its total acidity. A volume of 200 mL of NF permeate, exhibiting an initial pH of 2.5, was placed in a jacketed glass vessel and stirred continuously. The pH was measured using a Hanna pH209 pH meter. A 1.0 M NaOH solution was added stepwise in increments of 0.5 mL up to a total volume of 10 mL. After each addition, the system was allowed to reach a stable reading before recording the pH value. The collected data were used to construct the potentiometric titration curve.

The total acidity of the permeate was calculated from the titration curve by identifying the equivalent point, corresponding in this case to the volume of NaOH at which the major inflection in pH occurred. The total acidity was then determined according to:

$$\text{Total acidity (eq/L)} = \frac{C_{\text{NaOH}} \cdot V_{\text{NaOH,eq}}}{V_{\text{sample}}} \quad (1)$$

where C_{NaOH} (1 mol/L) is the concentration of the titrant, $V_{\text{NaOH,eq}}$ is the NaOH volume (L) at the equivalent point and V_{sample} is the volume of permeate titrated.

This procedure provided the quantitative acid content of the NF permeate and enabled the identification of pH intervals relevant to subsequent adsorption experiments.

5.2.2.2. Adsorption-desorption experiments

A preliminary batch test was carried out to evaluate the effect of resin dosage on pH variation and on the removal of UV-absorbing compounds from the OMW sample (NF permeate). Amberlyst® A26 strong-base anion exchange resin (OH^- form) was weighed separately in amounts of 1, 2, 3, 4, and 5 g. Each resin sample was enclosed in a permeable tea bag and conditioned in deionized water under stirring for 60 min prior to use, ensuring proper hydration and removal of residual storage solution while preventing mechanical damage of the resin particles and allowing mass transfer with the liquid phase. Five 100 mL beakers were prepared, each containing 60 mL of NF permeate (initial pH = 2.5, concentration of UV-absorbing compounds = 75.2 mg GAE/L). The pre-conditioned resin samples were then added to the respective beakers, corresponding to resin dosages of approximately 16.7, 33.3, 50.0, 66.7, and 83.3 g/L, respectively. The five systems were maintained under constant stirring at room temperature. A schematic representation of the experimental setup is shown in Figure 5.1 (a). Samples were collected at predetermined time intervals and analysed to measure pH and the concentration of UV-absorbing compounds. All experiments were performed under identical conditions, and results were reported as a function of contact time and resin dosage.

The removal efficiency (%) was determined by measuring the residual amount of the target species in the liquid phase, then calculating the adsorption percentage with the following equation:

$$\% \text{ ads} = \frac{C_0 - C_e}{C_0} \times 100 \quad (2)$$

where C_0 and C_e are the initial and equilibrium concentration (mg/L), respectively.

Column adsorption-desorption experiments (see Figure 5.1 b) were carried out using a glass column equipped with a porous frit at the bottom, with an internal diameter of 2.5 cm and a total length of 1 m. The column was fitted with a valve to allow flow rate regulation. The column packing was performed by directly adding the adsorbent material (~ 40 g of Amberlyst® A26, OH^- form) onto the porous support, resulting in a packed bed height of approximately 20 cm. This parameter was used to estimate the resin bed volume (BV ~100 mL) for subsequent process calculations. A layer of inert sand was placed on top of the packed bed in order to stabilize the upper surface and ensure uniform flow distribution. A peristaltic pump (Percom N-M) was used to feed the column. The flow rate was

controlled by adjusting the pump rotation speed (rpm), allowing precise regulation of the liquid flow through the column.

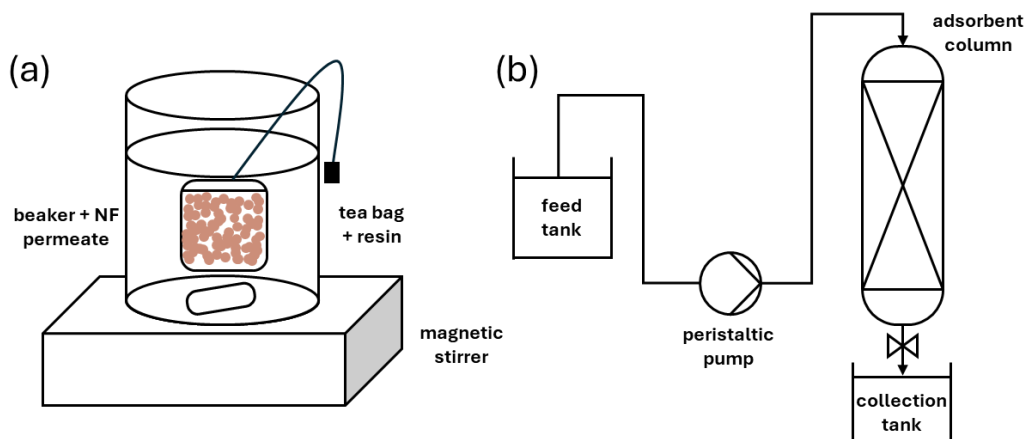


Figure 5.1: Experimental lab-scale set-up used for the (a) batch-test and (b) column adsorption-desorption experiments.

Prior to adsorption experiments, the packed column was conditioned by feeding deionized water at a flow rate of approximately 10 mL/min for 1 h, in order to properly settle and compact the resin bed. After complete elution of the deionized water, the feed was switched to the NF permeate, which was introduced from the top of the column, while the effluent was collected from the bottom. The inlet flow rate was controlled by the peristaltic pump and set equal to the outlet flow rate (approximately 15 mL/min), thus simulating continuous operation. Considering a resin bed volume of approximately 100 mL, the corresponding empty bed contact time ($EBCT = V/Q$) was approximately 6.7 min. Samples of the effluent were collected at predetermined volume intervals and analyzed in term of pH, EC and UV absorbance at 280 nm, as well as by HPLC analysis, in order to determine the concentration of target phenolic compounds in the collected fractions. The obtained data were used to construct breakthrough curves, defined as the variation of the concentration of UV-absorbing compounds in the effluent as a function of the treated volume. These curves allowed the identification of resin exhaustion point, corresponding to the condition in which the outlet concentration approached the inlet concentration (plateau region). All the effluent collected during the initial adsorption stage, corresponding to the continuous feeding of NF permeate through the packed-bed column before the breakthrough of UV-absorbing compounds, was grouped and referred to as the “zero fraction”. This stream therefore contains the components of the OMW-derived feed that were not retained by the resin, while the majority of aromatic compounds remained adsorbed within the resin bed.

Additional column experiments were carried out to evaluate the effect of feed pH on adsorption performance. The pH of the NF permeate (2.5) was adjusted to approximately 4 and 7 by addition of 1 M NaOH prior to column feeding. The experiments were performed under the same operating conditions described above, and breakthrough curves were obtained by monitoring the concentration of UV-absorbing compounds (expressed as GAE) as a function of the treated volume.

Following adsorption, desorption experiments were performed to recover the compounds retained on the resin. Different eluents were tested, including deionized water, methanol, and acetic acid, alone

or in different mixtures. The desorbed fractions were collected and characterized in terms of pH, EC, and UV absorbance at 280 nm, as well as by HPLC analysis, in order to determine the concentration of target phenolic compounds in the collected fractions. After adsorption-desorption experiments, the exhausted resin was regenerated using a 1 M NaOH solution. The regenerant was passed through the column in order to restore the OH^- form of the resin. Following regeneration, the column was rinsed with deionized until the effluent reached approximately neutral pH before subsequent use.

5.3. Results and Discussion

5.3.1. Batch adsorption test

The preliminary batch experiments were carried out to evaluate the effect of resin dosage on pH variation and on the removal of UV-absorbing compounds from the NF permeate.

As shown in Figure 5.2 (a), both the concentration of UV-absorbing compounds (left axis) and the pH (right axis) evolve over contact time as a function of resin dosage. The concentration reduction is more pronounced at higher resin dosage (83.3 g/L) showing the lowest residual concentration. Most of the adsorption occurs within the first hours of contact, after which the concentration tends to stabilize, indicating the approach to equilibrium conditions. At the same time, the pH increases sharply upon contact with the resin and then gradually stabilizes over time. The final pH strongly depends on the resin dosage, reaching progressively higher values as the amount of resin increases. This behavior is consistent with the basic nature of the resin in OH^- form, which progressively neutralizes the acidic species present in the solution. Figure 5.2 (b) reports the removal efficiency as a function of resin concentration showing a clear positive correlation. In particular, the highest resin concentration (83.3 g/L) leads to a removal of approximately 70%, confirming the strong influence of resin dosage on adsorption performance.

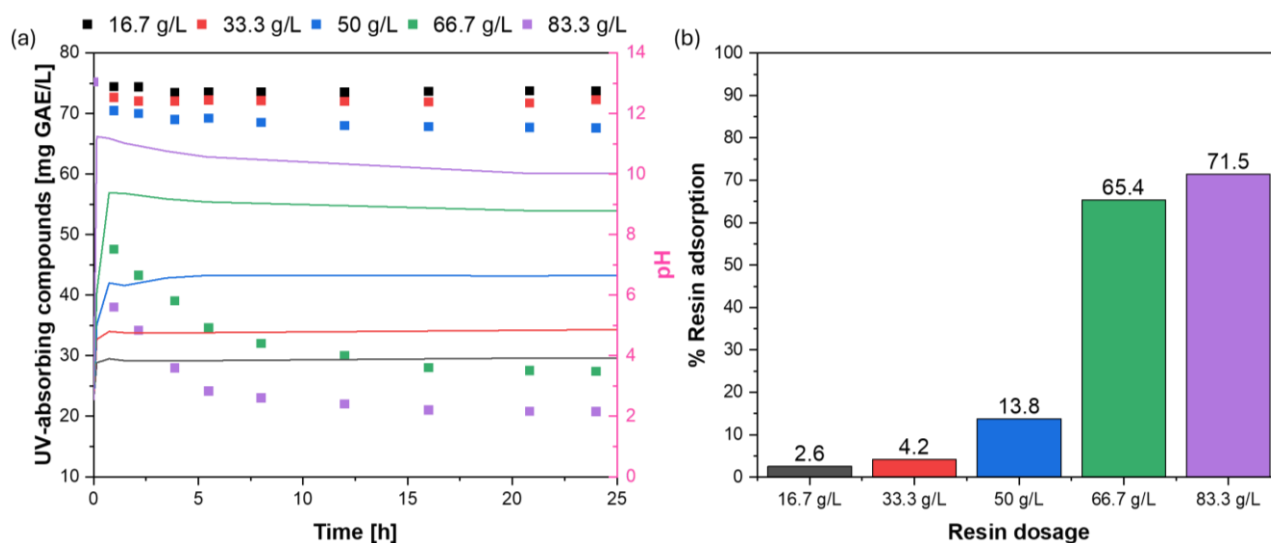


Figure 5.2: (a) Evolution of UV-absorbing compounds concentration (square) and pH (lines) over contact time for NF permeate samples (60 mL) in beakers containing increasing amounts of resin; (b) adsorption efficiency (%) (see Equation 1) as a function of resin dosage.

The relationship between pH and removal efficiency should therefore be interpreted considering the acid–base interactions occurring in the system. The anion exchange resin in OH^- form first neutralizes the acidic species present in the matrix and subsequently promotes the deprotonation of phenolic compounds, forming phenolate ions that can interact with the positively charged functional groups of the resin. When the resin dosage is insufficient, part of the acidic species and phenols remain protonated, buffering the solution at lower pH and limiting adsorption. Conversely, when the resin is present in excess, a larger fraction of phenolic compounds is converted into phenolate form, which favors their interaction with the resin and results in a more basic equilibrium pH. The observed increase in pH can therefore be attributed mainly to the formation of phenolate species in solution, together with a minor contribution from OH^- leakage from the resin. This interpretation is consistent with previous studies showing that the removal of phenolic compounds by anion exchange resins is strongly influenced by the acid–base speciation of the solute, with the deprotonated phenolate form being preferentially retained through electrostatic interactions with the positively charged functional groups of the resin [8,26]. For instance, tyrosol has a pK_a of approximately 10.2, meaning that at pH values above this threshold the phenolic group is predominantly present in its deprotonated form, which favors its interaction with the resin.

5.3.2. Packed bed resin column adsorption test

Packed-bed column experiments were performed to evaluate the dynamic adsorption behavior of the resin under continuous-flow conditions using the NF permeate at its natural pH (2.5). Figure 5.3 (a) reports the concentration of UV-absorbing compounds in the effluent as a function of the treated volume, while Figure 5.3 (b) shows the corresponding pH and electrical conductivity profiles.

At the beginning of the experiment, the concentration of UV-absorbing compounds in the effluent remained very low, indicating that the resin effectively retained the aromatic constituents of the permeate. This initial phase corresponds to the adsorption stage, during which the active sites of the resin were progressively occupied. As the treated volume increased, the effluent concentration exhibited a sharp rise, indicating the breakthrough region. The effluent concentration approached the inlet concentration at approximately 1000 mL, corresponding to the exhaustion volume of the column, i.e., the point at which the resin active sites are effectively saturated. Beyond this point, a plateau was observed, with the outlet concentration remaining nearly constant and comparable to the feed. This behavior confirms that the adsorption capacity of the resin had been exhausted and that no further significant retention occurred. The pH profile initially showed elevated values relative to the feed, followed by a gradual decrease toward acidic conditions. This behavior reflects the release of OH^- ions associated with the initial ion exchange and neutralization reactions occurring within the resin, followed by their progressive consumption as the exchange capacity of the resin decreases. Once the exchangeable capacity was depleted, the effluent pH converged to that of the feed. A similar trend was observed for electrical conductivity, which initially increased and then gradually approached the feed value. The initial conductivity increase can be attributed to the ion-exchange and neutralization reactions occurring within the resin. In particular, the exchange of acidic anions, such as HSO_4^- , SO_4^{2-}

and organic species, with OH^- released from the resin contributes to the increase in EC of the first collected fractions. The high mobility of OH^- ions, together with the transmission of dissolved cations through the column, likely contributes to the elevated conductivity observed during the initial stage of operation. As the exchange capacity of the resin becomes progressively exhausted, the conductivity stabilizes at values comparable to those of the feed, indicating that the system approaches steady-state conditions.

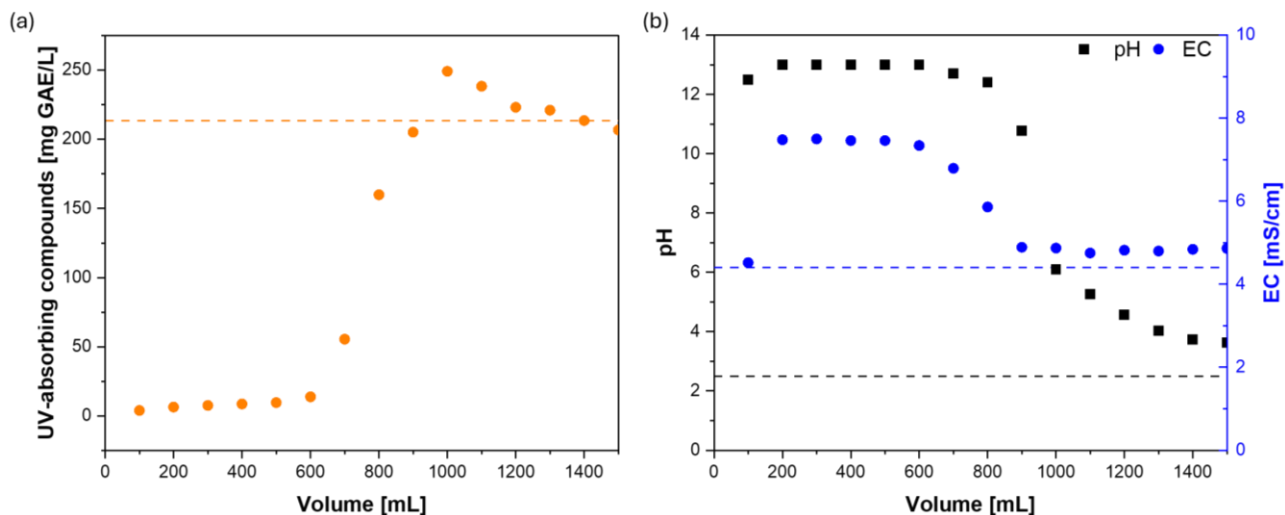


Figure 5.3: (a) UV-absorbing compounds concentration and (b) pH and electrical conductivity of the NF permeate fractions eluted from the packed-bed resin column. The horizontal dashed line indicates the feed (NF permeate) UV-280nm concentration (orange), pH (black) and electrical conductivity (blue).

The UV-based breakthrough behavior was further validated by HPLC analysis of the main target phenolic compounds. As shown in Figure 5.4, the breakthrough profiles of hydroxytyrosol, tyrosol, and glycol follow the same trend as the UV-absorbing fraction, confirming the reliability of UV monitoring at 280 nm for tracking column performance. Although UV quantification is based on gallic acid equivalents, the similar trends and comparable values suggest that the selected target compounds represent a major contribution to the overall UV signal at 280 nm and that their response are sufficiently consistent for comparative purposes.

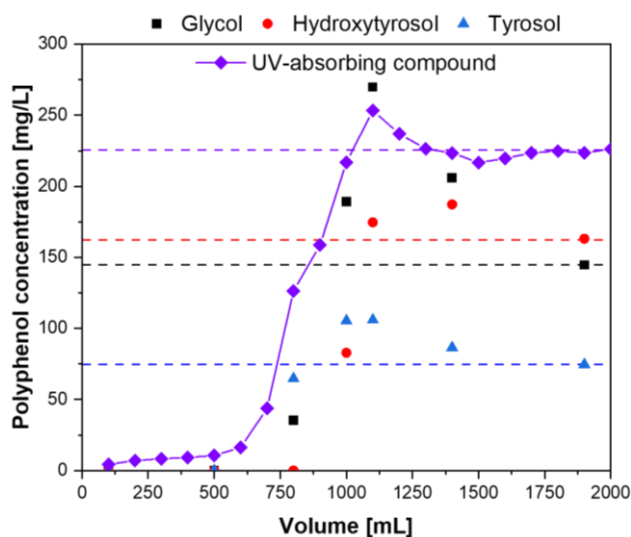


Figure 5.4: Breakthrough curve of the UV-absorbing and the three target compounds. The horizontal dashed lines indicates the feed (NF permeate) UV-280 nm (purple), glycol (black), hydroxytyrosol (red) and tyrosol (blue) concentration.

Overall, the breakthrough curve (outlet solute concentration as a function of eluted volume) provides a clear indication of the resin's dynamic performance and allows the identification of the exhaustion point, which is a key parameter for process design and scale-up.

5.3.2.1. Effect of feed pH on column adsorption performance

Although the packed-bed experiment at the natural pH of the permeate provides a clear baseline description of the resin's behavior, the evolution of pH and conductivity during the adsorption stage indicates that acid–base processes play a significant role in determining the availability of the active sites and the progression of breakthrough. Since the interactions of polyphenols and weak acids with polymeric resins are strongly pH-dependent, a deeper understanding of how the NF permeate responds to controlled pH changes was required.

For this reason, a potentiometric titration of the NF permeate was performed to characterize its acid–base properties, quantify its total acidity, and identify the pH range in which the main classes of dissolved compounds, particularly polyphenols, change their protonation state. These results form the basis for interpreting the adsorption behavior observed when feeding the column at different pH values.

Potentiometric titration

To investigate the acid–base behavior of the NF permeate and identify pH intervals potentially relevant to adsorption, a potentiometric titration was performed. A volume of 200 mL of permeate (initial pH 2.5) was titrated with 1.0 M NaOH, added in stepwise increments up to a total of 10 mL. The resulting pH values were used to construct the titration curve shown in Figure 5.5.

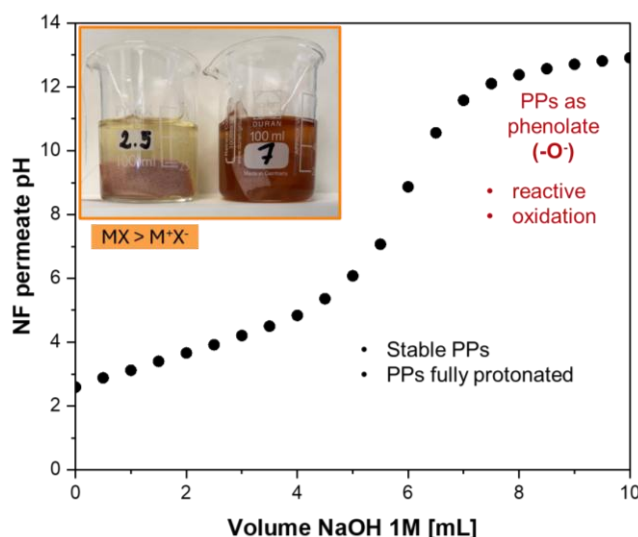


Figure 5.5: Potentiometric titration curve of the NF permeate (initial pH 2.5) obtained by incremental addition of 1.0 M NaOH up to a total of 10 mL.

The titration curve exhibits a broad buffering behavior extending approximately between pH 3 and 7, indicating the presence of multiple acidic species with different acid strengths. This behavior is consistent with the complex composition of OMW-derived streams, which contain a mixture of low-molecular-weight organic acids together with residual inorganic acidic species originating from prior

acidification with sulfuric acid and carried through the loose nanofiltration membrane (resulting in an electrical conductivity of approximately 4.5 mS/cm). However, these inorganic acidic species, such as bisulfate (HSO_4^-), are expected to be neutralized predominantly at the beginning of the titration, whereas the buffering capacity observed between pH 3 and 7 is mainly associated with the progressive neutralization of organic acids present in the NF permeate. [22,27].

The main inflection point occurred between 6 and 6.5 mL of NaOH, which was taken as the equivalent volume for determining the total acidity of the permeate. Total acidity was calculated using equation 1, where $C_{\text{NaOH}} = 1 \text{ mol/L}$, $V_{\text{NaOH,eq}} = 0.006\text{--}0.0065 \text{ L}$ and $V_{\text{sample}} = 0.2 \text{ L}$. The resulting total acidity was approximately 0.03 eq/L, consistent with the cumulative contribution of multiple weak organic acids together with inorganic bisulfate, providing a quantitative picture of the acid load that the resin encounters during adsorption. Beyond the quantification of acidity, the titration provided insight into the protonation state of polyphenols. Up to pH 6–7, phenolic hydroxyl groups (pK_a 8–11) remain fully protonated, and polyphenols therefore exist in their neutral form. Only above pH 8–10 does progressive phenolate formation occur, accompanied by a visible color transition (yellow to orange-brown), consistent with increased π -electron delocalization and with the onset of oxidative reactions in the presence of oxygen. Above pH 11, these processes accelerate significantly [28–31].

This characterization allowed identification of the pH intervals relevant to both the neutralization of acidic species and the speciation of phenolic compounds, providing a framework for interpreting the adsorption behavior observed in the packed-bed experiments.

Column adsorption performance

On the basis of the titration results, adsorption experiments were carried out by feeding the resin-packed column with NF permeate adjusted to three distinct pH values (2.5, 4, and 7) to assess how the protonation state of dissolved organic compounds and the overall acid–base composition of the feed influence the breakthrough behavior.

The experiment conducted at pH 7 immediately exhibited operational inconsistencies. The eluate developed a rapid and unstable pink–violet coloration upon exiting the column, preventing reliable quantification of UV-absorbing species at 280 nm. This behavior is consistent with the color changes observed during the alkalization of the NF permeate and can be attributed to the increased oxidative sensitivity of phenolic compounds under near-neutral conditions. Partial deprotonation of phenolic hydroxyl groups may promote the formation of quinones and other oxidation products, thereby altering the UV signal and preventing meaningful interpretation of the breakthrough behavior [32,33]. For these reasons, the pH 7 dataset was excluded from the comparative analysis.

Figure 5.6 reports the breakthrough curves obtained by monitoring the concentration of UV-absorbing species at 280 nm as a function of the eluted volume for the experiments performed at the natural pH of the NF permeate (2.5) and at pH 4.

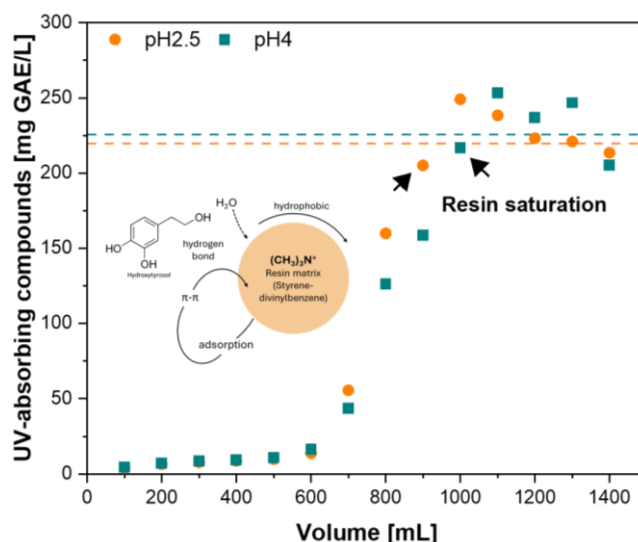


Figure 5.6: Breakthrough curves of UV-absorbing compounds (280 nm) obtained during packed-bed adsorption experiments using NF permeate at the natural pH (circles) and adjusted to pH 4 (squares). The horizontal dashed lines indicate UV-280nm concentration of feed at pH2.5 (orange) and at pH4 (dark cyan).

At both pH values, the initial effluent fractions exhibited low UV absorbance, indicating effective retention of aromatic compounds by the resin during the early adsorption stage. However, clear differences emerged in the progression of the breakthrough curves. At pH 4, the resin exhibited improved adsorption performance, as indicated by the delayed breakthrough and later saturation compared to pH 2.5. Under acidic conditions, classical ion-exchange interactions are limited because both organic acids and polyphenols are largely protonated and neutral. Adsorption is therefore primarily governed by non-electrostatic interactions with the polystyrene matrix of the resin, including: hydrophobic interactions, π - π interactions between aromatic structures, van der Waals forces [20,30,34]. Nevertheless, the strong-base resin in the OH^- form may locally generate alkaline conditions within the resin phase. Under these conditions, partial deprotonation of a fraction of phenolic compounds cannot be excluded, potentially leading to the formation of phenolate species capable of interacting electrostatically with the positively charged quaternary ammonium groups. Therefore, although non-electrostatic interactions are expected to dominate under the investigated bulk pH conditions, the coexistence of secondary ion-exchange contributions may also occur.

Nevertheless, the acid–base composition of the feed strongly influences how rapidly the resin’s active sites become consumed by neutralization reactions. [16]. This behavior can be interpreted considering the acid–base properties of the main compounds present in the NF permeate. Carboxylic acids typically exhibit pK_a values in the range of 4–5, whereas the phenolic hydroxyl groups of tyrosol, hydroxytyrosol and 3,4-dihydroxyphenylglycol present significantly higher pK_a values (approximately 9–10). Consequently, within the investigated pH range (2.5–4), phenolic compounds remain predominantly protonated and electrically neutral, while acidic species are responsible for most of the neutralization demand exerted on the resin in the OH^- form. According to the titration results, the permeate at pH 2.5 contains a higher load of titratable acidic species, both inorganic (e.g., bisulfate) and organic, than the permeate adjusted to pH 4. As a result, at pH 2.5 a larger fraction of the resin’s exchange capacity is initially consumed in neutralizing these acidic components before a

significant fraction of the resin capacity becomes available for the retention of aromatic compounds. Conversely, at pH 4 the overall acid load is lower, meaning that a smaller proportion of the resin's capacity is spent in neutralization, and a larger fraction of active sites remains available for adsorption of UV-absorbing phenolic compounds. This effect results in a delayed breakthrough and an apparently higher adsorption capacity at pH 4. In summary, within the acidic range investigated (pH 2.5–4), adsorption of aromatic compounds proceeds through a combination of non-electrostatic interactions and pH-dependent competition between inorganic acids, organic acids, and phenolic compounds for access to the resin's active sites.

5.3.3. Resin desorption results

To examine the influence of feed conditions and elution strategy on the behavior of the packed-bed system, four adsorption–desorption tests were carried out under different operating conditions. Table 5.2 summarizes the key parameters for each test, including: (i) the feed used (NF permeate at pH 2.5 or pH 4), (ii) the exhaustion volume, defined as the treated volume at which the effluent concentration approaches that of the feed ($C/C_0 \approx 1$), (iii) the eluent sequence, and (iv) the electrical conductivity (EC) measured in the fractions collected during each desorption step. The eluents were grouped into three classes: A (water), B1–B3 (hydroalcoholic mixtures, H₂O:MeOH 8:2, acidified with increasing acetic acid concentrations), and C1–C3 (acidified aqueous solutions). The EC values reported for the A, B and C fractions provide an indication of the ionic content detected during each elution step.

Table 5.2: Desorption conditions tested in this study, including solvent composition and acetic acid concentration.

	Test 1	Test 2	Test 3	Test 4
Feed	NF perm_pH 2.5	NF perm_pH 4	NF perm_pH 4	NF perm_pH 4
Exhaustion volume* [mL]	900	1000	800	800
Eluent	900 mL A + 900 mL B	300 mL A + 900 mL C	900 mL A + 900 mL C	900 mL A + 900 mL B
EC [μ S/cm] fraction	A) 500 B) 800-950	A) 312 C) 1100-1600	A) 200 C) 1100-1250	A) 200 B) 800-1100
A = H ₂ O; B = H ₂ O:MeOH (8:2) + AcOH, % v/v (B1=2.5% AcOH, B2=5% AcOH, B3=7.5% AcOH); C = H ₂ O+AcOH, % v/v (C1=2.5% AcOH, C2=5% AcOH, C3=7.5% AcOH)				

*feed volume at which the effluent concentration approaches the inlet concentration ($C/C_0 \sim 1$), indicating saturation of the adsorption sites of the resin. All the experiments were conducted on the same amount of resin (~100 mL)

Figure 5.7 reports the desorption profiles of the UV-absorbing compounds and target polyphenols in terms of concentration as a function of the eluted volume. All compounds exhibit a decreasing concentration with increasing eluted volume, confirming their progressive release from the resin.

At the beginning of the desorption step, the first collected fraction corresponds to the displacement of the residual NF permeate contained in the column void volume (column hold-up). Since the phenolic compounds detected in this fraction originate from the liquid phase remaining in the column

at the end of the adsorption step, rather than from actual desorption from the resin, this fraction was excluded from the recovery calculations. Desorption recoveries were therefore calculated only from fractions collected after the hold-up volume had been completely displaced. This distinction is essential to avoid overestimation of desorption efficiency.

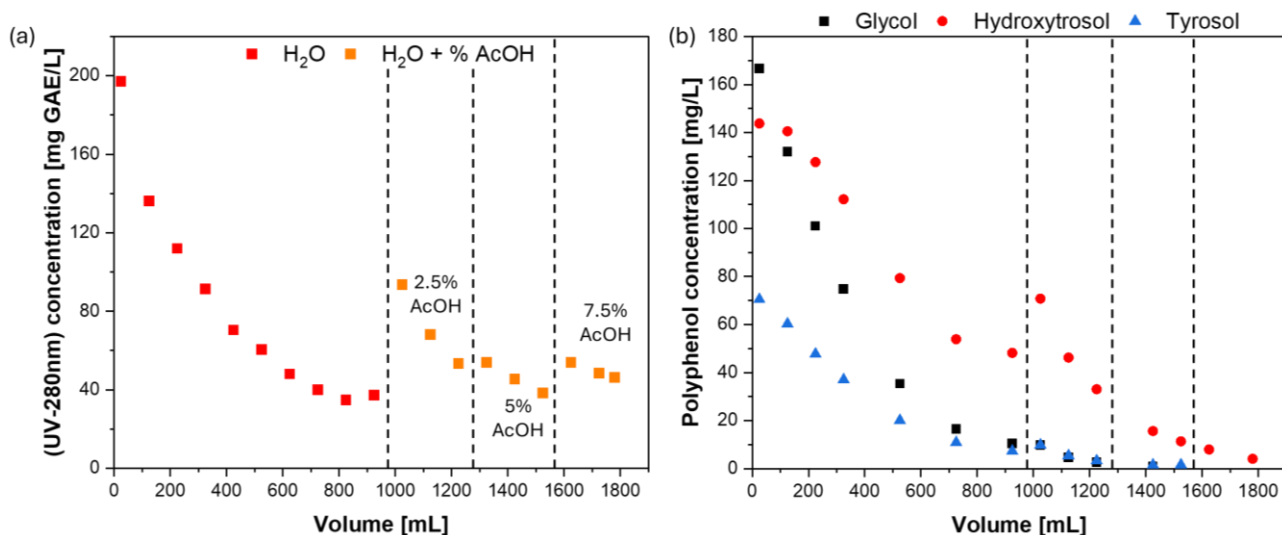


Figure 5.7: Desorption profiles of the UV-absorbing compound and target polyphenols in terms of concentration over eluted volume. First collected fraction (hold-up volume) wasn't considered as part of the desorption recovery calculation.

During the initial stage of desorption with water, a gradual decrease in phenolic compounds was observed, indicating the removal of species retained through relatively weak interactions with the resin matrix. This behavior suggests that adsorption was not exclusively governed by strong ion-exchange interactions, but also involved weaker interactions with the polymeric matrix, as previously discussed. The relatively low electrical conductivity of the water fractions further suggests that this step mainly displaced residual liquid contained in the column and recovered weakly retained aromatic compounds, with limited desorption of ionic species. The subsequent addition of acetic acid further enhanced the desorption of phenolic compounds and was accompanied by an increase in electrical conductivity, indicating the release of ionic species previously associated with the resin. This effect can be attributed to the decrease in pH, which promotes reprotonation of partially deprotonated species and weakens electrostatic interactions with the positively charged resin functional groups. At the same time, the acidic environment facilitates disruption of weaker adsorption interactions, promoting the release of retained phenolic compounds [35].

Recovery was calculated as the ratio between the total mass of compounds recovered during desorption and the initial mass fed to the column. The recovered mass was obtained by integrating the concentration of each fraction multiplied by its corresponding volume ($m = C \times V$), summing the contributions of all collected fractions (solvent A and solvent B/C). The initial mass was estimated as the product of the inlet concentration of the NF permeate and the exhaustion volume of the column.

$$Recovery(\%) = \frac{\sum_i C_i V_i}{C_0 V_{exh}} \times 100 \quad (3)$$

where C_i and V_i are the concentration and volume of each collected fraction, C_0 is the inlet concentration, and V_{exh} is the exhaustion volume. The overall recovery yields for each condition are summarized in Figure 5.8, where panel (a) shows the recovery of UV-absorbing compounds (expressed as % of gallic acid equivalents, GAE) and panel (b) the recovery of target phenolic compounds (expressed as %), determined by HPLC analysis. Recoveries are calculated on a mass basis (% w/w) relative to the initial mass fed to the column.

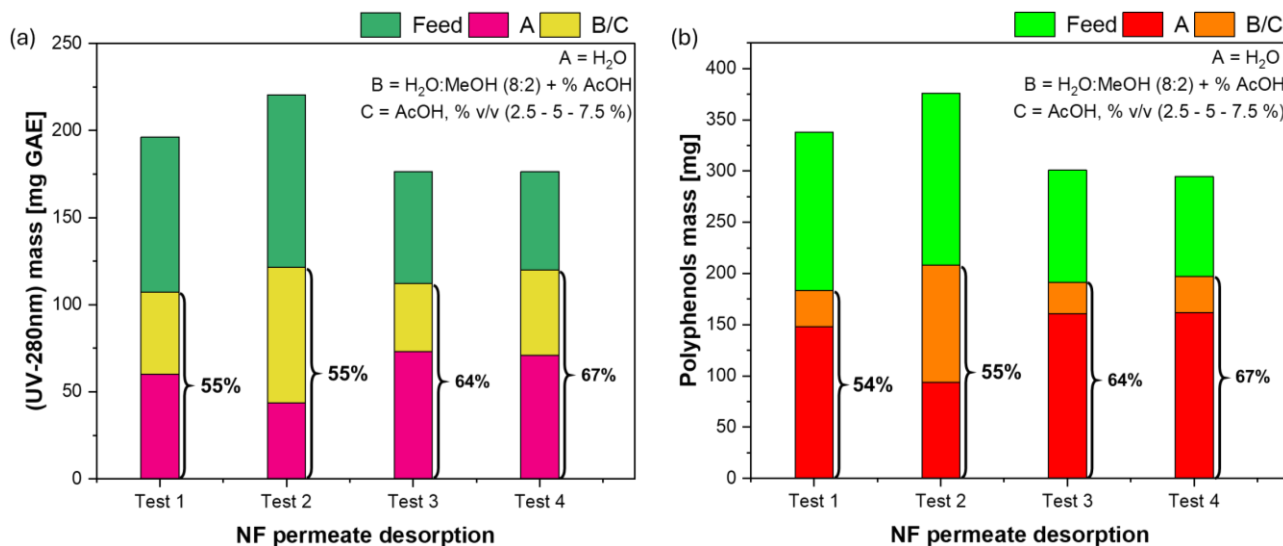


Figure 5.8: UV-absorbing and target polyphenols compounds recovery for each elution test. Feed bar represents the initial mass of polyphenols in the NF permeate prior to elution and correspond to the ideal value required to achieve 100% recovery. Bars A and B/C indicate the mass of polyphenols recovered using the different eluent solvents.

Among the tested conditions, increasing the volume of the initial water washing step (900 mL vs 300 mL) resulted in a higher overall recovery of phenolic compounds, highlighting that a fraction of the adsorbed phenolic compounds can already be recovered during the water elution step. Furthermore, the use of water–methanol mixtures in combination with acetic acid led to an additional improvement in desorption efficiency (polyphenols recovery of 67%). The presence of methanol likely enhances the solubility of phenolic compounds and weakens hydrophobic interactions with the resin matrix, promoting their release [16].

In addition, the effect of feed pH on the overall recovery was observed. Test 1, carried out using NF permeate at pH 2.5, showed a lower recovery than tests 2–4, which were performed at pH 4. This difference is more likely related to the adsorption stage rather than to the desorption step. At lower pH, a larger fraction of the resin exchange capacity is consumed by neutralization of acidic species present in the feed, reducing the effective adsorption capacity available for phenolic compounds. As a consequence, a higher fraction of phenols is not retained and is instead lost in the initial effluent “zero fraction”. Therefore, the lower recovery observed at pH 2.5 is attributed to a reduced initial adsorption of phenolic compounds, rather than to differences in desorption efficiency. At pH 4, the lower acid load reduces the consumption of resin capacity by neutralization and ion-exchange reactions, allowing a larger fraction of the resin to participate in phenolic retention. This interpretation is consistent with the breakthrough behavior observed under different pH conditions and with

previous studies reporting improved phenolic removal efficiency at higher pH values in resin-based processes [36]. The desorption profiles of UV-absorbing compounds were comparable to those of the target phenolics determined by HPLC, particularly hydroxytyrosol, which showed the highest recovery among the investigated compounds. This agreement confirms that UV monitoring can be used as a reliable proxy for phenolic behavior during desorption, although it remains a non-specific method.

To better visualize the recovery performance and the distribution of the three phenolic compounds among the different fractions, the results are reported in Figure 5.9 and Table 5.3 in terms of (i) overall recovery and (ii) distribution between water and subsequent solvent-assisted desorption steps.

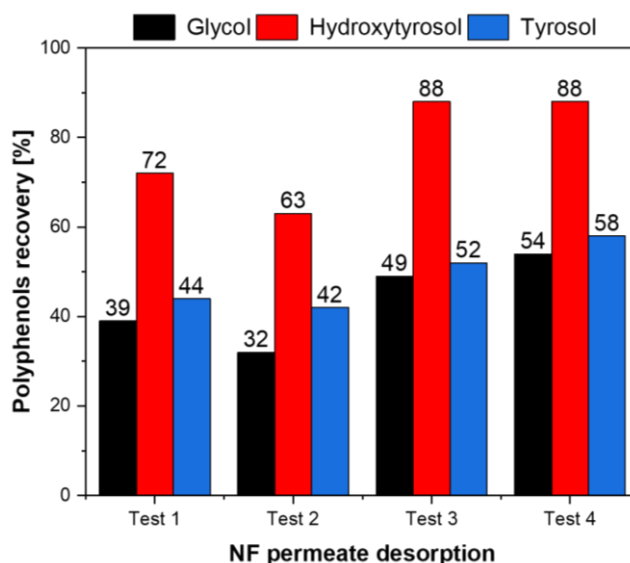


Figure 5.9: Glycol, hydroxytyrosol and tyrosol recovery for each elution test.

Table 5.3: Target polyphenols distribution in water.

% extract in H ₂ O	Glycol	Hydroxytyrosol	Tyrosol
Test 1 (900 mL)	97 %	81 %	94 %
Test 2 (300 mL)	55 %	32 %	47 %
Test 3 (900 mL)	96 %	79 %	92 %
Test 4 (900 mL)	97 %	82 %	94 %

The distribution of phenolic compounds among the different fractions shows that a significant portion of the total recovery occurs during the initial water washing step, while the addition of acid and organic solvent mainly contributes to the recovery of more strongly retained species. Overall, these results indicate that effective recovery of phenolic compounds can be achieved using predominantly water-based strategies, thereby reducing the need for organic solvents and supporting the development of a more sustainable and cost-effective process.

In contrast, previous studies on OMW treatment have often relied on organic solvents or non-aqueous processes to maximize polyphenol recovery, particularly when using hydrophobic resins. For instance, Frascari et al. [37] employed ethanol–water mixtures (up to 70% ethanol) to desorb

polyphenols from hydrophobic resins such as XAD16N and FPX66, achieving recoveries exceeding 90%, but highlighting the necessity of organic solvents to overcome strong hydrophobic interactions. Similarly, Zagklis et al. [15] utilized methanol or acetone to elute phenolics from XAD4 and XAD16 resins, obtaining 75–90% recovery, as pure water alone was insufficient to release strongly retained species. Pinelli et al. [38] also reported the use of acidified ethanol for desorption from IRA96 resin, achieving approximately 72% recovery and concluding that aqueous strategies alone were not sufficient for complete compound recovery.

These studies highlight the trade-off between process yield and solvent consumption and place the present results in the context of developing more sustainable recovery strategies. Although complete recovery was not achieved, the use of predominantly aqueous conditions represents a promising approach, particularly considering that OMW is a low-value waste stream and that process simplicity and economic feasibility are key factors for practical implementation.

5.3.3.1. Resin regeneration

The regeneration of the resin was evaluated over consecutive adsorption–desorption cycles. After each cycle, the resin was regenerated using 1 M NaOH, followed by rinsing with deionized water until neutral pH was reached. The breakthrough curves obtained after regeneration show that, after the first cycle, a shift toward lower treated volumes occurs, suggesting a reduction in adsorption capacity. However, after the second regeneration cycle, the breakthrough behavior remains stable, indicating that the resin reaches a steady performance after initial conditioning. Figure 5.10 reports the breakthrough curves obtained over three consecutive cycles. Further investigations are required to assess the long-term stability and lifetime of the resin, particularly in view of potential scale-up applications.

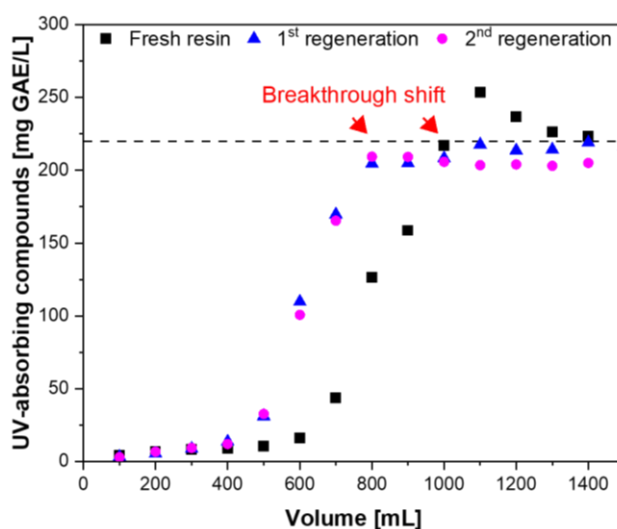


Figure 5.10: Breakthrough curve after test 2-3-4

5.3.3.2. Preliminary techno-economic assessment

Although a slightly higher recovery of phenolic compounds was achieved when using methanol-containing eluents, this option does not appear to be the most suitable, particularly from an environmental perspective. The use of organic solvents introduces additional constraints related to

solvent handling, recovery, and disposal, which may increase both operational complexity and environmental impact. In contrast, water-based extraction represents a more sustainable alternative. Moreover, from the perspective of potential industrial application, the direct recovery of phenolic compounds in aqueous solution is advantageous, as it avoids additional solvent removal steps and simplifies downstream processing.

To further support this consideration, a preliminary techno-economic comparison was carried out between the desorption strategies adopted in Test 3 and Test 4. The analysis was performed considering the treatment of 1 m³ of OMW using 40 kg of resin. Case study A involves water and acetic acid as eluents, whereas Case study B includes a water-methanol mixture with acetic acid. Common costs include the initial capital expenditure (CAPEX) for the resin (approximately €100/kg), corresponding to about €4000. The amortized cost per cycle decreases with increasing resin lifetime, ranging from €40 per cycle (100 cycles, conservative assumption) to €8 per cycle (500 cycles). Operating costs (OPEX) include solvent consumption, energy for pumping, and general plant overheads. Solvent costs were assumed to be €5/m³ for deionized water, €0.60/kg for acetic acid, and €0.80/kg for methanol (Case B) [38]. Disposal costs were not considered, as all eluted fractions are regarded as valuable product streams. The total operating cost was estimated to be approximately €50 per cycle for Case study A and €150 per cycle for Case study B, referred to the treatment of 1 m³ of feed. The main assumptions and cost items considered in the analysis are summarized in Table 5.4.

Table 5.4: Summary of capital (CAPEX) and operating (OPEX) expenditure for the two desorption scenarios.

Case study A		Case study B	
H ₂ O + % AcOH		H ₂ O + H ₂ O:MeOH (8:2) % AcOH	
CAPEX			
40 kg resin	€ 0.60 / kg	€ 4000 / N cycle	€ 40 N = 100 cycles € 8 N = 500 cycles
OPEX			
1.8 m ³ H ₂ O	€ 5 / m ³	1.6 m ³ H ₂ O	€ 5 / m ³
		140 kg MeOH	€ 0.80 / kg
47 kg AcOH	€ 0.60 / kg	47 kg AcOH	€ 0.60 / kg
Energy cost (pump)	€ 0.2 / m ³	Energy cost (pump)	€ 0.2 / m ³
Overhead	€ 10 / m ³	Overhead	€ 10 / m ³
€ 50 / cycle (per 1 m ³ feed)		€ 150 / cycle (per 1 m ³ feed)	

Based on experimental results, a polyphenol recovery yield of approximately 64% was obtained (≈ 190 g/m³ from an initial concentration of 300 g/m³). Assuming a market value of €500/kg, this corresponds to a potential revenue of about €120/m³ of treated feed. Overall, the elimination of methanol leads to a substantial reduction in operating costs while maintaining satisfactory recovery performance. Therefore, the water-based desorption strategy (Case study A) represents the most economically and environmentally sustainable option among those investigated.

5.4. Conclusions

This study investigated the removal and recovery of phenolic compounds from OMW nanofiltration permeate using a strong-base anion exchange resin (Amberlyst® A26, OH⁻ form). The results demonstrated that the resin enables selective retention of UV-absorbing compounds while simultaneously interacting with acidic inorganic and organic species, thus achieving an effective preliminary fractionation of the stream.

Batch experiments highlighted the strong influence of resin dosage on adsorption performance, with increasing removal efficiency observed at higher resin concentrations. The increase in pH was mainly attributed to acid–base interactions between the OH⁻ form of the resin and acidic species present in the permeate, leading to neutralization reactions and potentially promoting the partial formation of phenolate species under locally alkaline conditions. These findings indicate the coexistence of neutralization, ion-exchange, and adsorption phenomena.

Column experiments confirmed the effectiveness of the process under dynamic conditions. Breakthrough curves showed a well-defined adsorption front, and the good agreement between UV measurements and HPLC analysis demonstrated that UV monitoring can be used as a rapid proxy for phenolic concentration. Adsorption behavior was strongly influenced by pH. Under the acidic conditions investigated, phenolic compounds remain predominantly protonated and adsorption appears to be governed mainly by hydrophobic, π – π , and van der Waals interactions. The higher adsorption capacity observed at pH 4 was attributed to the lower consumption of resin capacity by acidic species, leaving a larger fraction available for aromatic compound retention.

Desorption studies demonstrated that a significant fraction of phenolic compounds can be recovered under relatively mild conditions. Water elution recovered a substantial portion of the retained compounds, while subsequent acid-assisted desorption further enhanced recovery. The addition of methanol provided only a limited improvement (+3%). A preliminary techno-economic assessment indicated that water-based desorption strategies are more advantageous than methanol-containing systems due to their lower operating costs.

Overall, the proposed approach enabled the selective enrichment and recovery of phenolic compounds from NF permeate while partially separating them from acidic and inorganic species present in the feed stream. The process can therefore be considered a promising refining step within integrated OMW valorization schemes, contributing to the production of phenolic-rich fractions suitable for further downstream applications. Future work should address long-term resin stability through repeated adsorption–desorption cycles, optimization of regeneration protocols, scale-up validation, and a more comprehensive techno-economic and environmental assessment. Particular attention should also be devoted to further elucidating the respective contributions of ion-exchange and non-electrostatic interactions to the overall adsorption mechanism.

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CHAPTER 6: REFINING OF OMW BY ELECTRODIALYSIS

6.1. Introduction

In the previous chapter, the removal of inorganic salts from olive mill wastewater (OMW) was investigated using anion exchange resins, with the aim of separating phenolic compounds from the saline fraction of the effluent. Ion exchange resins represent a well-established approach for the selective adsorption of charged species and have been widely applied in water treatment and purification processes [1–3]. However, several limitations may affect the large-scale implementation of this technology when applied to complex agro-industrial effluents such as OMW.

For example, Zagklis et al. [4] demonstrated that the regeneration and replacement of resins may significantly increase operational costs, especially when treating large volumes of wastewater. Resin saturation requires periodic regeneration using chemical reagents, which increases operating costs and generates additional chemical waste streams. These costs add to those already associated with the use of extraction solvents required for the recovery of phenolic compounds from the resin phase. Moreover, the adsorption efficiency of ion exchange resins strongly depends on the pH of the solution and, more generally, on the complex composition of the wastewater matrix [5]. Under highly acidic conditions, most phenolic compounds remain predominantly in their neutral form, which limits their interaction with anion exchange resins [6]. In addition, the presence of inorganic acids may reduce the availability of active sites for phenolic adsorption. As a consequence, fewer phenolic compounds can be retained by the resin and effectively separated from inorganic salts. Furthermore, the complex composition of OMW may promote fouling phenomena and irreversible adsorption of organic matter onto the resin structure, progressively decreasing the availability of active sites and thus reducing the long-term adsorption capacity of the material [1,2,7].

In the previous chapter, the application of the anion exchange resin Amberlyst A26 to OMW enabled a maximum polyphenol recovery of approximately 64% with reduced salt content. Although this recovery may appear moderate, it represents a promising result considering the highly complex composition of OMW, which is currently treated as a waste stream with essentially no commercial value.

In this context, preserving bioactive compounds while reducing the high salt content of OMW represents a key challenge, as it would simultaneously enable the recovery and valorization of phenolic compounds while mitigating the environmental impact associated with the disposal of this highly polluting effluent. Electrodialytic processes, already widely applied in the food industry as well as in water treatment and resource recovery processes, therefore offer a promising perspective. Indeed, these processes have been successfully used for desalination of brackish water, whey demineralization, wine tartrate removal, organic acid recovery, wastewater treatment, while minimizing environmental impact [8–11].

Electrodialysis (ED) is a mature membrane-based separation technology that uses an externally applied electric field to drive the selective migration of ions through ion-exchange membranes (IEMs) [12–14]. Ion-exchange membranes contain fixed charged groups that selectively allow the transport of counter-ions while excluding co-ions through electrostatic interactions, a phenomenon commonly referred to as Donnan exclusion [15].

The simplest unit of ED is a cell pair (c.p.), consisting of four key elements: a diluting compartment (diluate), a cation exchange membrane (CEM), a concentrating compartment (concentrate), and an anion exchange membrane (AEM). These compartments are formed by placing a spacer between the membranes, which defines the intermembrane distance and promotes turbulence [16–18]. When a direct electric current is applied, as shown in Figure 6.1, the electrodes drive the migration of ions through selective ion exchange membranes: cations move toward the cathode through CEMs, and anions move toward the anode through AEMs. As a result, ions are removed from the diluate compartments and accumulate in the adjacent concentrate channels, enabling desalination or selective ion removal from the feed solution [19,20].

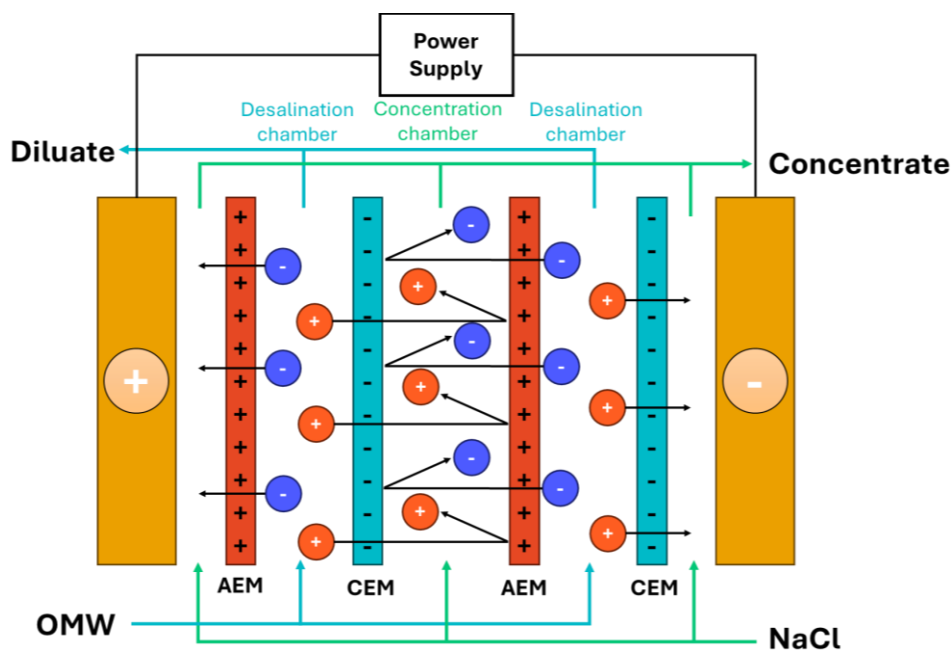


Figure 6.1: Illustration of the electrodialysis process applied to desalination.

During the electrodialysis process, ion transport across the membranes is governed by several coupled mechanisms. In addition to the migration of ions driven by the applied electric field, mass transfer is also influenced by diffusion caused by concentration gradients and convection induced by the hydrodynamic conditions within the flow channels. These transport phenomena collectively determine the overall efficiency of ion removal in electrodialysis systems [17,21,22].

Near the membrane surface, the formation of a diffusion boundary layer (DBL) leads to concentration gradients between the bulk solution and the membrane interface. As ions are transported through the membranes, the concentration in the diluate side decreases while it increases in the concentrate side, giving rise to concentration polarization. This phenomenon limits mass transfer and plays a key role

in defining the operational limits of the process [23,24]. When the applied current exceeds a critical threshold known as the limiting current density, the ion concentration at the membrane surface approaches depletion. Under these conditions, undesirable phenomena such as water dissociation, increased electrical resistance, and reduced current efficiency may occur, negatively affecting process performance and energy consumption [19,25].

Over the years, several configurations of electrodialysis have been developed to address different separation objectives and process requirements. In addition to conventional electrodialysis (ED), which is primarily used for desalination and ion removal, other variants have been introduced, including electrodialysis reversal (EDR) [26], reverse electrodialysis (RED) [27], selective electrodialysis (SED) [28], and bipolar membrane electrodialysis (BMED) [29]. In EDR, the polarity of the electrodes is periodically reversed in order to reduce membrane fouling and scaling during long-term operation. RED exploits the salinity gradient between two solutions to generate electrical energy. SED employs membranes with enhanced ion selectivity to preferentially separate specific ions, while BMED uses bipolar membranes to generate acids and bases from corresponding salts through water dissociation at the membrane interface. These technologies expand the applicability of electrodialysis to a wide range of industrial processes including water desalination, wastewater treatment, chemical production, and resource recovery [30–32].

Despite its advantages, the performance of electrodialysis systems can be significantly affected by membrane fouling and scaling, particularly when treating complex solutions containing organic matter, suspended solids, or multivalent ions. Fouling typically results from the accumulation or adsorption of organic compounds, colloidal particles, or inorganic precipitates on the membrane surface or within the membrane structure, leading to increased electrical resistance, reduced permselectivity, and decreased process efficiency. [31,33]. Several strategies have been proposed to mitigate membrane fouling during electrodialysis operation. Among them, EDR represents a widely adopted approach in large-scale desalination systems. In EDR processes, the polarity of the electrodes is periodically reversed, causing ions to migrate in the opposite direction through the membrane stack. This periodic switching reduces the buildup of inorganic scaling and organic deposits, allowing longer operational cycles and improved process stability [34,35]. Other approaches included optimized hydrodynamic conditions, membrane surface modification, chemical cleaning procedures, and, in particular, the application of pulsed electric field operation [24,31]. These strategies aim to improve mass transfer near the membrane surface, reduce concentration polarization, and minimize the adsorption of organic compounds on ion-exchange membranes [36,37].

When treating agro-industrial effluents, membrane fouling is often associated with the presence of natural organic matter such as phenolic compounds, proteins, and other natural aromatic molecules. These compounds may interact with ion-exchange membranes through electrostatic interactions, hydrogen bonding and hydrophobic interactions, leading to partial blockage of active sites and progressive deterioration of membrane performance during operation [8,38,39].

Among agro-industrial waste streams, OMW represents one of the most challenging matrices due to its high organic load, elevated salinity and significant concentration of phenolic compounds. Shestakov et al. [40] investigated the application of ED to real OMW samples, highlighting the potential of integrating ED with RO to improve the overall separation efficiency. Other promising approaches include pH-controlled desorption and membrane selection based on polymer chemistry, which can influence the interactions between polyphenols and ion-exchange membranes [39,41]. In particular, aromatic membranes tend to exhibit stronger adsorption of phenolic compounds due to π - π interactions, whereas aliphatic membranes may facilitate their desorption [42]. Understanding these interactions is therefore essential for designing ED systems capable of balancing salt removal with polyphenol preservation.

Electrodialytic systems are widely used in several industrial fields, including water desalination, wastewater treatment, and the food industry. Typical applications include desalination of seawater and brackish water [20,26], treatment of industrial effluents and landfill leachates [34], as well as food processing operations such as wine tartrate stabilization [43], whey demineralization [44] and purification of sugars such as lactose and galactose [45]. Additional applications include biotechnology and pharmaceutical processes, such as purification of amino acid solutions, recovery of proteins and production of organic acids and bases [46]. For example, Luiz et al. [47] demonstrated the application of electrodialysis for the desalination of bio-refinery streams, achieving up to 96% salt removal with minimal organic losses (0.3-6.3%). In the food sector, Bazinet et al. [9] reported the potential of BMED for several dairy application, including the production of lactic acid from whey product fermentation, the production of caseinates, and the fractionation of whey proteins. Since electrodialysis has proven to be an efficient, chemical-free, and highly selective separation technology with low energy consumption for low-salinity streams, it was selected for the demineralization of olive mill wastewater.

In this context, the present study investigates the application of electrodialysis to real samples of pretreated OMW, with the dual objective of (i) selectively removing salts to reduce environmental impact and facilitate downstream processing, and (ii) preserving valuable polyphenolic compounds, thereby maintaining the antioxidant potential of the stream. By evaluating different ion-exchange membrane pairs, operational parameters (applied voltage, flow rate and circulation mode), total specific energy consumption and membrane fouling behavior, this work aims to establish a framework for optimizing electrodialysis performance in complex agro-industrial effluents.

These experiments were performed during a research stay at Lund University, under the supervision of Dr. Ahmet Halil Avci and Prof. Frank Lipnizki.

6.2. Materials and Methods

6.2.1. Materials

Olive mill wastewaters coming from three-phase system olive oil production were supplied by Frantoio Rocca Danilo (Tovo San Giacomo, Italy). The raw effluent was subsequently pretreated by acidification, followed by a coupled microfiltration and nanofiltration stage. The NF permeate was used as feed solution for diluate compartment in the electrodialysis experiments. The main physicochemical characteristics of the NF permeate are summarized in Table 6.1.

Table 6.1: Main characteristics of diluate (NF permeate).

Parameter	Value
pH	2.5
Electrical conductivity (EC)	~ 6 mS/cm
Total polyphenol content (TPC)*	500.0 ± 60.1 mg GAE/L
Polyphenol concentration**	313.0 ± 12.6 mg/L

* obtained with Folin-Ciocalteu method **determined by HPLC analysis

The concentrate solution was prepared by dissolving NaCl in deionized water (0.8 µS/cm) to obtain a 0.05 M solution. The electrolyte circulating in the electrode compartments consisted of 0.15 M sodium sulfate. All analytical reagents were purchased from Merck (Sweden). NaOH, HCl (Merck, Sweden) and Ultrasil 110 (Ecolab AB, Sweden) were used for membrane cleaning procedures.

Folin–Ciocalteu phenol reagent, sodium carbonate and gallic acid used were purchased from Merck (Sweden). 3,4-dihydroxyphenylglycol, hydroxytyrosol, tyrosol were used as standard for HPLC calibrations. Acetonitrile and trifluoroacetic acid were used for HPLC mobile phase preparation. All the chemicals were purchased from Sigma Aldrich (Milan, Italy).

Several ion-exchange membranes supplied by PCCell GmbH (Heusweiler, Germany) were investigated in this study. The tested membranes included standard homogeneous membranes PC-SA (AEM) and PC-SK (CEM); monovalent selective membranes PC-MVA (AEM) and PC-MVK (CEM), organic anion exchange membranes PC-100D, PC-200D, PC-400D, and sulfonic acid-based PC-MTE (CEM). Their main structural and electrochemical properties are summarized in Table 6.2, which was supported by the manufacturer (PCA GmbH, Heusweiler, Germany).

Table 6.2: Electrochemical and physical properties of ion exchange membranes^a.

Membrane	Permselectivity (-) ^b	Resistance [Ωcm^2]	Water content (wt%)	Ion exchange capacity [meq/g]	Thickness [μm]
PC-MTE	>0.94	~ 4.5	-	~ 1.8	220
PC-SA	>0.95	~ 1.8	~ 14	~ 1.2	100-110
PC-SK	>0.95	~ 2.5	~ 9	~ 3.0	100-120
PC-MVA	-	~ 5	-	-	100-110
PC-MVK	-	~ 6	-	-	100-120
PC-100 D	>0.94	~ 5	~ 50	~ 1.2	100-160
PC-200 D	>0.91	~ 2	~ 40	~ 1.24	180-220
PC-400 D	>0.86	~ 10	~ 48	~ 0.66	160-200

^aData taken from PCCell GmbH's technical datasheet. ^bMeasured in 0.1/0.5 KCl solutions.

6.2.2. Methods

6.2.2.1. Physicochemical analyses

Voltage, current, charge, temperature and electrical conductivity were continuously monitored online using sensors connected to the PCCell PC Frontend software. The pH was measured using a Thermo Scientific™ Orion Star™ A211 Benchtop pH Meter.

The total polyphenol (TP) contents in OMW were determined using the Folin–Ciocalteu (FC) spectrophotometric method [48]. Absorbance was measured at 750 nm and quantified using a calibration curve prepared with gallic acid standard solutions in the concentration range of 5–100 mg/L ($R^2 = 0.999$). Results were expressed as mg of gallic acid equivalent for OMW liter (mg GAE/L). Target polyphenols quantification was carried out by HPLC analysis. It was performed with a Shimadzu system (LC-10ATvp pump, SPD-10Avp detector, $\lambda = 280$ nm), equipped with a Synergi Hydro-RP column (150 × 3 mm, 4 μ m) and a pre-column filter. The mobile phase consisted of H₂O + 0.1% trifluoroacetic acid (A) and acetonitrile (B), with a 46 min gradient program. Injection volume: 5 μ L; flow rate: 0.7 mL/min; column temperature: 23–25 °C. Calibration curves are reported in the Supplementary Material.

6.2.2.2. Electrodialysis equipment and membranes

Experiments were carried out on a bench scale electrodialysis system supplied by PCCell GmbH (Heusweiler, Germany). The ED setup consists of a DC power supply (Votcraft PPS-16005), an ED stack (PCCell ED 64002), two feed cylinders and an electrolyte tank. Three centrifugal pumps equipped with flowmeters, pressure gauges and regulating valves allowed accurate control of the flow rates in each circuit. The ED stack was assembled with five CEMs and four AEMs, separated by polypropylene spacers (0.45 mm). Each membrane had an active surface area of 0.0064 m². A meshed titanium anode coated with Pt/Ir and a AISI 316 stainless steel cathode were used as electrodes. The electrode compartments were filled with 0.15 M Na₂SO₄ electrolyte solution, ensuring stable electrical conductivity while minimizing pH fluctuations and gas evolution at the electrodes. The electrode compartments were sealed with CEM end-membranes (PC-MTE, sulfonic acid based). The stack was mechanically compressed using four corner bolts to ensure proper sealing and membrane alignment. Figure 6.2 shows the schematic layout of the laboratory electrodialysis setup.

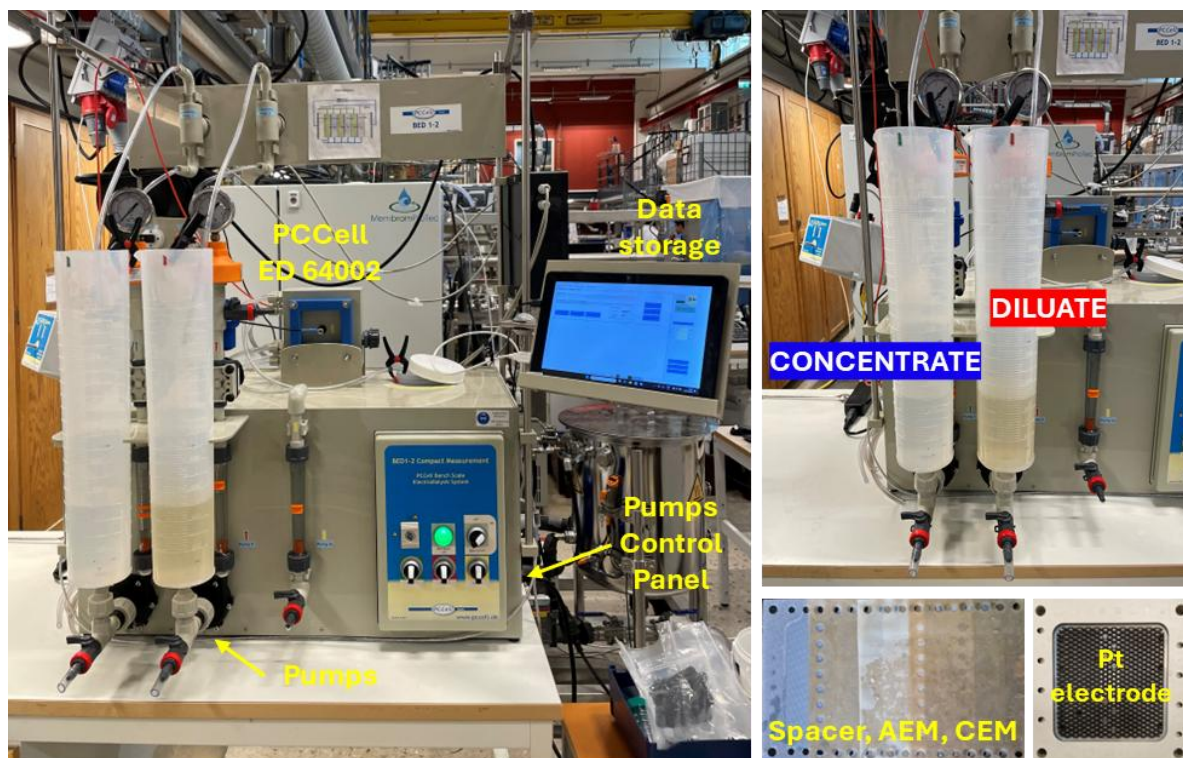


Figure 6.2: Laboratory scale of ED system and cell equipment.

6.2.2.3. Experimental procedures

Electrodialysis experiments were performed using the NF permeate described in Section 6.2.1 as diluate feed, while a NaCl solution was used as concentrate and a 0.15 M Na₂SO₄ solution circulated in the electrode compartments as electrolyte.

Two operating configurations were investigated: batch and semi-batch modes. Batch experiments were first performed as preliminary tests to screen the different membrane pairs and evaluate process efficiency. Based on these results, the most promising configuration was further investigated under semi-batch conditions in order to better simulate continuous industrial operation.

In batch mode, both the diluate and concentrate streams were continuously recirculated to evaluate membrane performance and identify the configuration providing the highest demineralization efficiency while minimizing polyphenol losses.

In semi-batch mode, designed to better simulate industrial operation, the process was continued until the target electrical conductivity (EC) of the diluate was reached (process efficiency > 90%). At that point, part of the desalted OMW was removed and replaced with fresh NF permeate. This procedure was repeated three times during each experiment. In parallel, salt accumulation in the concentrate was controlled using a similar bleed-and-feed strategy, in which part of the enriched concentrate was periodically replaced with fresh NaCl solution.

The performance of the electrodialysis process was evaluated in terms of demineralization efficiency, polyphenol retention in the diluate, and polyphenol adsorption onto the anion-exchange membranes (AEMs).

The demineralization efficiency (W) was calculated from the electrical conductivity (EC) variation of the diluate according to Equation 1:

$$W = \frac{EC_0 - EC_f}{EC_0} \times 100 \quad (1)$$

where EC_0 and EC_f are the electrical conductivity of the diluate at the beginning and at the end of the ED process.

The loss of polyphenolic compounds (PP_{loss}) from the diluate was determined according to Equation (2):

$$PP_{loss} = \frac{C_{feed} - C_{dil}}{C_{feed}} \times 100 \quad (2)$$

where C_{feed} is the initial polyphenol concentration in the feed solution and C_{dil} is the polyphenol concentration measured in the diluate at the end of the ED experiment.

Polyphenol adsorption onto the anion-exchange membranes was estimated from the mass balance between diluate and concentrate streams according to Equation (3):

$$PP_{ads} = 100 - \frac{C_{dil} + C_{conc}}{C_{feed}} \times 100 \quad (3)$$

where C_{conc} represents the polyphenol concentration in the concentrate compartment.

The operating conditions applied during batch and semi-batch tests are summarized in Table 6.3. Before each experiment, the system was filled, deaerated and allowed to reach stable flow conditions. The process was started by switching on the power supply and applying the selected constant voltage. Samples were periodically collected from all streams during the experiments. Flow rates of the diluate, concentrate and electrolyte solutions were fixed at the beginning of each run.

Table 6.3: Main operating parameters applied during ED experiments.

Parameter	Value
Applied voltage	5 or 10 V (constant)
Active cells	5 cell pairs (c.p.)
Tested membranes	SA+SK, MVA+MVK, 100D/200D/400D+SK
Flow rate	10 or 20 L/h (diluate and concentrate), 150 L/h (electrolyte)
Duration	60-100 min (batch); 4h (semi-batch)

For each membrane pair, a reference experiment was carried out using NaCl solutions in both the diluate and concentrate compartments, with electrical conductivity values adjusted to match those used during OMW desalination tests. After each OMW experiment, the NaCl run was repeated to verify the reproducibility of the conductivity profiles and eventually evaluate membrane fouling.

When the original diluate flow rate could not be restored, membrane cleaning was performed. The cleaning protocol consisted of an alkaline wash (Ultrasil 110, 0.05 wt%), followed by water rinsing, acidic washing with HCl (0.05 wt%), a second water rinse, alkaline washing with NaOH (0.05 wt%), and a final rinse with deionized water.

6.2.2.4. Energy consumption

The total specific energy consumption (E_{total} , kWh/m³) in the recirculating ED tests results from two main contributions, as shown in Equation 4: the electrical energy supplied to the ED stack (E_{ED} , kWh), associated with ionic transport and ohmic losses within the system, and the hydraulic energy required for solution recirculation (E_{pumps} , kWh), related to the recirculation of the diluate, concentrate, and electrolyte solutions through the stack [12].

The E_{ED} depends directly on the applied voltage (U), the electric current (I), and the duration of the process (Δt), as expressed in Equation 5. Conversely, the E_{pumps} , is governed by the solution flow rate (Q), the pressure drops across the cell (ΔP), the operating time (t) and the hydraulic efficiency of the pump (η), as shown in Equation 6.

$$E_{total} = \frac{E_{ED} + E_{pump}}{V_{treated}} \quad (4)$$

$$E_{ED} = U \cdot I \cdot \Delta t \quad (5)$$

$$E_{pump} = \frac{Q \cdot \Delta P \cdot t}{\eta} \quad (6)$$

6.3. Results and Discussion

Two experimental configurations were investigated: (i) preliminary batch recirculation tests aimed at screening the performance of different ion-exchange membranes in terms of desalination rate, polyphenol losses, energy consumption and fouling phenomena; and (ii) a semi-batch configuration designed to simulate continuous operation, where the effect of the diluate and concentrate flow rate (10–20 L/h) was also evaluated.

6.3.1. Electrodialysis performance in batch-mode

Preliminary experiments were carried out in batch mode using the standard ion-exchange membrane pair SA and SK. Figure 6.3 shows the evolution of electrical conductivity of the diluate (red) and concentrate (blue) during electrodialysis. Solid lines correspond to tests performed at a constant voltage of 5 V, whereas dashed lines represent the experiments conducted at 10 V. In all cases, the diluate and concentrate flow rates were maintained at 20 L/h.

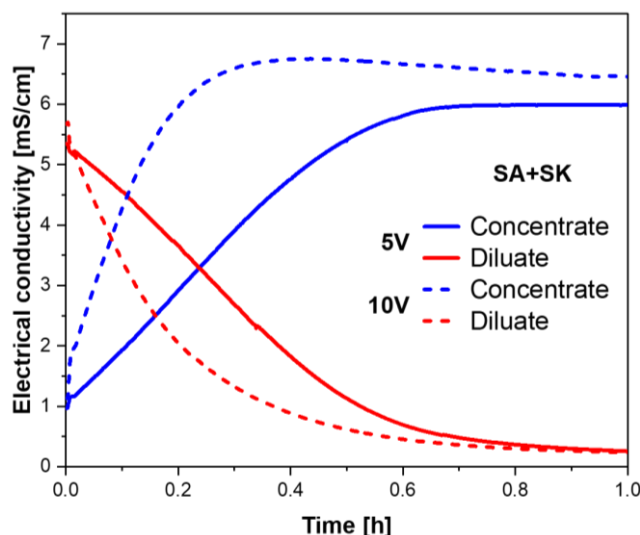


Figure 6.3: Evolution of EC in the diluate (red) and concentrate (blue) during electrodialysis using the standard membrane pair SA+SK. Solid lines represent experiments performed at 5 V, while dashed lines correspond to 10 V operation.

The decrease in diluate conductivity and the corresponding increase in concentrate conductivity clearly demonstrate the progressive transfer of ions through the ion-exchange membranes. When operating at 5 V (solid lines), the conductivity of the diluate decreased from 5.35 to 0.185 mS/cm, corresponding to a desalination efficiency of 96% after approximately 1.2 h.

Increasing the applied voltage to 10 V (dashed lines) significantly accelerated ion transport, allowing a comparable desalination level to be achieved in approximately 1 h. Moreover, the concentrate conductivity reached a plateau more rapidly at 10 V, indicating faster ion accumulation in the concentrate compartment as a result of the higher electric driving force. However, operating at higher voltages may promote undesirable phenomena such as water splitting, increased ohmic losses, and potential membrane degradation [19]. Therefore, subsequent experiments were mainly conducted at 5 V in order to compare the performance of different membrane pairs under stable operating conditions.

In addition to the standard SA and SK membrane pair, four additional AEM/CEM combinations were tested, which differ mainly in their selectivity, thickness and resistance (see Table 2, section 6.2.1). Figure 6.4 reports the evolution of the electrical conductivity of the diluate during electrodialysis for the five membrane pairs tested (SA+SK, MVA+MVK, 100D+SK, 200D+SK and 400D+SK), operating at a constant voltage of 5 V and a flow rate of 20 L/h.

To facilitate comparison among membranes with different initial conductivity values, the data were normalized with respect to the initial conductivity of the diluate, as described in Equation 4:

$$EC(t) = \frac{EC_t - EC_f}{EC_0 - EC_f} \quad (4)$$

where EC_0 and EC_f represent the initial and the final conductivity of the diluate and EC_t is the conductivity measured at time t . This normalization allows a direct comparison of the desalination

kinetics of the different membrane pairs independently of the absolute conductivity values of the feed solution. For clarity, only the conductivity evolution of the diluate stream is reported.

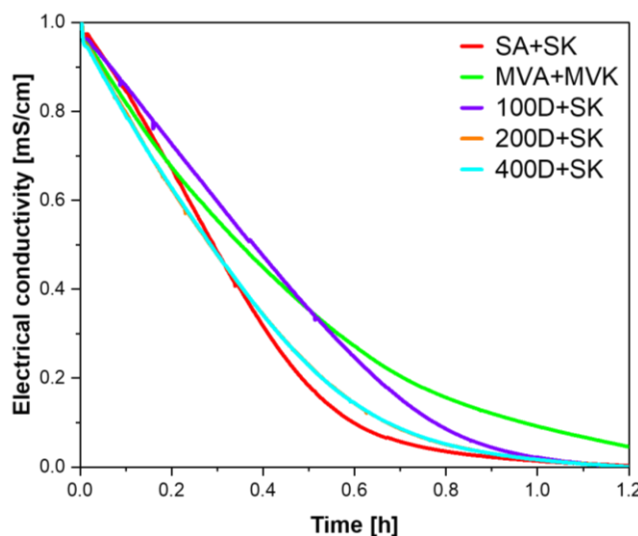


Figure 6.4: Evolution of normalized diluate EC of the five membrane pairs (5V, 20 L/h).

As shown in Figure 6.4, the membrane pairs exhibited different desalination kinetics during the electrodialysis process. Among the tested configurations, SA+SK and 400D+SK showed the fastest decrease in normalized conductivity, indicating a higher ion transport rate. In contrast, the MVA+MVK membrane pair displayed the slowest desalination kinetics. This behavior can be attributed to its monovalent-selective structure, which limits the transport of divalent ions such as sulfates that are abundant after the nanofiltration with a loose NF membrane, described in Chapter 4. Despite these differences in kinetics, all membrane pairs were able to achieve a high desalination efficiency.

Table 6.4 reports the initial and final electrical conductivity values together with the desalination efficiency obtained for each membrane pair.

Table 6.4: Diluate results of the ED tests with the different AEM/CEM pairs, maintaining constant flow rates (20 L/h), stream composition, and operating temperature (25 °C).

AEM/CEM	Δt (h)	EC_0 [$\mu S/cm$]	EC_f [$\mu S/cm$]	W (%)
SA+SK (5V)	1.2	5353	185	96.5
SA+SK (10V)	1.0	5680	238	95.8
MVA+MVK	1.4	6192	606	90.2
100D+SK	1.2	6398	512	92.0
200D+SK	1.2	6611	380	94.3
400D+SK	1.2	6316	299	95.3

Based on the electrical conductivity data of the diluate, a kinetic analysis was performed to evaluate the desalination rate during the electrodialysis process. The linear relationship between $\ln(EC_f/EC_0)$ and time confirmed that the demineralization process followed a pseudo-first-order kinetic model for all membrane configurations operated at 5V ($R^2 = 0.97\text{--}0.99$). This indicates that ion removal was mainly governed by a combination of electromigration and diffusion mechanisms, with the

demineralization rate being proportional to the residual ionic concentration in the diluate [17,21]. Among the tested pairs, SA+SK and 400D+SK exhibited the highest apparent rate constants (3.1 and 2.8 min^{-1} , respectively), suggesting faster ionic transport and higher desalination efficiency. Conversely, the monovalent-selective (MVA+MVK) membrane pair showed the slowest kinetics (1.6 min^{-1}), reflecting the resistance to ionic diffusion, particularly for divalent ions. For the SA+SK pair operated at 10 V, the conductivity decay followed a trend closer to a second-order kinetic model, with a higher apparent rate constant (4.5 min^{-1}). This behavior can be attributed to the transition toward overlimiting current conditions, where additional transport mechanisms such as electroconvection and water splitting contribute to the overall ion transport [49].

The superior performance of the SA+SK membrane pair can be attributed to its relatively low electrical resistance and high permselectivity, which promote efficient ion transport across the membrane stack and reduce ohmic losses during electrodialysis. Membranes characterized by lower resistance generally facilitate faster electromigration of ions and therefore higher desalination rates under constant voltage operation, as widely reported in electrodialysis systems treating multi-ionic solutions [50,51]. The relatively high kinetic constant observed for the 400D+SK pair suggests that this membrane configuration can also sustain efficient ion transport despite its higher water uptake and different structural characteristics. Membranes with a more open polymer structure may facilitate ionic mobility within the membrane phase, particularly in complex matrices containing both inorganic ions and organic compounds. Similar behavior has been observed in electrodialysis systems designed for the transport of larger organic anions and complex species, where membrane structure and hydration properties strongly influence ion mobility [52]. Conversely, the slower desalination kinetics observed for the monovalent-selective MVA+MVK membrane pair can be explained by the intrinsic selectivity of these membranes. Monovalent-selective membranes are designed to preferentially transport monovalent ions while restricting the passage of multivalent ions such as sulfates. Since NF permeate contains a significant fraction of divalent ions, this selective transport behavior limits the overall ion flux and results in slower conductivity decay in the diluate. Similar effects have been reported in multicomponent electrodialysis systems, where the presence of divalent ions reduces desalination efficiency when monovalent-selective membranes are used [53].

Overall, these results highlight the strong influence of membrane physicochemical properties on electrodialysis performance. Membranes combining low electrical resistance and moderate selectivity tend to provide the best compromise between ion transport efficiency and desalination kinetics, whereas highly selective membranes may reduce overall ion flux when treating complex wastewater streams containing multivalent ions.

6.3.2. Polyphenols reduction

Leaving aside the 10 V test, when considering only EC and desalination rates, the most performant membrane pairs are SA+SK and 400D+SK. However, it is also important to evaluate which membranes better retain polyphenols in the diluate stream, as their high antioxidant activity is essential for the potential valorization of OMW.

Table 6.5 reports the percentage of residual EC (% EC residual = 100-W) together with the percentage of polyphenol losses determined by the Folin–Ciocalteu (FC) colorimetric method for total polyphenols and by HPLC for the three main polyphenols present in OMW: 3,4-dihydroxyphenylglycol, hydroxytyrosol, and tyrosol. Two equations (2 and 3) were used to describe the polyphenol losses from OMW and their adsorption onto the anion-exchange membranes, particularly the AEMs.

Table 6.5: Percentage of EC residual and of polyphenols losses calculated by Folin–Ciocalteu and HPLC.

AEM+CEM (5 V)	% EC residual	Folin–Ciocalteu (FC)		HPLC	
		% PPs lost from diluate	% PPs lost by AEM ads	% PPs lost from diluate	% PPs lost by AEM ads
SA+SK	3.5	6	4	1	0.5
MVA+MVK	9.8	11	8	5	2.5
100D+SK	8.0	9	7	1.5	0.5
200D+SK	5.7	11	7	2	0.5
400D+SK	4.7	31	10	14	1

The results demonstrated that both total and target polyphenol losses during electrodialysis were generally limited for most membrane pairs, although significant differences were observed depending on the membrane characteristics. The lowest polyphenol losses were observed for SA+SK and 100D+SK, whereas the 400D+SK pair exhibited the highest losses. The discrepancy between the values obtained by the Folin–Ciocalteu assay and HPLC analysis is inherent to the analytical methods themselves, since the FC method measures the overall reducing capacity of the sample and therefore accounts for the total phenolic fraction, whereas HPLC quantifies only the three target polyphenols considered in this study. Polyphenols that were not retained in the diluate may either migrate into the concentrate compartment or be adsorbed onto the anion-exchange membranes during the electrodialysis process. Considering also the conductivity results, the optimal performance was achieved with SA+SK membranes, which combine rapid ion removal with high antioxidant retention.

The mechanisms underlying polyphenol loss can be explained by two distinct processes: (i) migration of charged phenolics into the concentrate stream and (ii) adsorption onto the AEM surface [8]. At the typical acidic pH of the NF permeate (~3-4), most phenolic compounds are present predominantly in their neutral form because phenolic hydroxyl groups (pKa ~ 9–10) remain protonated. Under these conditions, polyphenols are only weakly influenced by the electric field and therefore do not migrate efficiently toward the concentrate compartment. Nevertheless, they can interact with anion-exchange membranes through π – π stacking, hydrogen bonding and hydrophobic interactions with the polymer matrix, leading to adsorption-driven fouling phenomena [10,39]. However, during electrodialysis local pH variations may occur at the membrane interface due to concentration polarization and partial water splitting phenomena. These local changes can promote the deprotonation of ionizable organic compounds present in the OMW matrix, including phenolic species, generating negatively charged phenolate ions that may migrate across the AEM together with inorganic anions [19].

These trends align with membrane properties reported in the PCA Membrane Handling Guide (Table 2). In electrodialysis systems, membrane performance is strongly influenced by structural and electrochemical parameters such as water uptake, permselectivity and electrical resistance. High water uptake generally increases the swelling of the polymer matrix and the channel size (looser membranes), facilitating the transport of both counter-ions and organic species across the membrane. Conversely, membranes characterized by lower water uptake typically exhibit a more compact structure with narrower ion channels, enhancing permselectivity and reducing organic losses. Membrane electrical resistance also plays a key role in determining ion transport efficiency, as lower resistance promotes faster electromigration and more efficient desalination under constant voltage conditions [51,54]. The SA anion-exchange membrane combines high permselectivity, relatively low water uptake and low electrical resistance, enabling efficient salt removal while limiting polyphenol loss. In contrast, the 400D AEM, characterized by higher water uptake and lower permselectivity, likely possesses a more open polymer structure that facilitates the migration of organic molecules, which may explain the higher polyphenol losses observed in this configuration.

Overall, the results suggest that polyphenol losses during electrodialysis arise from a combination of mechanisms, including migration of negatively charged phenolic species into the concentrate stream and adsorption phenomena occurring at the membrane surface. The relative contribution of these mechanisms depends on several factors, including solution pH, membrane selectivity and structural properties. Under acidic conditions, adsorption onto AEMs tends to dominate because most phenolic compounds remain largely unionized. Conversely, when local pH increases near the membrane surface due to concentration polarization or water splitting, partial deprotonation of phenolic hydroxyl groups may occur, promoting their migration across the membrane. Membrane structure also plays an important role, since looser polymer matrices with higher water uptake may facilitate the transport of organic species, whereas more compact membranes favor selective ion transport.

Considering both desalination efficiency and polyphenol retention, the SA+SK membrane pair provided the most favourable compromise, allowing rapid conductivity reduction while minimizing losses of antioxidant compounds. Similar phenomena have been reported in phenolic-rich matrices treated by electrodialysis, such as wine, where polyphenol losses are associated with both adsorption onto AEMs and migration of ionized species [10,41]. In a broader food-processing context, Barone et al. [55] also showed that ED can selectively modulate the composition of complex protein-based matrices, although in that case the objective was the removal rather than the preservation of phenolic compounds. Overall, these findings highlight the potential of electrodialysis for the selective manipulation of ionic species in complex agro-industrial streams while preserving valuable bioactive compounds, especially with high-permselectivity membranes.

6.3.3. Energy consumption and total cost

At industrial or pilot scale, the total energy consumption is typically dominated by the electrical power required to drive ion transport through the membranes. However, at laboratory scale the contribution of pumping energy cannot be neglected. Due to the relatively small volumes treated, even modest

hydraulic energy inputs may become significant when normalized per unit volume of treated solution [56].

The total operating cost, expressed in [€/m³], was estimated assuming an electricity price of 0.20 €/kWh [57]. Figure 6.5 compares the total energy consumption and the corresponding cost for each membrane pair tested under recirculating ED conditions. The blue bars represent the total energy consumption, divided into the electrical contribution of the ED cell (E_{ED} , red pattern) and the hydraulic contribution of the pumps (E_{pumps} , green pattern). The purple symbols refer to the total operating cost.

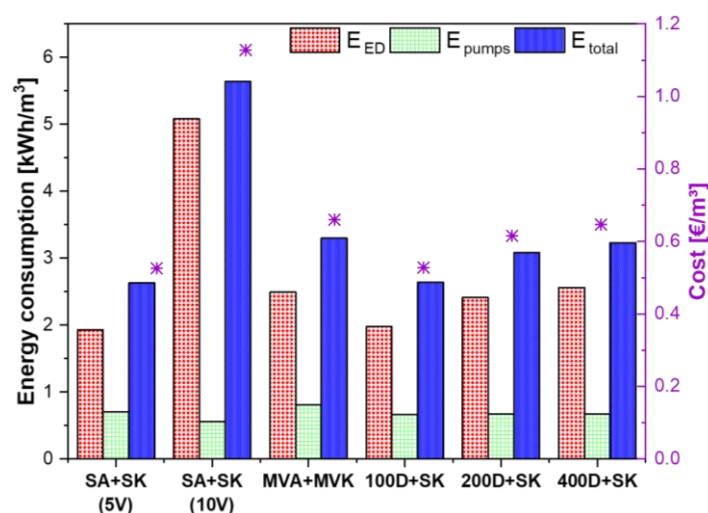


Figure 6.5: Energy consumption and total cost per membrane pairs.

In general, the total specific energy consumption was mainly associated with the energy supplied to the electrodialysis stack, with the pumping energy contributing on average about 20%. The pumping cost remained nearly constant since all experiments were carried out for the same operating time, the energy cost associated with the ED process varied depending on the membrane pair used. The standard membrane pair (SA+SK) was the most efficient in terms of both energy consumption and total cost. Increasing the applied voltage to 10 V led to a marked rise in E_{ED} , resulting in an almost double increase in the energy cost. Although the operating time decreased for the test conducted at 10 V, this reduction did not compensate for the higher overall energy consumption. This behavior is typical of a system operating beyond the limiting-current regime [58], where ion-depleted layers form near the membrane surface. Such conditions lead to an increase in local resistance and ohmic drop, higher electrode overpotentials, and the onset of electrolysis and concentration polarization phenomena. As a result, the fraction of current effectively contributing to ion transport decreases, while the portion dissipated as heat increases. At the laboratory scale, where only a limited number of cell pairs are used and the feed solutions exhibit relatively low conductivity, the optimal operating voltage for OMW treatment is around 5 V, at which the system functions in a sub-limiting regime, ensuring a good compromise between desalination rate and energy efficiency. These results confirm that operating at moderate voltages maximizes ion-transport efficiency while minimizing ohmic losses, in agreement with previous literature observations [12,17].

6.3.4. Semi-batch process

Figure 6.6 shows the evolution of the electrical conductivity of diluate and concentrate during the semi-batch process, using the standard SA+SK membrane pair. The influence of flow rate was evaluated by performing ED tests at 20 and 10 L/h.

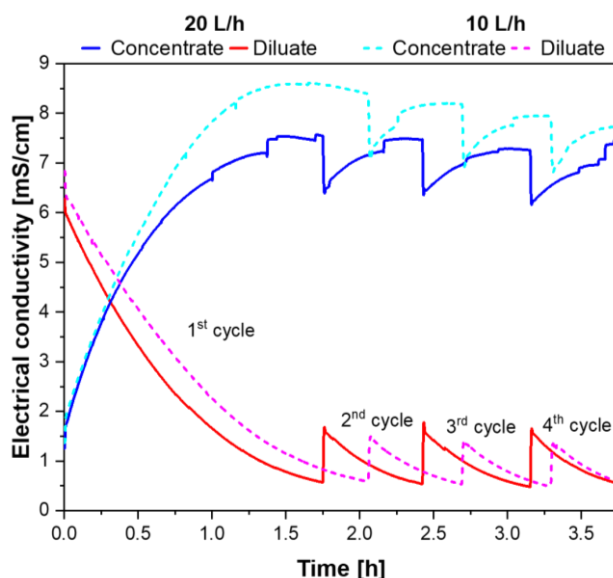


Figure 6.6: (a) Electrical conductivity of diluate and concentrate during semi-batch ED using SA+SK membrane pair at different flow rate (10-20 L/h).

To simulate a quasi-continuous operating regime, a bleed-and-feed configuration was implemented. Once a defined desalination efficiency was reached, a fraction of the diluate (demineralized OMW) was withdrawn and replaced with an equal volume of fresh feed (OMW). Simultaneously, a corresponding portion of ion-enriched concentrate was removed and replenished with a fresh NaCl solution. This substitution was repeated three times after the first cycle, allowing control of both diluate and concentrate EC within a manageable range and extending the operating time. This approach enabled the evaluation of ion transport stability and fouling phenomena under prolonged operation.

When operating at 20 L/h, the target conductivity in the diluate was reached faster than at 10 L/h, and polyphenol losses were also reduced. These results may appear counterintuitive, as higher flow rates generally imply shorter residence times near the membrane surface, which theoretically should reduce the transport of species across the membrane. The reduced loss of polyphenols observed at higher flow rates can be attributed to the limited diffusional contact time between phenolic compounds and the membrane surface, which decreases adsorption onto the anion-exchange membrane. Conversely, ion transport was enhanced at higher flow rates, with faster desalination kinetics. This suggests that the hydrodynamic conditions near the membrane surface play a crucial role in modulating ion migration. Due to the presence of spacers and the imposed solution flow, the bulk solution inside the compartments is generally well mixed, while concentration gradients are confined to a relatively thin diffusion boundary layer adjacent to the membrane surface [12]. Increasing the solution velocity

promotes the continuous renewal of the solution near the membrane interface, maintaining higher ionic concentrations and sustaining stronger electric field gradients across the membranes. As demonstrated by Pawlowski et al. [59], higher flow rates reduce the thickness of the diffusion boundary layer in spacer-filled channels and mitigate concentration polarization phenomena, thereby enhancing mass transfer and improving electrodialysis performance. Therefore, the improved desalination efficiency observed at 20 L/h can be attributed to the reduction of concentration polarization and the enhancement of ion transport near the membrane surface. At the same time, the shorter contact time between polyphenols and the membrane likely limits adsorption phenomena, contributing to the lower polyphenol losses observed at higher flow rates.

6.3.4.1. Fouling study with NaCl solutions

Figure 6.7 shows the evolution of electrical conductivity in the diluate and concentrate compartments during ED tests performed with NaCl solutions, designed to reproduce the conductivity values used in the OMW/NaCl experiments. The aim was to isolate the effect of organic fouling by comparing system performance before OMW treatment (continuous lines), after OMW exposure (dashed lines), and after a basic–acid–basic cleaning protocol (dotted lines).

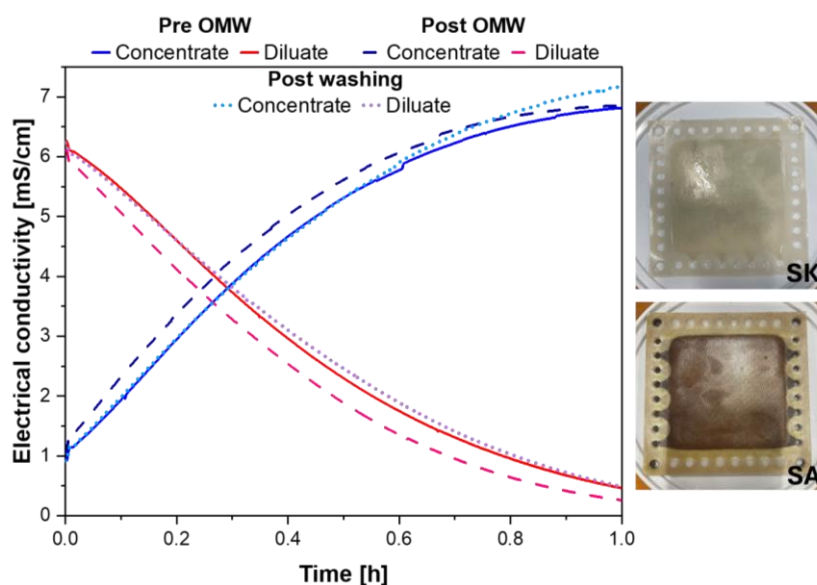


Figure 6.7: EC profiles of NaCl reference trial before and after OMW ED test and after cleaning procedure, together with representative images of CEM and fouled AEM.

During the preliminary screening trials conducted in batch recirculation mode, the EC profiles of the NaCl diluate and concentrate overlapped before and after operating the ED unit with OMW for approximately one hour, and the flow rate of both streams remained unchanged. This indicates that short-term exposure to OMW did not significantly affect the ion transport properties of the membranes.

In semi-batch case, however, after a longer exposure time, an unexpected behavior was observed. After extended electrodialysis operation with real OMW followed by a simple water rinse (dashed lines), the subsequent NaCl test showed an apparent enhancement of ion transport: the diluate conductivity decreased faster and the concentrate conductivity increased more rapidly than before

OMW exposure. This behavior, which contrasts with the expected fouling-induced decline in performance, can be explained by the findings of Bdiri et al. [60], who demonstrated that polyphenolic fouling may form colloidal deposits within the meso–macroporous domains of anion-exchange membranes, altering the apparent inter-gel structure of the polymer matrix. Partial removal or rearrangement of these deposits after rinsing may transiently increase membrane hydration and effective ionic mobility within the membrane phase, resulting in a temporary enhancement of ion transfer. The subsequent acid–base cleaning step restored the original conductivity profiles, confirming that this effect was associated with reversible structural reorganization rather than permanent membrane regeneration.

Nevertheless, the extended operating time likely promoted the deposition and adsorption of organic matter, particularly on the anion-exchange membrane. Visual inspection supports this interpretation: the cation-exchange membrane (SK) remained relatively unaffected, whereas the anion-exchange membrane (SA) exhibited visible brown deposits, consistent with polyphenol adsorption. Although these observations strongly suggest that organic fouling was the dominant mechanism under the investigated conditions, the relative contribution of organic deposition and inorganic scaling could not be quantified within the scope of the present work. Future investigations should therefore include physicochemical and electrochemical characterization of fouled membranes in order to better understand membrane stability and performance in complex OMW matrices and to discriminate between the respective contributions of organic fouling and inorganic scaling phenomena.

A comparison with electrodialysis studies performed on food highlights how the nature of the treated matrix strongly influences fouling behaviour [31]. In whey processing, fouling is generally more predictable and mainly associated with protein deposition and mineral scaling, which can typically be controlled through periodic alkaline and acidic cleaning cycles. In contrast, matrices rich in aromatic organic compounds, such as wine, fruit juices or phenolic streams, tend to promote fouling dominated by polyphenol adsorption and hydrophobic interactions with the membrane surface. Fouling in OMW treatment is more complex and less reversible, as it is primarily driven by the adsorption of phenolic compounds onto AEMs, resulting in stronger chemical interactions and potential structural modifications of the membrane polymer matrix.

6.4. Conclusions

This study demonstrated that electrodialysis can be effectively applied to pretreated olive mill wastewater to achieve significant salinity reduction while minimizing the loss of valuable polyphenolic compounds. The choice of membrane pair was shown to be critical: standard SA+SK and 100D+SK configurations achieved the best balance between conductivity reduction and phenolic retention, whereas 400D+SK resulted in the highest polyphenol leakage due to its looser structure, higher water uptake, and lower permselectivity. The semi-batch configuration highlighted the importance of hydrodynamic conditions. Higher flow rate (20 L/h) led to two key benefits. Firstly, it accelerated desalination rate by maintaining elevated current densities. Secondly, it mitigated polyphenol losses. This was likely due to shorter residence times and higher turnover of more

conductivity-rich liquid near the membrane surface. This dual benefit underscores the role of flow management in optimizing ED performance. Fouling analysis suggested that organic deposition, particularly of phenolics, preferentially occurs on anion exchange membranes, with visual evidence of brown deposits on SA membranes. Nonetheless, the applied cleaning sequence (basic–acid–basic) restored membrane performance to initial levels, indicating the feasibility of routine regeneration protocols. Overall, ED emerges as a viable, chemical-free, and environmentally friendly technology for OMW valorization. By selecting suitable membranes and operating conditions, it is possible to combine efficient demineralization with preservation of antioxidant rich phenolic compounds. As a next step, efforts will focus on closing the treatment cycle within a renewable and circular framework. In this regard, the salt enriched concentrate can be considered a secondary raw material, that can potentially undergo evaporation or crystallization to recover inorganic salts and reduce waste. Furthermore, validation on a pilot scale is needed to assess long-term performance, fouling dynamics, and the integration of ED with complementary technologies, such as electrodialysis reversal (EDR). This could ensure continuous operation, reduce the cleaning frequency and extend membrane durability. Future studies should also include physicochemical and electrochemical characterization of fouled membranes in order to better understand membrane stability and to distinguish between the respective contributions of organic fouling and inorganic scaling under realistic OMW processing conditions.

Inspired by more mature industrial applications, particularly the dairy sector, where ED is already implemented on large scale, the future goal is to evaluate the economic feasibility and industrial scalability of this process. The ultimate vision is to design an ED based treatment process that can transform the OMW management from a disposal problem into a resource recovery opportunity. This would enable olive mill operators not only to reduce environmental impact and treatment costs, but also to generate value-added products capable of offsetting operational expenses.

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CHAPTER 7: PRELIMINARY ASSESSMENT OF POTENTIAL APPLICATIONS OF RECOVERED OMW FRACTIONS

7.1. Introduction

The treatment strategy developed in Chapters 3–6 enabled the progressive fractionation of olive mill wastewater through a combination of physicochemical pretreatments and membrane-based separation processes, including microfiltration, nanofiltration, reverse osmosis, adsorption resins, and electrodialysis. This approach enabled not only the purification of the aqueous stream but also the recovery of several streams characterized by different compositions and properties.

Beyond the purification of the aqueous phase, the proposed treatment chain generates multiple fractions that can potentially be valorized within a circular and biorefinery-oriented framework. In particular, the acidification step produced a solid sludge fraction, while subsequent membrane and refining processes yield streams enriched in polyphenols and other bioactive constituents.

Given their different compositions, these fractions may be suitable for a variety of applications. Therefore, this chapter aims to provide a preliminary assessment of the potential uses of the recovered products. The investigated applications include enzymatic lipophilization of phenolic compounds, the use of the solid sludge as a substrate for black soldier fly larvae (BSFL) growth, cytocompatibility evaluation on human keratinocyte cells, phytotoxicity tests on plant seeds, and the development of preliminary cosmetic formulations based on phenolic-rich fractions.

Table 7.1 summarized the main characteristic and investigate application for the products selected from the valorization processes.

Table 7.1: Overview of the recovered OMW fractions and investigated potential applications.

Product	Main characteristics	Investigated application
Sludge post acidification	Organic-rich solid fraction	BSFL feed supplement, Phytotoxicity
NF permeate post resin	Desalted phenolic fraction	Enzymatic Lipophilization, Cyto- and phytotoxicity
RO retentate	Rich in target polyphenols	Cosmetics, Cyto- and phytotoxicity
DF retentate	High MW bioactive molecules	Cyto- and phytotoxicity

7.2. Enzymatic Lipophilization of Phenolic Compounds

7.2.1. Objective

Phenolic compounds such as hydroxytyrosol and tyrosol are among the most valuable bioactive molecules present in olive mill wastewater and olive-derived by-products, due to their well-known antioxidant and biological activities [1–3]. Thanks to these properties, they have attracted considerable interest for potential applications in the food, cosmetic and nutraceutical sectors.

However, despite their high bioactivity, the relatively high polarity of these molecules can limit their direct use in lipid-based systems. In particular, their limited solubility in lipid media and their moderate chemical stability can restrict their incorporation into formulations such as emulsions, cosmetic products or lipid-based delivery systems [4]. For this reason, several studies have investigated strategies aimed at increasing the lipophilicity of these phenolic compounds in order to expand their potential applications.

A widely explored approach consists in the esterification of the primary alcoholic group of hydroxytyrosol or tyrosol, leading to the formation of lipophilic derivatives. Among the possible derivatives, fatty acid esters of these phenolic compounds have attracted considerable attention due to their improved compatibility with lipid matrices and their potential use as antioxidant additives.

Both chemical and enzymatic methods have been investigated for the synthesis of such derivatives. Chemical esterification strategies have been reported for the preparation of lipophilic hydroxytyrosol derivatives, for example through the reaction of tyrosol or hydroxytyrosol with acyl chlorides under acidic conditions, yielding regioselective esterification at the alcoholic group [5]. For instance, Alcudia González et al. [6] patented a procedure based on acid-catalyzed esterification or transesterification of hydroxytyrosol using methyl or ethyl esters of fatty acids (C4–C18) in the presence of *p*-toluenesulfonic acid, obtaining the corresponding esters in satisfactory yields (62–86%). Similar approaches have also been applied to phenolic-rich extracts obtained from olive by-products, demonstrating that lipophilization can be successfully performed on complex mixtures rather than only on pure compounds [7].

In recent years, enzymatic approaches have gained increasing attention as greener and more selective alternatives to conventional chemical synthesis. Lipases have been widely employed as biocatalysts for the esterification or transesterification of phenolic alcohols with fatty acids or their esters. Several studies have shown that lipase-catalyzed reactions are typically regioselective, occurring preferentially at the primary alcoholic group of hydroxytyrosol. Among the different enzymes investigated, immobilized lipase from *Candida antarctica* (CALB) has consistently demonstrated high catalytic efficiency in the synthesis of lipophilic antioxidants [8–10]. In particular, Torres de Pinedo et al. [9] successfully synthesized hydroxytyrosol esters with both saturated and unsaturated fatty acids using immobilized lipase CALB, obtaining yields ranging from 43% to 94%. In their study, the ethyl esters of fatty acids were used both as substrates and as reaction medium, allowing solvent-free reactions. More recent studies have confirmed the effectiveness of CALB for the preparation of

hydroxytyrosol and tyrosol esters with various fatty acids, highlighting the potential of these derivatives as antioxidant additives for the stabilization of lipid-based systems [4,11]. Comprehensive reviews have further summarized the growing interest in enzymatic synthesis of lipophilic antioxidant and antimicrobial compounds derived from phenolic molecules [12].

Based on these considerations, enzymatic lipophilization was investigated in this work as a strategy to modify phenolic compounds recovered from OMW fractions. Model substrates, namely tyrosol and hydroxytyrosol, were initially used to evaluate the feasibility of the enzymatic esterification reaction. Subsequently, the same approach was applied to the phenolic fraction obtained after nanofiltration and desalination treatments, in order to assess the potential production of lipophilic derivatives starting from recovered OMW phenols. Therefore, enzymatic lipophilization reactions were carried out using immobilized *Candida antarctica* lipase B and vinyl palmitate as acyl donor. CALB was selected as biocatalyst due to its well-established catalytic efficiency and high regioselectivity in esterification reactions involving phenolic alcohols. Vinyl palmitate was used as acyl donor to introduce a long-chain fatty acid moiety into the phenolic molecules. The use of vinyl esters is commonly preferred in enzymatic transesterification reactions because the vinyl alcohol generated during the reaction rapidly tautomerizes to acetaldehyde, which irreversibly shifts the reaction equilibrium toward ester formation.

7.2.2. Methodological Approach

Enzymatic lipophilization reactions were carried out through lipase-catalyzed transesterification using immobilized lipase B from *Candida antarctica* (CALB, Novozym® 435) as biocatalyst and vinyl palmitate as acyl donor. The reactions were performed in tetrahydrofuran (THF) as solvent in the presence of molecular sieves in order to remove residual water and favor the esterification equilibrium. The reaction mixtures were maintained under nitrogen atmosphere and stirred at 40 °C using a thermostated system.

The progress of the reactions was monitored by TLC and the identification of the products were monitored using nuclear magnetic resonance (NMR) spectroscopy and high-performance liquid chromatography (HPLC). Additional analytical data and experimental details are provided in the Supporting Information. NMR analysis was used to confirm the formation of the corresponding fatty acid esters and to qualitatively assess the conversion of the starting phenolic compounds, as well as to identify lipophilic derivatives present in the fractions obtained from the OMW-derived phenolic stream. HPLC analysis was instead used for quantitative purposes. In addition to the calibration curves generated using commercial standards of tyrosol and hydroxytyrosol, new calibration curves were established for the isolated palmitate esters, enabling accurate quantification and, consequently, determination of the distribution of phenolic compounds and their lipophilized derivatives in the OMW-derived fractions. Calibration curves and regression parameters are reported in the Supporting Information.

For the model substrates, the reaction yield was calculated gravimetrically after purification of the isolated products according to the following equation:

$$Yield(\%) = \frac{m_{product}}{m_{theoretical}} \times 100 \quad (1)$$

where $m_{product}$ is the mass of the isolated ester and $m_{theoretical}$ is the theoretical mass calculated from the initial amount of phenolic substrate.

7.2.2.1. Reactions with model compounds

The feasibility of the lipophilization reaction was first evaluated using tyrosol and hydroxytyrosol (Sigma-Aldrich) as model substrates. Tyrosol and hydroxytyrosol were reacted with 1.5 equivalents of vinyl palmitate (VP) under the conditions described above to obtain the corresponding fatty acid esters. After completion of the reaction, the products were purified using chromatographic techniques. Tyrosol palmitate was isolated by silica gel column chromatography, whereas hydroxytyrosol palmitate was purified by preparative thin-layer chromatography (TLC). The enzymatic transesterification reaction leading to the formation of tyrosol and hydroxytyrosol palmitate is schematically illustrated in Figure 7.1.

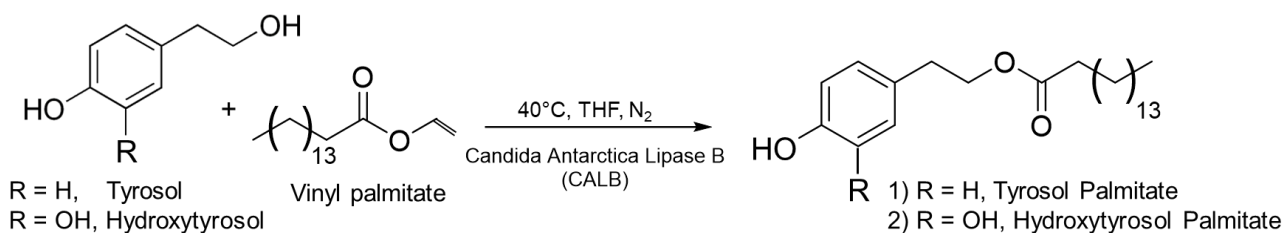


Figure 7.1: Enzymatic transesterification of tyrosol and hydroxytyrosol with vinyl palmitate catalyzed by Candida antarctica lipase B (CALB), leading to the formation of the corresponding 1) tyrosol palmitate and 2) hydroxytyrosol palmitate.

7.2.2.2. Reaction on phenolic fraction recovered from OMW

After verifying the reaction on model compounds, the same approach was applied to the phenolic fraction recovered from the nanofiltration permeate after desalination by adsorption resin. Prior to the reaction, the fraction was freeze-dried in order to remove residual water and obtain a solid material suitable for the transesterification reaction. Following the enzymatic reaction, the crude material was extracted with diethyl ether. This step allowed the recovery of the lipophilic products while leaving most of the highly hydrophilic compounds originally present in the fraction in the aqueous phase.

The organic phase was subsequently purified by silica gel column chromatography, and the collected fractions were monitored by TLC in order to identify and isolate the lipophilized phenolic derivatives. The obtained fractions were further analyzed by NMR spectroscopy to identify the lipophilic derivatives present, while HPLC analysis was used to quantify the distribution of phenolic compounds and their corresponding palmitate esters.

7.2.3. Result and Implications

7.2.3.1. Model enzymatic lipophilization

Experiments carried out using model phenolic compounds showed that both enzymatic reactions proceeded efficiently under the selected conditions. The reaction progress was monitored by TLC, which allowed verification of the disappearance of the starting substrates and the formation of the corresponding palmitate esters. After 48 hours, nearly complete conversion of the starting phenols was observed and confirmed by NMR spectroscopy, reaching approximately 90% for (1) tyrosol palmitate and 100% for (2) hydroxytyrosol palmitate. Following purification, the isolated yields were 60% for compound (1) and 27% for compound (2), respectively. The moderate to relatively low yields can mainly be attributed to losses during the purification steps, although the crude reaction mixtures appeared relatively pure. The purified products obtained from these reactions were subsequently used as reference compounds for HPLC analysis. In addition, these experiments confirmed that the enzymatic acylation occurs selectively at the primary alcoholic group of the substrates, while the phenolic hydroxyl groups remain unaffected.

These results are consistent with previous studies on lipase-catalyzed acylation of hydroxytyrosol and related phenolic compounds. In particular, Torres de Pinedo et al. [9] reported the efficient synthesis of hydroxytyrosol esters using immobilized lipase from *Candida antarctica* (CALB), obtaining yields ranging from 43 to 94% depending on the fatty acid substrate and reaction conditions. Similarly, Alcudia González et al. [6] described the preparation of lipophilic tyrosol and hydroxytyrosol esters in 65–86% yields, demonstrating the feasibility of producing phenolipids with enhanced solubility in lipid systems. In another study, enzymatic esterification of tyrosol and hydroxytyrosol catalyzed by CALB led to conversions up to 97%, confirming the high catalytic efficiency of this enzyme in the synthesis of lipophilic phenolic derivatives [10].

7.2.3.2. Enzymatic lipophilization of phenolic compounds recovered from OMW

The enzymatic lipophilization reaction was subsequently applied to the phenolic fraction recovered from the OMW treatment chain. Prior to the reaction, 300 mL of the aqueous fraction obtained after resin treatment were freeze-dried, yielding 390 mg of solid material. HPLC analysis of this material indicated the presence of approximately 30 mg of hydroxytyrosol, 6 mg of tyrosol, and 17 mg of 3,4-dihydroxyphenyl glycol. Vinyl palmitate was added in a threefold excess relative to the mass of the lyophilized material in order to promote the transesterification reaction, together with CALB B and the other components required.

After 48 h of reaction and extraction with diethyl ether, the crude mixture was analysed by NMR. The spectra revealed signals attributable to lipophilized phenolic derivatives together with a large excess of unreacted vinyl palmitate. Notably, no signals attributable to carbohydrate-derived compounds were detected, suggesting that highly hydrophilic species had been effectively retained during the previous resin treatment or during extraction with diethyl ether after lipophilization. The crude extract (approximately 1.2 g) was subsequently purified by silica gel column chromatography, yielding four main fractions. According to NMR spectra, the first fraction contained mostly unreacted

vinyl palmitate; the second was predominantly pure 1; the third contained 2 and possibly palmitic acid; and the fourth contained small amounts of 2, potentially palmitoyl glycol and palmitic acid. Fractions 2 and 3 were further analysed by HPLC to quantify the lipophilized phenolic derivatives 1 and 2 present after chromatographic purification. The main quantitative results are summarized in Table 7.2.

Table 7.2: Quantification of lipophilized phenolic derivatives in chromatographic fractions obtained after enzymatic lipophilization of OMW-derived phenols.

Fraction	Main compounds (NMR)	HPLC concentration (ppm)	Estimated content in fraction (%)	Apparent yield (%)
F2	Product (1)	47.1 ± 0.7	~ 9 (3.7 mg)	23%
F3	Product (2)	430.7 ± 5.6	~ 63 (27 mg)	35%

The results indicate that tyrosol palmitate represented approximately 9% of fraction 2, corresponding to about 3.7 mg of product, whereas hydroxytyrosol palmitate accounted for approximately 63% of fraction 3, corresponding to about 27 mg of product. When compared with the theoretical maximum obtainable from the initial amounts of tyrosol and hydroxytyrosol present in the fraction, these values correspond to apparent recoveries of approximately 23% and 35%, respectively. However, due to the complexity of the OMW-derived matrix and the possible presence of co-eluting acylated compounds, these values should be considered as indicative rather than exact quantitative yields. From a process optimization perspective, the results also suggest that reducing the amount of vinyl palmitate used in the reaction could simplify downstream purification. In addition, the use of an aqueous sodium bicarbonate solution during the extraction step may help remove palmitic acid and improve the separation of the lipophilized phenolic derivatives.

Despite the moderate apparent recoveries observed for the OMW-derived fraction, the formation of lipophilic derivatives confirms that enzymatic acylation can also be successfully applied to phenolic compounds recovered from complex matrices. Similar strategies have been reported for the lipophilization of hydroxytyrosol-rich fractions obtained from olive by-products [7] and for the esterification of tyrosol and hydroxytyrosol extracted from olive mill wastewater using immobilized lipases [13]. In addition, enzymatic acylation of hydroxytyrosol directly in olive mill wastewater has been demonstrated using engineered acyltransferases, although lower conversions were observed due to the complexity of the wastewater matrix [14]. More recently, the esterification of hydroxytyrosol-rich extracts has been proposed to improve the oxidative stability of edible oils, highlighting the potential application of lipophilic phenolic derivatives as antioxidants in lipid-based systems [15]. The introduction of a long fatty acid chain increases the hydrophobic character of the phenolic molecules, improving their stability and solubility in lipid phases, which may help in separating these lipophilized phenols from other more hydrophilic organic compounds. As a result, these lipophilized derivatives can more effectively exert their antioxidant activity in lipid-based environments, enabling their use in a wide range of formulations such as edible oils, emulsions, nutraceutical preparations, and cosmetic products.

7.3. Use of Sludge as Black Soldier Fly Larvae Feed Supplement

7.3.1. Objective

The acidification step applied to olive mill wastewater generates a solid sludge fraction rich in suspended organic matter. As discussed in Chapter 3, this fraction represents a potentially valuable by-product of the treatment process and may be suitable for different valorization pathways.

One promising strategy for the valorization of organic residues is the use of black soldier fly larvae (*Hermetia Illucens*), which are widely recognized for their ability to convert a wide range of organic substrates into valuable biomass. BSFL can efficiently transform agro-industrial by-products and organic waste streams into protein- and lipid-rich biomass while simultaneously reducing the volume of organic residues.

Several studies have demonstrated the feasibility of rearing BSFL on agro-industrial residues and food waste streams. For example, grape pomace derived from the wine industry has been evaluated as a substrate for BSFL growth [16]. In addition, Meneguz et al. [17] evaluated the growth of BSFL on different organic wastes and agro-industrial by-products, including fruit and vegetable residues, brewery by-products, and winery by-products, demonstrating their potential for waste bioconversion.

However, the nutritional composition of these substrates can vary considerably depending on the type of waste, making it difficult to directly compare growth performance among different studies [18]. In many cases, individual waste streams do not provide a nutritionally balanced diet for optimal larval development. For example, substrates such as rice straw or fruit and vegetable waste have been shown to negatively affect larval growth and bioconversion efficiency when used alone due to their poor nutritional composition [19,20]. For this reason, many studies have investigated the formulation of diets based on mixtures of different residues in order to achieve a more balanced nutrient composition. For example, Bellezza Oddon et al. [21] evaluated several isonutrient diets formulated with multiple agro-industrial by-products and observed high survival rates (> 92 %) and emergence rates (> 94 %), with the best larval growth obtained in diets containing mixtures of brewers' spent grain, fruit waste, and vegetable residues.

Within the olive oil production chain, previous studies have evaluated the use of solid residues such as olive pomace, olive cake, and olive leaves as feeding substrates for BSFL. These by-products are generally incorporated into mixed diets, since their use as the sole substrate may lead to nutritionally unbalanced diets. For example, olive pomace has been successfully included in larval diets at levels up to approximately 50% of the substrate without significantly affecting larval survival [22], although higher inclusion levels tend to reduce larval growth and bioconversion efficiency. Similarly, olive leaves have been incorporated at levels of around 10–15% of the diet, above which negative effects on larval performance have been reported [23].

Based on the above consideration, the objective of this experiment was to evaluate the potential use of the sludge fraction obtained from the acidification of OMW as a dietary component for black

soldier fly larvae. In particular, the study aimed to assess the effects of increasing inclusion levels of olive sludge in the larval diet on survival, growth performance, and substrate bioconversion efficiency.

7.3.2. Experimental Approach

The feeding trial with black soldier fly larvae was performed by Dr. *Sara Bellezza Oddon* at the Department of Agricultural, Forest and Food Sciences, University of Turin, using the sludge fraction obtained from the acidification of OMW described in Chapter 3.

7.3.2.1. Diets preparation

Experimental diets were prepared by incorporating different levels of olive sludge (OS) into a standard Gainesville (GA) diet. The GA diet consisted of 50% wheat bran, 30% alfalfa meal and 20% corn meal, according to the formulation proposed by Hogsette et al. [24], and was hydrated with tap water to reach approximately 30% dry matter. The sludge fraction was added to the GA diet at different inclusion levels (Table 7.3). Each experimental unit consisted of 300 g of substrate placed in plastic containers (12 × 12 × 5 cm) in order to maintain a sufficient substrate depth and prevent rapid water evaporation.

Table 7.3: Diets prepared with different levels of olive sludge inclusion (OG).

Diets	Inclusion level %		Larval biomass (g)		
	GA	OS	GA	OS	Total (g)
1	100	0	300	0	300
2	80	20	240	60	300
3	60	40	180	120	300
4	40	60	120	180	300
5	20	80	60	240	300
6	0	100	0	300	300

7.3.2.2. Colony

Black soldier fly larvae were obtained from a colony maintained at the experimental facilities of the Tetto Frati research center (Carmagnola, Italy). Eggs collected over a 24 h period were incubated under controlled conditions (75% relative humidity) until hatching. After hatching, larvae were reared on the control GA diet for six days.

At the beginning of the experiment, 200 larvae (six days old, average individual weight 0.003 g) were counted individually and inoculated into each container containing the experimental substrates. The containers were then placed in climate-controlled chambers at 28.5 °C and 60% relative humidity.

7.3.2.3. Larval growth performance and bioconversion efficiency

After eight days of feeding (corresponding to 14 days of larval age), larvae were separated from the substrate to evaluate growth performance and survival. Larvae were washed with warm water, dried

with absorbent paper, counted and weighed using a precision balance. The residual substrate weight was also recorded.

From the number of larvae and the larval biomass weight, the following parameters were calculated:

- Survival rate (SR %)

$$SR (\%) = \frac{\text{estimated number of larvae at the end of the experiment}}{\text{estimated number of larvae at the end of the experiment}} \times 100 \quad (2)$$

- Growth rate (GR, g/day)

$$GR (\text{g/day}) = \frac{\text{final average larval weight} - \text{initial average larval weight}}{\text{number of feeding days}} \quad (3)$$

- Specific growth rate (SGR, %/day)

$$SGR (\%/day) = \frac{\ln(\text{final average larval weight}) - \ln(\text{initial average larval weight})}{\text{number of feeding days}} \times 100 \quad (4)$$

- Bioconversion efficiency (BER, %)

$$BER (\%) = \frac{\text{final larval biomass} - \text{initial larval biomass}}{\text{initial substrate weight} - \text{residual substrate weight}} \times 100 \quad (5)$$

- Substrate reduction rate (RR, %)

$$RR (\%) = \frac{\text{initial substrate weight} - \text{residual substrate weight}}{\text{residual substrate weight}} \times 100 \quad (6)$$

- Waste reduction index

$$WRI = \frac{RR}{\text{number of feeding days}} \quad (7)$$

7.3.3. Result and Valorization Potential

The results of the feeding trial are summarized in Figure 7.2 and Table 7.4. Data from the treatment containing 100% olive sludge were not included in the analysis, as larvae escaped from the substrate shortly after inoculation, indicating that this substrate was not suitable as the sole feeding source.

Overall, the average individual larval weight increased in all treatments during the experimental period. However, larval weight and survival decreased progressively at higher sludge inclusion levels ($\geq 60\%$), while low inclusion ($\leq 20\%$) supported performance comparable to the control diet. The chemical characterization of the sludge (Chapter 3) provides a mechanistic explanation for these trends: although SEM–EDX indicated mostly inorganic species that are not inherently detrimental for BSFL, sequential extraction, FT-IR and GC–MS analyses revealed a matrix rich in ethanol-extractable lipid-like and aromatic compounds but deficient in readily assimilable proteins and fermentable carbohydrates. This nutritionally unbalanced profile, divergent from macronutrient ranges known to sustain optimal BSFL development [25–29], likely underlies the impaired growth, survival and BER at higher inclusion levels. Conversely, low inclusion remains feasible because the base diet compensates for these deficiencies, suggesting that the sludge could be more effectively valorized when blended with protein- or carbohydrate-rich organic by-products to restore nutritional balance.

CHAPTER 7: PRELIMINARY ASSESSMENT OF POTENTIAL APPLICATIONS OF RECOVERED OMW FRACTIONS

Similar observations have been reported in previous studies evaluating the use of agro-industrial by-products in BSFL diets. For example, Ramzy et al. [30] investigated the use of olive pomace residue (OPR) as a substrate for black soldier fly larvae, testing inclusion levels of 25, 50, and 75% in mixtures with wheat bran. Although the larvae were able to convert the substrate into insect biomass, higher pomace inclusion levels increased the lignin content of the diet and resulted in a longer larval development time. More broadly, many organic residues perform better in blends than alone because formulations allow nutrient balancing [21]. Consistently, Gold et al. [18] showed that waste-based formulations (vs single wastes) improved larval performance by meeting protein/carbohydrate/lipid requirements.

At the end of the experiment, the bioconversion efficiency of the larvae was calculated using the BER index, which accounts for substrate consumed and the production of larval biomass, exuviae and frass, as recommended by Bosch et al. [31]. In line with growth and survival, BER decreased at higher sludge inclusions.

Table 7.4: Comparison between average weight of *Hermetia Illucens* (HI) larvae, mortality, and substrate reduction indices in the different treatments.

Diet (GA:OS) % wt	1 (100:0)	2 (80:20)	3 (60:40)	4 (40:60)	5 (20:80)
Average larval weight (g)	0.133	0.068	0.036	0.019	0.015
Substrate weight (g)	53.1	82.5	87.1	99.8	112.1
Survival rate (%)	95.5	100	92	86	25
Growth rate (g/day)	0.13	0.07	0.04	0.02	0.01
Specific growth rate (%)	13	7	4	2	1
Bioconversion efficiency (%)	25.46	13.6	6.73	3.29	0.77
Substrate reduction rate (%)	0.82	0.72	0.71	0.66	0.62
Waste reduction index	0.12	0.10	0.10	0.10	0.09

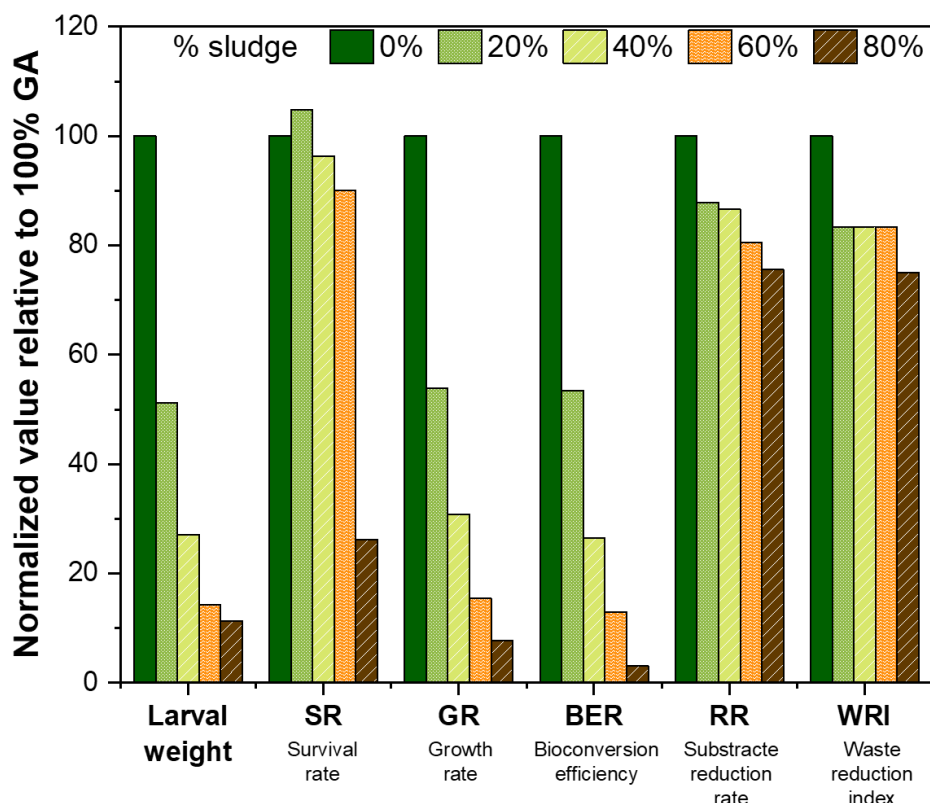


Figure 7.2: Normalized values of larval indices relative to 100% GA in the different treatments.

Overall, these results indicate that olive sludge derived from OMW treatment cannot be used as the sole feeding substrate for BSFL. However, its incorporation at low inclusion levels appears feasible and could represent a potential valorization pathway for this fraction. Future studies should further investigate the performance of black soldier fly larvae reared on diets containing lower inclusion levels of olive sludge, particularly within the range of 1–10% and evaluate the growth performance and BER under larger-scale rearing conditions.

From a broader perspective, the use of black soldier fly larvae for the treatment and valorization of organic residues is increasingly recognized as a promising strategy within circular bioeconomy frameworks. Insect-based bioconversion systems enable the transformation of low-value organic by-products into valuable biological resources, contributing to waste reduction and resource recovery. Through this process, organic residues can be converted into larval biomass that may subsequently be processed into insect-derived ingredients for animal feed, such as protein-rich insect meal and lipid fractions [32]. In this context, the integration of BSFL-based bioconversion into the OMW treatment chain could represent an additional strategy for the sustainable management and valorization of sludge fractions generated during wastewater processing.

7.4. Phytotoxicity Assessment

7.4.1. Objective

Olive mill wastewater and its derived fractions are widely recognized as complex agro-industrial effluents due to their high organic load and the presence of various bioactive compounds. These

streams typically contain large amounts of organic matter, suspended solids, mineral salts, and phenolic compounds, which can pose environmental and agronomic challenges if discharged without appropriate treatment. In particular, the high chemical oxygen demand (COD), acidic pH, and phenolic content of raw OMW may negatively affect soil properties, microbial activity, and plant development [33–36].

Among the different constituents of OMW, phenolic compounds are often considered the main contributors to its phytotoxicity. These molecules can interfere with plant metabolic processes, inhibit seed germination, and reduce root elongation. Several studies have reported inhibitory effects of OMW on plant germination and early seedling development. For example, Saadi et al. [37] reported significant reductions in germination and root growth of cress (*Lepidium sativum*) exposed to untreated OMW, while Abou- Hassan et al. [38] observed marked inhibition of seed germination and seedling growth in maize (*Zea mays* L.). Similarly, Bouhia et al. [39] highlighted that the phytotoxicity of olive mill residues is largely related to the presence of phenolic compounds, including *p*-coumaric acid, gallic acid, oleuropein, hydroxytyrosol, and tyrosol, which are known to interfere with seed germination and early plant development.

However, the agronomic effects of OMW are strongly dependent on its concentration and on the type of treatment applied. Several studies have shown that dilution or appropriate treatment can significantly reduce its phytotoxicity and, in some cases, even promote plant growth. For instance, Enaime et al. [40] reported that undiluted OMW strongly inhibited maize and tomato germination, whereas dilution markedly reduced phytotoxic effects and restored seed viability. Similarly, Rusan et al. [41] demonstrated that untreated OMW inhibited barley (*Hordeum vulgare* L.) seed germination, while diluted or treated effluents allowed plant emergence and early growth.

Recent research has also highlighted the potential of treatment technologies to transform OMW from a phytotoxic waste into a valuable resource for agricultural applications. Biological or physicochemical treatments can reduce organic load and phenolic content, thereby decreasing phytotoxicity. For instance, Hanafi et al. [42] reported that a combined electrocoagulation and biological treatment significantly reduced COD and phenolic content in OMW, leading to a germination index of approximately 106% in *Triticum durum* seeds, indicating the complete removal of phytotoxicity and a possible fertilizing effect. In addition to conventional treatments, membrane-based processes have recently attracted considerable attention for the management and valorization of OMW. Membrane fractionation can generate streams with markedly different biological properties depending on their phenolic content. For example, Popolizio et al. [43] evaluated the phytotoxicity of retentates obtained from nanofiltration and reverse osmosis of olive mill wastewater and reported strong inhibition of seed germination in several plant species, including *Lepidium sativum*, *Solanum lycopersicum*, and *Triticum turgidum*. At higher concentrations, these polyphenol-rich retentates caused near-complete inhibition of germination, suggesting their potential use as natural bioherbicides.

Similarly, the biological properties of OMW-derived sludge have been shown to vary widely depending on its composition and treatment history. Recent studies reported germination index values ranging from strongly inhibitory conditions to phytostimulant responses exceeding 100%, highlighting the potential of treated sludge as a soil amendment or organo-mineral fertilizer. For instance, Bouhia et al. [44] demonstrated that microbial conditioning of OMW sludge combined with rock phosphate can significantly reduce pollutant loads and produce a semi-liquid organo-mineral fertilizer suitable for agricultural applications, while Martínez-Gallardo et al. [45] reported phytostimulant responses in germination tests depending on the physicochemical characteristics of the sludge and its associated microbial communities.

Overall, these studies highlight the dual agronomic role of OMW and its derived fractions: while raw or polyphenol-rich streams may exert strong phytotoxic effects that could potentially be exploited for bioherbicidal applications, appropriately treated or diluted fractions may become valuable agricultural inputs, such as irrigation water, soil amendments, or biostimulants. In this context, evaluating the phytotoxic potential of the fractions obtained during the OMW treatment chain is essential to assess their possible agricultural applications. Therefore, the objective of this study was to investigate the phytotoxic effects of selected OMW-derived fractions using seed germination tests with *Lepidium sativum*, to provide preliminary insights into their potential use in agricultural systems.

7.4.2. Experimental Approach

The experiments were conducted at the Plant Pathology Laboratory, Section Microbiology and Molecular Biology, Centro di Sperimentazione e Assistenza Agricola (CerSAA, Albenga), under the supervision of Dr. Anna Lanteri and Dr. Federico Tinivella.

Phytotoxicity was assessed by analyzing the seed germination and root length of *Lepidium sativum* seeds, following the method described by Zucconi et al. [46] and consistent with the UNICHIM 1651:2003 standard [47], with minor modifications. The phytotoxicity tests were performed on both liquid samples (NF permeate, RO retentate, and DF retentate) and on a treated solid matrix (sludge obtained after the acidification step). The preparation of the liquid samples included an initial microfiltration using a sterile syringe filter (0.2 μm , cellulose acetate). Serial dilutions were then prepared with deionized water to obtain concentrations of 1, 5, 10, 25, 50, and 100% (v/v). The solid sludge (60% moisture content (w/w) determined using a Tecna 145 moisture analyser equipped with a Sartorius weighing system) was dispersed in deionized water to obtain a 10% (w/v) suspension. The suspension was mixed for 30 min under magnetic stirring and subsequently filtered through filter paper to remove coarse particles. The resulting extract was then microfiltered using a sterile syringe filter (0.2 μm , cellulose acetate). Serial dilutions were prepared with deionized water to obtain the same concentration range used for the liquid samples.

Briefly, 2 mL of each sample were added to filter paper placed in petri dishes (90 mm diameter). Subsequently, 10 *Lepidium sativum* seeds were distributed on the filter paper. The petri dishes were then covered with their lids to prevent evaporation and contamination. Plates containing distilled

water were used as the control. Ten replicate plates were prepared for each concentration to guaranty reproducibility. All plates were incubated for 72 h at room temperature in darkness. After incubation, the number of germinated seeds in the samples and in the control were recorded for each experimental set. A seed was considered germinated when the radicle protrusion was visible. The root length of the sample and the root length of the control were measured for each experimental set. The root length was measured from the tip of the primary root to the base of the hypocotyl. The germination index (GI) was calculated according to Zucconi et al. [46] using the following equation:

$$GI\% = \frac{G_s \times L_s}{G_c \times L_c} \times 100 \quad (8)$$

where L_s is the mean root length (cm) in the sample, G_s is the percentage of seeds germinated in the sample, L_c is the mean root length in the control (cm), and G_c is the percentage of seeds germinated in the control. GI values lower than 50% indicate phytotoxic effects, values between 50% and 80% indicate moderate phytotoxicity, while GI values higher than 80% indicate the absence of phytotoxic effects. Values exceeding 100% may indicate phytostimulant activity.

Statistical differences among the tested concentrations were evaluated using one-way analysis of variance (ANOVA). The significance of the effect of concentration on the germination index was assessed using the F statistic and the associated p-value. When significant differences were detected, Tukey's post-hoc test was applied to determine significant differences between concentration levels. followed by Tukey's post-hoc test to determine significant differences between concentration levels. Differences were considered statistically significant at $p < 0.05$. All statistical analyses were performed using Origin software (OriginLab Corporation, Northampton, MA, USA).

7.4.3. Result and Interpretation

The phytotoxicity of the OMW-derived fractions was evaluated by measuring the germination index (GI) of *Lepidium sativum* seeds exposed to increasing concentrations of the tested samples. The results obtained for the sludge extract and the membrane-derived fractions are reported in Figure 7.3.

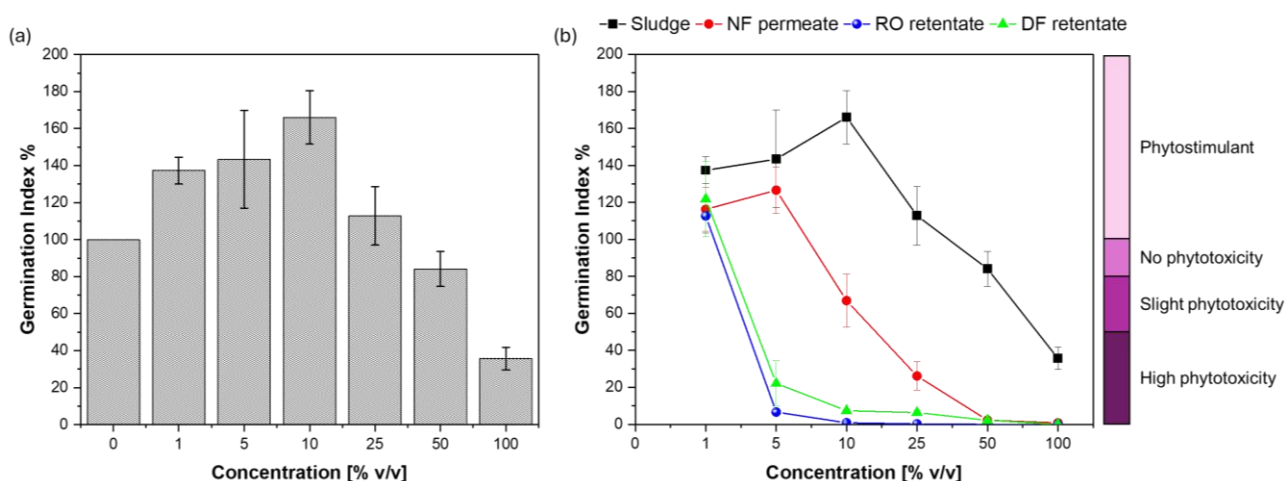


Figure 7.3: Germination index (GI) of *Lepidium sativum* seeds exposed to increasing concentrations of OMW-derived fractions. (a) sludge extract and (b) comparison among sludge and membrane-derived fractions (NF permeate, RO retentate and DF retentate). Error bars represent standard deviation (n = 10).

As shown in Figure 3, all tested fractions exhibited a clear concentration-dependent effect on seed germination. In general, low concentrations of the samples produced a stimulatory effect, whereas increasing concentrations resulted in a progressive reduction of the GI, indicating the onset of phytotoxic effects.

The sludge extract showed the lowest phytotoxicity among the tested matrices. At low concentrations (1–10%), the sludge fraction exhibited a marked biostimulant effect, with GI values exceeding 100% and reaching a maximum of approximately 166%. Increasing sludge concentrations progressively reduced the GI, with significant inhibition observed only at the highest concentrations tested. One-way ANOVA confirmed a highly significant effect of concentration on the germination index ($F = 98.68$, $p < 0.001$).

A stronger phytotoxic response was observed for the membrane-derived fractions. In the case of the NF permeate, low concentrations (1–5%) produced a moderate stimulatory effect, whereas concentrations $\geq 10\%$ resulted in a sharp decrease in GI values. At concentrations $\geq 25\%$, the germination index dropped well below 50%, indicating strong phytotoxicity. Statistical analysis confirmed highly significant differences among treatments ($F = 356.55$, $p < 0.001$).

The RO retentate showed an even more pronounced inhibitory effect. Although the lowest concentration (1%) exhibited a slight stimulatory effect ($GI \approx 113\%$), a drastic reduction of germination was already observed at 5%, where the GI dropped to approximately 6%. At concentrations $\geq 10\%$, seed germination was almost completely inhibited. The ANOVA analysis confirmed the strong effect of concentration ($F = 2126.57$, $p < 0.001$).

Similarly, the DF retentate displayed a pronounced phytotoxic behavior. While a stimulatory effect was observed at 1% ($GI \approx 122\%$), the germination index sharply decreased at higher concentrations, reaching values below 25% already at 5%. Concentrations $\geq 10\%$ caused near-complete inhibition of seed germination. One-way ANOVA confirmed significant differences among concentrations ($F = 320.47$, $p < 0.001$).

The concentration-dependent phytotoxic behavior observed in this study is consistent with several reports on olive mill wastewater and its derivatives. Numerous studies have shown that raw or concentrated OMW fractions can strongly inhibit seed germination and root development due to the presence of phenolic compounds and other bioactive molecules [38,39]. In particular, high phenolic concentrations have been associated with severe reductions in germination index values in different plant species. Conversely, several studies have also reported that diluted OMW fractions may exert stimulatory or neutral effects on plant germination and growth, highlighting the strong dependence of phytotoxicity on concentration and treatment conditions [41,48]. Similar trends were observed in the present study, where low concentrations of the investigated fractions promoted seed germination, whereas higher concentrations caused strong inhibitory effects. The sludge extract was the fraction that exhibited the most pronounced phytostimulant properties in the present study. This observation is consistent with the findings of Martínez-Gallardo et al., who reported that OMW-derived sludge

may display highly variable biological activity, with germination index values ranging from strongly inhibitory conditions to phytostimulant responses exceeding 120%, depending on sludge composition. Moreover, the pronounced phytotoxicity observed for the more concentrated membrane-derived fractions is consistent with previous studies showing that membrane processes can concentrate phenolic compounds and enhance the biological activity of OMW-derived streams [43].

Representative images of the germination tests are shown in Figure 7.4, clearly illustrating the progressive inhibition of seed germination and root development with increasing concentration of the tested samples. The visual inspection of the plates confirms the trends observed in the germination index analysis.

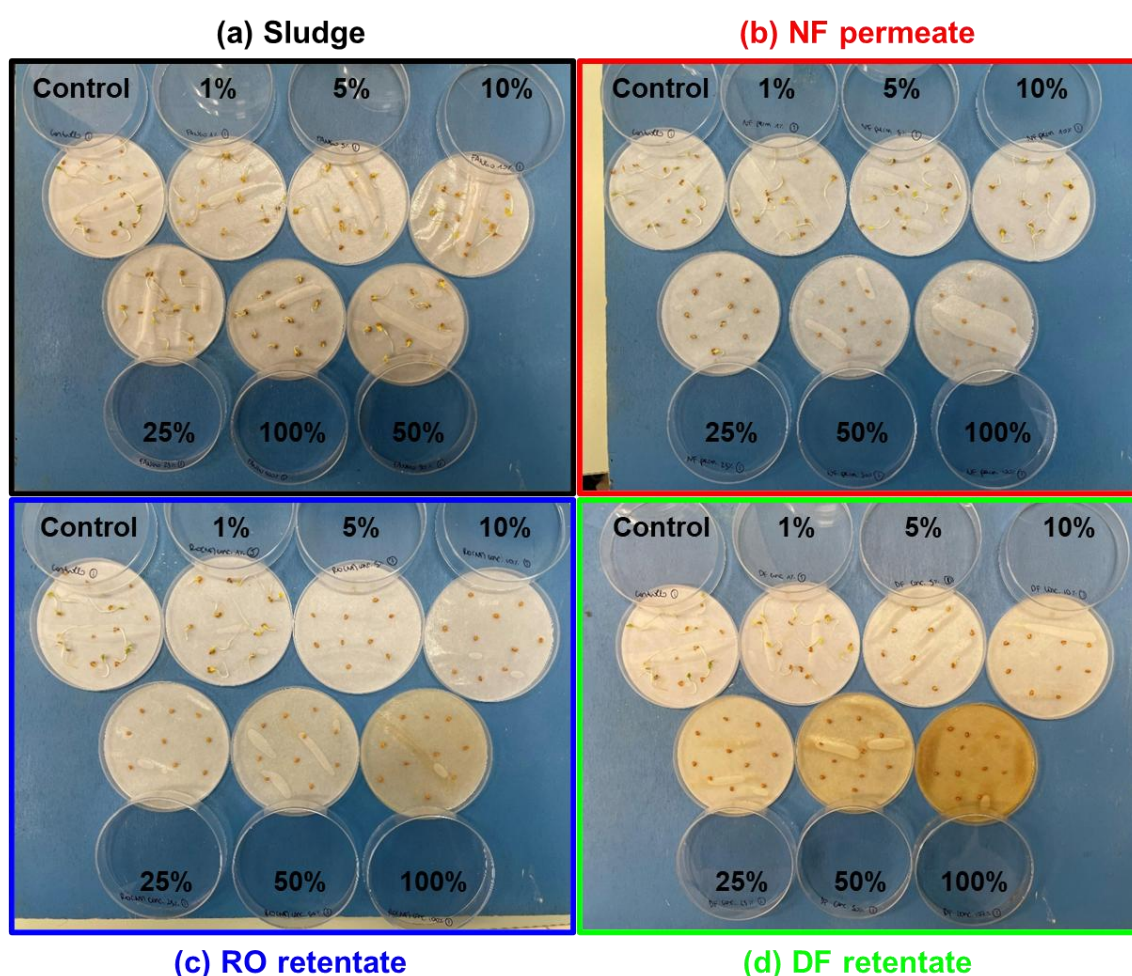


Figure 7.4: Representative images of *Lepidium sativum* seed germination after 72 h of exposure to increasing concentrations (0–100%) of OMW-derived fractions: (a) sludge extract, (b) NF permeate, (c) DF retentate, and (d) RO retentate

From an application perspective, these findings suggest that appropriately diluted fractions may potentially be explored as plant biostimulants, whereas more concentrated streams could find application as natural bioherbicides due to their strong inhibitory effects on seed germination.

However, in order to confirm these trends and better assess their agronomic relevance, it is necessary to further scale the investigation under more realistic growth conditions. Future studies should therefore evaluate the effects of the most promising OMW-derived fractions through pot or soil-based

experiments, following standardized protocols such as the OECD 208 Terrestrial Plant Test [49]. In these experiments, diluted liquid fractions or solid matrices could be incorporated into soil at concentrations selected based on the results of the germination assays. At least two plant species representing monocotyledons and dicotyledons (e.g., *Hordeum vulgare* and *Brassica rapa* or *Lepidium sativum*) should be evaluated over a 14–21 days growth period.

These experiments would allow verification of the stimulatory or inhibitory effects observed in the germination assays under soil conditions and help identify fractions suitable for potential agronomic applications, either as plant biostimulants at low concentrations or as natural bioherbicides at higher concentrations.

7.5. Cytocompatibility Assessment on Human Keratinocytes

7.5.1. Objective

Olive mill wastewater contains a complex mixture of organic compounds, including phenolic substances that are responsible for many of its biological properties. These compounds have been extensively investigated due to their well-known antioxidant, anti-inflammatory, and antimicrobial activities, which have attracted interest for potential applications in the food, pharmaceutical, and cosmetic sectors [34,36,50].

Polyphenols are also known to exert several biological effects on skin physiology. Recent reviews have highlighted that phenolic compounds can interact with skin cells by modulating oxidative stress, inflammation, and cellular signaling pathways, contributing to protective and bioactive effects in cutaneous tissues [51]. However, the biological activity of polyphenols is often concentration-dependent, and higher concentrations may lead to adverse cellular responses, including cytotoxic effects. [52,53]. For this reason, evaluating the cytotoxicity of OMW-derived fractions represents an important step when considering potential applications involving human exposure, such as agricultural formulations, cosmetic ingredients, or environmental products. Among the different cellular models available, the human keratinocyte cell line HaCaT is widely used to investigate the effects of bioactive compounds on skin cells and to evaluate potential cytotoxic responses associated with natural extracts or environmental samples.

Based on these considerations, the objective of this study was to investigate the cytotoxic effects of selected fractions obtained during the OMW treatment process on human keratinocytes (HaCaT cell line), in order to provide preliminary information on their biological safety and potential compatibility with applications involving human exposure.

7.5.2. Experimental Approach

The cytotoxicity assays were performed by Dr. *Giulia De Negri Atanasio* and Prof. *Elena Grasselli* at the Department of Earth, Environmental and Life Sciences, University of Genoa, using the human keratinocyte cell line HaCaT as an in vitro model to evaluate the biological effects of three different

fractions obtained from the OMW treatment process described in Chapter 4: diafiltration retentate, reverse osmosis retentate, and nanofiltration permeate.

Cell viability was assessed using the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), a colorimetric method widely used to evaluate cellular metabolic activity as an indicator of cell viability [54]. Cells were exposed to increasing concentrations of the extracts (0.005, 0.01, 0.02, 0.03, 0.04, 0.06, 0.08, and 0.1 mg GAE/mL). After 24 hours of incubation, cell viability was determined by measuring the reduction of MTT to formazan crystals by metabolically active cells. Untreated cells were used as the control condition, and their viability was set as 100%. Results were expressed as percentage of cell viability relative to the control and reported as mean $\pm$ standard deviation. The concentrations of the tested samples were expressed as gallic acid equivalents (GAE), calculated from total phenolic content previously determined by the Folin–Ciocalteu method using calibration curves prepared with gallic acid as standard (Supporting material).

7.5.3. Result and Discussion

The cytotoxic effects of the investigated OMW-derived fractions on human keratinocytes are shown in Figure 7.5.

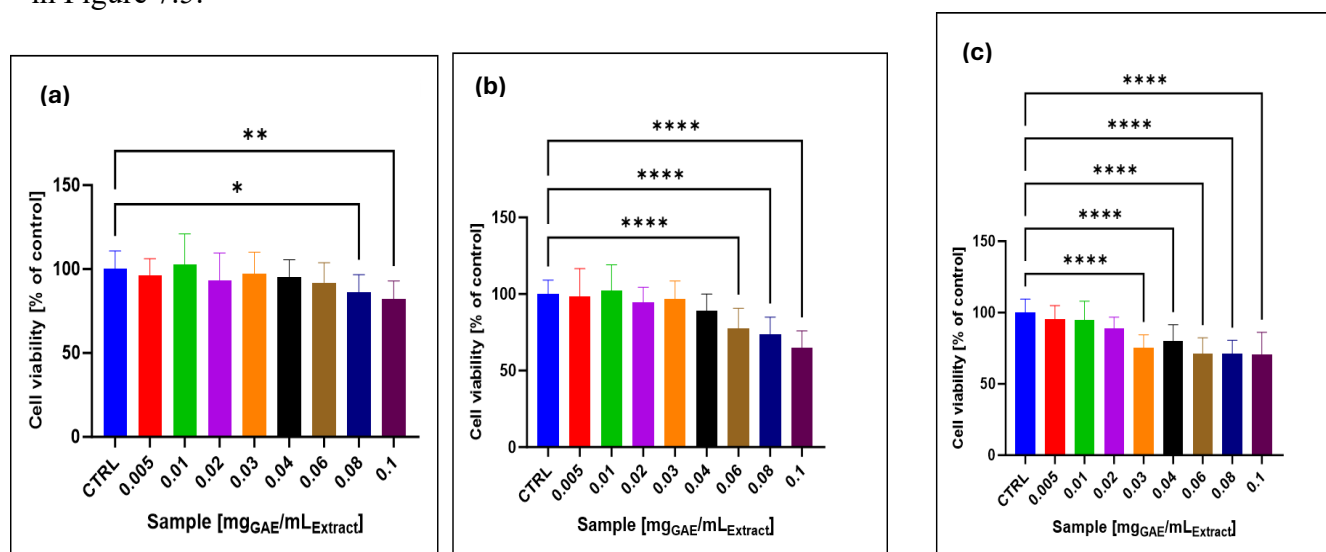


Figure 7.5: Cytotoxic effects of OMW-derived fractions on human keratinocytes (HaCaT cell line) evaluated by MTT assay after 24 h exposure. Samples tested include (a) diafiltration retentate, (b) reverse osmosis retentate, (c) nanofiltration permeate. Cell viability is expressed as percentage relative to untreated control cells. Results are reported as mean $\pm$ SD. Statistical significance was determined relative to control (**** $p \leq 0.0001$, *** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$).

Overall, the tested samples exhibited a concentration-dependent effect on cell viability. At the lowest concentrations (0.005–0.02 mg GAE/mL), all fractions showed cell viability values comparable to the untreated control, indicating the absence of significant cytotoxic effects under these conditions.

As the concentration increased, a gradual reduction in cell viability was observed for all tested samples. This trend was particularly evident for the fractions obtained after nanofiltration and reverse osmosis which permeate and retentate, respectively, showed statistically significant reductions in cell viability at higher concentrations (0.03–0.04 mg GAE/L). In contrast, the diafiltration retentate showed a less pronounced effect on cell viability across the tested concentration range, suggesting a

lower cytotoxic potential compared with the other fractions. These differences may be related to variations in the chemical composition of the fractions rather than to their total phenolic content. In complex polyphenolic extracts, the biological response often depends on the specific mixture of secondary metabolites present, as well as on their concentration [55]. Moreover, the biological activity of phenolic compounds is strongly influenced by their chemical structure, which may determine their antioxidant or cytoprotective effects in cellular systems [56]. Similar concentration-dependent responses have also been reported in studies investigating the effects of polyphenolic compounds on skin cells. Polyphenols may exert protective or stimulatory effects at low concentrations, while higher concentrations may interfere with cellular metabolism and reduce cell viability [51].

From an application perspective, the absence of strong cytotoxic effects at lower concentrations suggests that appropriately diluted OMW-derived fractions may be compatible with applications involving potential human exposure. In particular, such fractions could be considered for use in agricultural formulations or environmental products within circular bioeconomy strategies. However, further investigations would be necessary to better understand the biological effects of these extracts, including studies on additional cell models, longer exposure times, and a more detailed characterization of the compounds responsible for the observed cellular responses.

7.6. Preliminary Cosmetic Formulations Using Phenolic Fractions

7.6.1. Objective

Olive mill wastewater has attracted increasing interest as a potential source of bioactive compounds for high-value applications. Although traditionally considered an environmental pollutant due to its high organic load and phenolic content, OMW is now recognized as a rich reservoir of phenolic compounds that can be recovered and valorized within circular bioeconomy strategies [33,57,58]. In particular, several studies have highlighted that OMW may contain higher concentrations of polyphenols than olive oil itself, making it a promising raw material for the extraction of biologically active molecules.

Among the most abundant phenolic compounds present in OMW are hydroxytyrosol, tyrosol, oleuropein, verbascoside, caffeic acid, and ferulic acid. Due to their strong antioxidant capacity, anti-inflammatory, antimicrobial, and photoprotective activities, olive-derived polyphenols have been widely investigated as functional ingredients for skin care formulations, including anti-aging and photoprotective products [50,51,59,60]. In particular, hydroxytyrosol has been widely recognized as one of the most potent natural antioxidants and has been reported to protect skin cells from oxidative stress and UV-induced damage.

Recent research has therefore explored the potential use of OMW-derived phenolic extracts in cosmetic and dermatological formulations. These compounds have been shown to reduce reactive oxygen species (ROS) formation in skin cells, inhibit enzymes involved in skin aging such as collagenase and elastase, and contribute to improved skin protection and regeneration [61–63]. In

addition, the antimicrobial activity of OMW polyphenols may contribute to the development of formulations aimed at improving skin microbiological balance.

From a technological perspective, the recovery and concentration of these bioactive compounds can be efficiently achieved through membrane-based processes, which allow the separation of OMW into fractions with different chemical compositions and biological properties. Previous studies have demonstrated that membrane fractionation of OMW can produce phenolic-rich fractions with significant antioxidant activity and potential applicability in nutraceutical and cosmeceutical products [64]. For example, Russo et al. [65] reported that membrane-based purification processes enabled the recovery of phenolic-rich fractions with total phenolic contents ranging from approximately 1.5 to 15 g GAE/L, together with significant antioxidant and antimicrobial activities, highlighting the potential of these fractions in (phyto)pharmaceutical field.

For these reasons, several studies have explored the incorporation of OMW-derived phenolic extracts into different cosmetic formulations, including creams, serums, gels, and nanoemulsions, in order to enhance the stability, bioavailability, and skin penetration of the active compounds. Such approaches aim to exploit the beneficial biological properties of these molecules while simultaneously contributing to the sustainable valorization of olive oil industry by-products [66,67].

In this context, the present study aimed to explore the potential cosmetic applications of phenolic-rich fractions obtained from the OMW treatment process developed in this work. Two formulation types were selected in order to explore different cosmetic delivery systems: an antioxidant hydrogel and a phenolic serum. These systems were designed to evaluate the feasibility of incorporating OMW-derived phenolic extracts into cosmetic matrices while maintaining suitable physicochemical properties and antioxidant activity.

7.6.2. Methodological Approach Phenolic Gel & Antioxidant Serum

The preparation and characterization of the cosmetic formulations were carried out in collaboration with the Department of Pharmacy (DIFAR), University of Genoa. The experimental work was performed under the supervision of Prof. *Eleonora Russo* and Prof. *Carla Villa*, using phenolic-rich fractions obtained from the OMW treatment process described in the previous chapters.

Two different OMW samples, both derived from RO retentate, were prepared for cosmetic formulations. One underwent ED desalinization to reduce high salt content typical of RO concentrates, while the second was freeze-dried to yield a stable, phenolic-rich powder: the liquid was frozen at $-80\text{ }^{\circ}\text{C}$ and lyophilized under vacuum until complete dehydration. The obtained liquid and dry powder were stored in airtight containers protected from light and humidity until use. The target polyphenol concentration (3,4-dihydroxyphenylglycol, hydroxytyrosol and tyrosol) and antioxidant activity of the two treated samples are summarized in Table 7.5.

Table 7.5: Target polyphenol concentration and antioxidant activity of the treated OMW samples used for cosmetic formulations.

	Target polyphenol (mg/L)	Antioxidant activity (%)
Desalinated RO retentate	4800 ± 240	90
Freeze-dried RO retentate	4898 ± 245	95

DPPH (2,2-diphenyl-1-picrylhydrazyl), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 3,4-dihydroxyphenylglycol, hydroxytyrosol and tyrosol standards were purchased from Sigma-Aldrich (Milan, Italy). Propylene glycol (99.88% purity), Sodium Hyaluronate (Medium MW), and hydroxyethyl cellulose (HEC, Natrosol 250 HX PHARM, high viscosity) were purchased respectively from La Farmochimica S.r.l. (Genova, Italy), ACEF S.p.A (Piacenza, Italy), Ashland (USA). All other reagents were of analytical grade and used as received. Purified water was used throughout the study.

7.6.2.1. Preparation of OMW-Based Hydrogel

The hydrogel formulation was prepared using hydroxyethyl cellulose (HEC) as gelling agent.

Briefly, HEC (2% w/w) was gradually added to the desalinated RO retentate under continuous magnetic stirring to avoid aggregation and ensure homogeneous dispersion. The system was allowed to hydrate until complete swelling and gel formation. The final hydrogel was left to equilibrate for 24 h at room temperature before further analyses.

7.6.2.2. Preparation of OMW-Based Serum

The serum formulation consisted of a propylene glycol-based system containing OMW and hyaluronic acid as functional excipients.

A 1% (w/w) hyaluronic acid aqueous solution was prepared and allowed to fully hydrate under gentle stirring. Separately, lyophilized OMW was dispersed in propylene glycol at a concentration of 1% (w/w) until complete solubilization and homogeneous distribution were achieved. The two preparations were subsequently mixed in a 1:1 weight ratio under continuous stirring. As a result, the final concentrations of both hyaluronic acid and lyophilized OMW in the serum were 0.5% (w/w). The obtained formulation was stirred until a uniform and visually homogeneous serum was formed and was then stored in light-protected containers at room temperature until further characterization.

7.6.2.3. Physicochemical Characterization

The prepared formulations were evaluated for:

- Appearance and homogeneity (visual inspection).
- pH measurement at 25 ± 0.5 °C using a calibrated digital pH meter (Jenway 3510 Standard Digital pH Meter, UK).
- Viscosity/rheological behaviour. Rheological measurements carried out using a rotational viscometer (Vis-costar-R, FUNGILAB S.A., Sant Feliu de Llobregat, Spain) equipped with a coaxial cylinder system (bob and cup geometry) and spindle R5. All measurements were performed at 25 ± 0.5 °C. Flow behavior was evaluated by recording shear stress as a function of

shear rate over a range of 1–100 s⁻¹. The viscosity was determined from the resulting flow curves.

- (iv) Chemical composition. Target polyphenol concentration was measured using HPLC analysis and antioxidant activity using DPPH antioxidant assay [68].

Each analysis was performed in triplicate.

7.6.3. Results of OMW-Based Formulations

7.6.3.1. Organoleptic properties

Both formulations appeared homogeneous and free of visible aggregates. The hydrogel exhibited a semi-solid consistency with an amber coloration typical of phenolic-rich OMW fractions. The serum showed a slightly viscous texture with uniform yellowish coloration. No phase separation was observed immediately after preparation. Representative images of the formulations are shown in Figure 7.6. An exploratory test involved the addition of fragrances, which were successfully and homogeneously incorporated into both formulations.



Figure 7.6: Representative images of OMW-based formulations.

7.6.3.2. pH

The pH values of the hydrogel and serum were 4.45 ± 0.05 and 4.35 ± 0.05 , respectively, falling within the physiological skin compatibility range (approximately 4–5.5), suggesting good suitability for topical application without significant risk of skin irritation [69].

7.6.3.3. Rheological behavior

The hydrogel exhibited a non-Newtonian pseudoplastic behavior typical of cellulose-based gels, with viscosity (η) decreasing as shear rate increased, as shown in Figure 7.7.

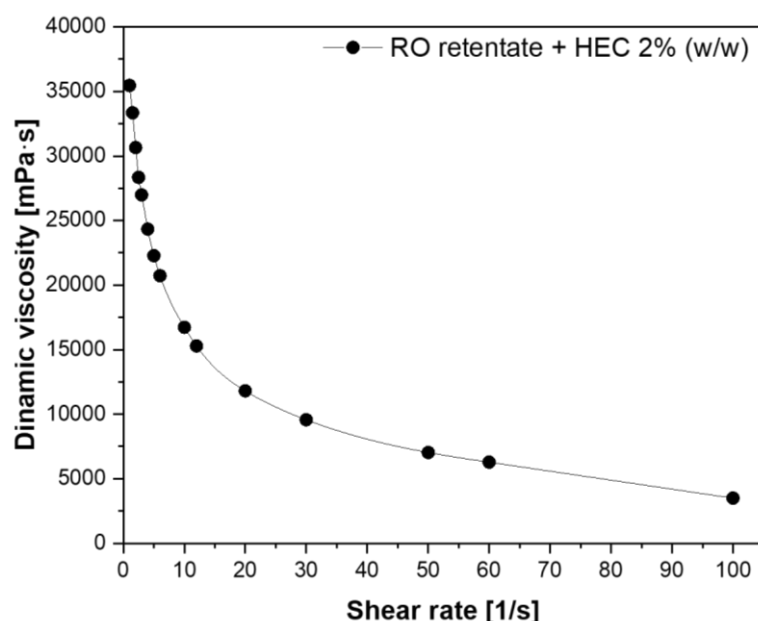


Figure 7.7: Viscosity behaviour of hydrogel formulation (RO retentate + 2% HEC) with increasing shear rate.

The serum showed lower viscosity values ($\eta = 500 \text{ mPa s}$) compared to the hydrogel, consistent with its intended cosmetic application as a lightweight and easily spreadable topical formulation. Rheological analysis demonstrated a Newtonian flow behavior, with viscosity remaining constant over the investigated shear rate range.

The incorporation of RO retentate did not significantly compromise the structural integrity of either system.

7.6.3.4. Antioxidant Activity

The antioxidant activity of both formulations, evaluated by the DPPH radical scavenging assay, was maintained after incorporation of the excipients employed in the hydrogel (hydroxyethyl cellulose) and in the serum (hyaluronic acid and propylene glycol) confirming their functional compatibility with the OMW phenolic fraction.

7.6.3.5. Discussion

The present study aimed to develop two topical formulations, a hydrogel and a serum, incorporating a highly concentrated stream of bioactive phenolic compounds obtained after the membrane processes (RO retentate). The choice of formulation strategy was based on the intended cosmetic application and on the physicochemical properties of OMW.

The hydrogel system, structured with hydroxyethyl cellulose, provided a semi-solid matrix suitable for prolonged skin contact. The pseudoplastic behavior observed is advantageous for topical administration, as it allows easy spreading under shear stress while maintaining consistency at rest.

Conversely, the serum formulation, based on propylene glycol and hyaluronic acid, was designed to enhance skin hydration and promote rapid absorption. Propylene glycol may also contribute to improved solubilization and potential penetration of phenolic compounds.

Importantly, the incorporation of RO retentate did not significantly alter the stability or rheological properties of the formulations, indicating good compatibility between the extract and the excipient matrices. The maintenance of antioxidant activity suggests that OMW retains its functional properties after formulation. Similar results have been reported for cosmetic formulations incorporating extracts derived from olive oil industry by-products [59]. For example, Nunes et al. [67] demonstrated the successful incorporation of phenolic-rich extracts into oil-in-water creams, showing stable pH, appropriate rheological behavior, and preserved antioxidant activity after formulation. These findings are consistent with the results obtained in the present study, where OMW-derived phenolic fractions were successfully incorporated into hydrogel and serum systems without compromising their physicochemical properties or antioxidant functionality. This finding supports the potential valorization of olive mill wastewater as a sustainable and cost-effective source of bioactive compounds for dermocosmetic applications.

Overall, both formulations demonstrated suitable physicochemical characteristics highlighting their potential for further dermatological evaluation through in vitro skin models and in vivo cosmetic testing.

7.7. Overall Valorization Strategy

The results presented in this chapter demonstrate that the different fractions obtained along the olive mill wastewater treatment chain can be effectively valorized through several potential applications, highlighting the versatility of these streams and their relevance within circular bioeconomy strategies.

The enzymatic lipophilization experiments showed that phenolic compounds recovered from OMW can be successfully converted into more lipophilic derivatives through CALB-catalyzed transesterification with vinyl palmitate. Model reactions performed with pure tyrosol and hydroxytyrosol confirmed the feasibility of the process, achieving conversions close to completion after 48 h and isolated yields of approximately 60% for tyrosol palmitate and 27% for hydroxytyrosol palmitate. When the reaction was applied to phenolic fractions recovered from OMW, lipophilic derivatives were also successfully obtained. Although the complexity of the matrix limited an exact quantitative evaluation, these results demonstrate that enzymatic modification can be applied directly to OMW-derived phenolic fractions, generating molecules with improved lipophilicity and potential applications as lipid-soluble antioxidants in food, cosmetic, or nutraceutical systems.

The potential use of another fraction generated during OMW treatment, namely the sludge obtained after acidification, was investigated through a feeding trial with black soldier fly larvae (*Hermetia illucens*). The results showed that increasing sludge inclusion levels negatively affected larval growth and survival. However, diets containing low inclusion levels (20%) showed larval survival comparable to the control diet, indicating that this fraction can potentially be incorporated into larval diets when properly balanced with other substrates. These findings suggest that insect-based bioconversion could represent a promising strategy for the valorization of this residual fraction,

enabling the conversion of organic waste into protein- and lipid-rich insect biomass that may subsequently be used for animal feed applications.

Phytotoxicity tests performed using *Lepidium sativum* further highlighted the concentration-dependent biological effects of OMW-derived fractions. The sludge extract showed marked phytostimulant activity at low concentrations, with germination index values exceeding 160% at 10% dilution, while inhibitory effects were observed at higher concentrations. Similarly, membrane-derived fractions displayed distinct behaviors: the nanofiltration permeate showed stimulatory effects at low concentrations but became inhibitory at higher levels, whereas reverse osmosis and diafiltration retentates exhibited strong phytotoxicity even at relatively low concentrations. These results indicate that the different fractions generated during the treatment process may have distinct agricultural applications, ranging from potential plant biostimulants at low concentrations to natural bioherbicides at higher concentrations.

The biological safety of selected membrane fractions was also evaluated through cytotoxicity assays using the human keratinocyte HaCaT cell line. The results showed that, at lower concentrations (0.005–0.02 mg GAE/mL), all tested samples exhibited cell viability values comparable to untreated controls after 24 h exposure, suggesting limited cytotoxic effects under these conditions. Although higher concentrations showed a moderate reduction in viability, the overall results indicate that diluted OMW-derived fractions may be compatible with applications involving potential human exposure, supporting their possible use in agricultural, environmental, or cosmetic formulations.

Finally, the feasibility of incorporating phenolic-rich OMW fractions into cosmetic products was explored through the development of two topical formulations: an antioxidant hydrogel and a phenolic serum. Both formulations exhibited suitable physicochemical properties, including pH values within the physiological skin range (4.35–4.45), good rheological behavior, and homogeneous appearance. Importantly, the antioxidant activity of the phenolic fraction was preserved after formulation, confirming the compatibility of the excipient matrices with the recovered bioactive compounds. These results demonstrate the potential of OMW-derived phenolic extracts as sustainable ingredients for dermocosmetic formulations.

Overall, the results obtained in this chapter highlight how the different fractions generated during the OMW treatment process can be directed toward diverse valorization pathways, including enzymatic modification of phenolic compounds, insect-based bioconversion of organic residues, agricultural applications, and cosmetic formulations. Although the experiments presented here represent preliminary investigations, they provide valuable insights into the multifunctional potential of OMW-derived streams.

Future research should focus on further optimization and scaling of these applications. In particular, the lipophilization process could be optimized to improve yields and simplify downstream purification, while the insect feeding trials could be expanded to larger-scale systems and to lower sludge inclusion levels. Similarly, phytotoxicity studies should be extended to soil-based experiments

and different plant species to better assess agronomic performance under realistic conditions. Finally, additional biological and stability studies would be required to fully evaluate the safety and efficacy of OMW-based cosmetic formulations.

Taken together, these results demonstrate that olive mill wastewater should no longer be considered solely as a problematic waste stream but rather as a valuable source of bioactive compounds and functional materials. By integrating different valorization strategies within a cascading approach, OMW treatment chains can contribute to the development of more sustainable and circular management of olive oil industry by-products.

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CHAPTER 8: TECHNO-ECONOMIC ASSESSMENT OF OMW TREATMENT AND VALORIZATION STRATEGIES

8.1. Preliminary considerations

The present analysis aims to evaluate the technical and economic feasibility of different treatment strategies for olive mill wastewater, with particular focus on their applicability within the Ligurian context. The assessment does not rely exclusively on purely economic indicators but also considers a set of operational and contextual factors that are essential for the practical implementation of treatment technologies.

In particular, the analysis takes into account several aspects such as ease of operation and plant management, which is particularly relevant for small-scale and family-run olive mills. Additional elements include process flexibility, defined as the ability to handle fluctuations in wastewater composition and flowrate, as well as the management of generated streams, including both liquid and solid fractions. Plant footprint and spatial requirements are also considered, especially in geographically constrained regions such as Liguria, together with the potential for integration within existing olive mill infrastructures or within centralized processing facilities.

Within this framework, the objective of this chapter is to identify treatment configurations that are not only technically feasible, but also operationally manageable, and economically sustainable within the structural characteristics of the regional olive oil sector.

Most treatment technologies for OMW share a common underlying objective: the separation of the wastewater into two main streams with different compositions and potential values. Typically, the treatment process results in a high-volume liquid fraction with reduced organic load, corresponding to clarified or treated water, and a low-volume concentrated fraction or sludge containing most of the organic matter and bioactive compounds originally present in the wastewater [1,2]. This separation represents a key step in enabling both environmental compliance and resource recovery. Traditionally, the concentrated fraction has been regarded as a waste stream requiring disposal. However, such an approach is increasingly incompatible with circular economy principles, since it leads to the loss of potentially valuable resources. From an alternative perspective, these streams may be valorized through several pathways [3,4]. Examples include the production of fertilizers or soil amendments, the development of biopesticides or phytosanitary products, the extraction of bioactive compounds for cosmetic, nutraceutical, or biotechnological applications, and their use as substrates for insect biomass production [5–7]. Similarly, the clarified effluent obtained after treatment may follow different pathways depending on its quality. Possible options include discharge into receiving water bodies, further treatment prior to discharge into municipal wastewater treatment plants, agricultural

reuse (for example in fertigation systems), or internal reuse within the olive mill for washing olives or cleaning processing equipment [8,9].

To estimate the economic performance of the proposed treatment configurations, several assumptions were adopted. A reference treatment capacity of 20 m³/day was considered, with an annual operating period of 150 days/year, reflecting the seasonal nature of olive oil production. Under these conditions, the total annual volume of OMW treated corresponds to approximately 3000 m³/year. The economic evaluation was carried out assuming a constant interest rate of 10% and a plant lifetime of 10 years, in line with typical techno-economic assessments for small-scale wastewater treatment systems [10].

It is important to emphasize that the present analysis should be regarded as a preliminary feasibility assessment rather than a detailed engineering design. Consequently, the results presented in this chapter should be interpreted as order-of-magnitude estimates, intended to support technology comparison and strategic decision-making. Further refinement would require pilot-scale validation, site-specific operational data, and detailed process modelling.

Finally, particular attention was given to the operational constraints of olive mills. During the milling season, operators are primarily focused on olive processing activities and have limited time to manage complex treatment systems. For this reason, technologies implemented directly at the mill level should be simple, robust, and highly automated, allowing the system to operate with minimal supervision.

8.2. Description of the developed processes

The treatment and valorization strategy investigated in this work is based on a multi-stage process aimed at progressively separating and concentrating the different components present in OMW. The overall process combines relatively simple pre-treatment operations with more advanced separation and purification techniques, enabling both wastewater stabilization and recovery of valuable compounds.

Several process units can be integrated within the proposed treatment chain, each fulfilling a specific function in the overall separation and valorization strategy.

Acidification represents a first pretreatment step aimed at destabilizing colloidal structures and promoting the separation of suspended solids through sedimentation. This step reduces the particulate load entering downstream membrane processes and may therefore contribute to limiting fouling phenomena. Microfiltration (MF) can be applied as an additional clarification step in order to remove residual suspended particles and improve feed quality prior to high-pressure membrane processes. Reverse osmosis (RO) can subsequently be used to significantly reduce the volume of the wastewater by producing a purified water stream and a concentrated retentate enriched in dissolved organic compounds.

Further fractionation of the concentrated streams can be achieved through nanofiltration combined with diafiltration (NF/DF). These processes enable a selective separation of phenolic compounds

based on molecular size and charge, allowing the production of phenol-enriched fractions with different compositions.

In order to improve the quality of the recovered streams, additional purification steps may also be considered. Demineralization of phenolic concentrates can be achieved through electrodialysis or adsorption on ion-exchange resins, which allow the removal of dissolved salts that may otherwise limit the applicability of the recovered fractions. An additional upgrading strategy investigated in this work involves the enzymatic lipophilization of phenolic compounds. Through enzymatic esterification reactions, hydrophilic phenols can be converted into lipophilic derivatives with improved solubility in lipid matrices, potentially expanding their applicability in cosmetic or nutraceutical formulations.

Within this broader framework, the streams generated during treatment should not necessarily be regarded as waste streams. Solid fractions and concentrated liquid streams may instead represent intermediate products that can be further valorized in different sectors, including agriculture (fertilizers, soil amendments or biopesticides), animal feed production, or cosmetic and nutraceutical applications.

Based on the laboratory experiments performed in this work, combined with data available in the literature, a techno-economic assessment was developed focusing primarily on the pretreatment stage and membrane-based processes. Additional considerations related to downstream valorization strategies are discussed in Chapters 5 and 6, where the selection of suitable desorption solvents for resin-based recovery of phenolic compounds and the energy requirements of electrodialysis tests were evaluated.

Although enzymatic lipophilization showed promising results at laboratory scale, the process is currently characterized by a relatively low technological readiness level. For this reason, it was not included in the core treatment scenarios considered in the techno-economic analysis, but should rather be regarded as a potential future development for increasing the value of OMW-derived phenolic compounds.

8.3. Cost Estimation Methodology

The economic evaluation of the proposed treatment and valorization strategies was performed through a preliminary techno-economic assessment, aimed at estimating the order of magnitude of the main investment and operating costs associated with the different process configurations.

Given the exploratory nature of the present work, the analysis does not aim to provide a detailed engineering cost estimation, but rather to compare alternative treatment scenarios and evaluate their potential feasibility within the context of small olive mills and centralized processing facilities.

8.3.1. Reference Plant Capacity

In order to enable comparison between different treatment configurations, a reference treatment capacity was defined, as shown in Table 8.1.

Table 8.1: Operating conditions assumed to compare the different configurations.

Parameter	Value
Treatment capacity	20 m ³ OMW/day
Operating period	150 days/year , 8 h/day
Annual treated volume	3000 m ³ OMW/year

These values reflect the seasonal nature of olive oil production, where OMW generation occurs mainly during the milling campaign. Economic performance is expressed as specific treatment cost per cubic meter of wastewater treated (€/m³).

8.3.2. Cost Categories

The total treatment cost was estimated by considering two main cost components, as reported in the Sandia National Laboratories TEA guidelines [11]:

- capital expenditure (CAPEX), corresponding to the investment required for the installation of the treatment system;
- operating expenditure (OPEX), including the annual costs associated with plant operation.

8.3.2.1. Capital Costs (CAPEX)

Capital costs include the purchase and installation of the main process equipment, such as acidification tanks, sedimentation units, membrane filtration systems, pumps, piping, auxiliary equipment and installation costs.

Since the plant investment is distributed over the lifetime of the installation, the capital cost was annualized using the capital recovery factor (CRF), according to the following equation:

$$CRF = \frac{i(1+i)^n}{(1+i)^n - 1} \quad (1)$$

where: *i* is the annual interest rate and *n* is the plant lifetime in years.

In this analysis, an interest rate of 10% and a plant lifetime of 10 years were assumed, which correspond to typical values used in techno-economic assessments for small-scale industrial systems.

The annualized capital cost was then calculated multiplying CAPEX x CRF. Finally, the capital cost per unit of treated OMW was obtained by dividing the annualized investment cost by the annual treated volume.

8.3.2.2. Operating Costs (OPEX)

Operating cost include all expenses associated with the routine operation of the treatment system. The following cost categories were considered: chemical reagents (acid consumption), electricity for pumping, mixing, and membrane processes, membrane replacement and cleaning chemicals, labour costs (plant operation and supervision), maintenance and auxiliary materials, handling and temporary storage of concentrated streams.

In the present analysis, the sludge produced during sedimentation and the concentrates generated during membrane processes were not considered as waste requiring disposal, but rather as potentially valorisable streams.

The total annual operating cost was obtained by summing all individual contributions and was subsequently expressed as specific cost per cubic meter of treated OMW (€/m³).

8.4. Economic Evaluation of Treatment Scenarios

This section provides a preliminary techno-economic evaluation of the main process steps considered in the proposed OMW treatment and valorization strategy. The objective is not to perform a detailed engineering cost analysis but rather to identify the main capital and operating cost drivers associated with the different treatment stages.

The analysis focuses on two main process units:

- acidification and sedimentation (pretreatment)
- membrane filtration (MF/NF/DF/RO)

Costs were estimated using experimental data obtained in this work, literature data, and order-of-magnitude engineering estimates commonly used in techno-economic assessments of wastewater treatment systems.

8.4.1. Acidification and Sedimentation

Based on the experimental results presented in Chapter 3, starting from 1000 L of OMW and adding 1.5 L of H₂SO₄ (98%), it is possible to obtain approximately 920 L of clarified liquid and 80 L of wet sludge, corresponding to about 30 kg of dry solids. The process configuration considered for this pretreatment stage is relatively simple and consists of the following units: acid storage and dosing system, mixing tank for acidification, sedimentation tank (lamellar settler), sludge drainage bed for solid-liquid separation, pumps and basic instrumentation.

The main operating cost associated with this pretreatment stage is the consumption of sulfuric acid, labour and electrical energy required for mixing, acid dosing and sludge extraction. Table 8.2 reports an estimate of the main operating costs associated with this pretreatment stage, expressed per cubic meter of treated OMW assuming an annual treated volume of 3000 m³/year.

Table 8.2: Main OPEX for acidification-sedimentation pre-treatment.

Item	Unit consumption	Unit cost	Cost per m ³ OMW
H ₂ SO ₄ (98%)	2.8 kg/m ³	0.50 €/kg [12]	1.40 €/m ³
Electric energy	0.45 kWh/m ³	0.20 €/kWh [13]	0.09 €/m ³
Labour*	4 h/day	20 €/h [10]	4 €/m ³

* Decrease significantly in systems with a higher degree of automation.

Capital costs were estimated using order-of-magnitude engineering estimates based on typical equipment costs for wastewater treatment systems. The cost of the sedimentation tank was estimated using the cost–capacity scaling relationship commonly known as the six-tenths rule [14,15]:

$$C = a V^b \quad (2)$$

where C represent the cost (€), V is the tank volume (m^3) and a , b are empirical coefficients. For water-containing tanks typical values of $a = 4000$ e $b = 0.6$ can be assumed [14,15]. Considering a treatment capacity of $20 \text{ m}^3/\text{day}$, corresponding to $2.5 \text{ m}^3/\text{h}$ over 8 operating hours per day, and assuming a sedimentation time of 2 h, the resulting tank volume is approximately 5 m^3 . Using the above correlation, the estimated cost of the tank is approximately 10,500 €. However, the tank represents only a portion of the total investment associated with this pretreatment stage. Additional components such as piping, lamella plates, pumps, dosing and mixing systems, instrumentation, electrical installation, and other auxiliary equipment contribute significantly to the overall installed cost. Since these items do not scale proportionally with tank volume and typically account for a substantial share of the capital expenditure in small treatment units, the total cost of the pretreatment system was estimated by applying an order-of-magnitude installation factor of 2–3 times the tank cost. Based on this approach, the total pretreatment CAPEX is approximately 26,000 €.

Suppose a plant lifetime of 10 years and an interest rate of 10%, the capital recovery factor (CRF) is 0.1627 which results in an annualized CAPEX of 4300 €/year. Considering an annual treated volume of $3000 \text{ m}^3/\text{year}$, the corresponding specific capital cost is 1.40 €/m^3 .

Overall, the estimated costs indicate that acidification–sedimentation is a relatively simple and economically accessible pretreatment step. In terms of operating costs, sulfuric acid consumption represents the main direct process cost (0.56 €/m^3), while electricity consumption remains relatively low due to the limited energy requirements of mixing and pumping operations. Neglecting labour costs, since the olive mill operator could directly manage the system during the milling season, the resulting OPEX value is comparable with those reported in the literature for coagulation–flocculation–sedimentation processes used in industrial wastewater treatment, where operating costs are largely dominated by chemical consumption and typically range between about 0.7 and 1.4 €/m^3 depending on the type and dosage of the coagulant [16].

8.4.2. Membrane Treatment

Membrane filtration represents the core treatment step of the proposed OMW valorization strategy. Following the pretreatment stage (acidification and sedimentation), membrane processes are used to remove residual suspended solids, concentrate organic compounds, and reduce the volume of wastewater requiring further treatment. In this study, all cross-flow membrane processes investigated during the experimental work are considered, including MF with ceramic and polymeric membrane, NF/DF and RO with spiral wound modules.

From an economic perspective, the capital cost of membrane processes is mainly represented by the membrane modules, to which additional costs must be added, including membrane housings, pumps,

pipings and valves, pressure and flow sensors, installation and auxiliary equipment. The membrane area required for each process was estimated from the experimental permeate flux values obtained in Chapters 3 and 4, assuming a feed flow rate of 2.5 m³/h (20m³/day per 8h/day). The membrane area was calculated according to:

$$A = \frac{Q}{J} \quad (3)$$

where A is the membrane area (m²), Q is feed flow rate (m³/h) and J is the permeate flux (m³/m²h). For NF and RO, the calculation of membrane area must also account for the recovery ratio, i.e. the fraction of feed converted into permeate (Q_p/Q_{feed}), which was in the range of 0.7-0.8.

The estimated membrane areas and the corresponding membrane costs are reported in Table 8.3, calculated from the experimental permeate flux values assuming a treatment capacity of 2.5 m³/h.

Table 8.3: Estimated membrane area and corresponding membrane module cost for the different membrane processes considered.

Membrane	Unit consumption [m ²]	Unit cost [\$/m ²]	Cost per membrane [€]
Ceramic MF	32	500-1000 [17]	13,856-27,712
Polymeric MF	85	20-200 [18]	1,472-14,722
NF/DF	100	50-200 [19]	4,330-17,320
RO	88	100-500 [19]	7,621-38,104

The comparison of membrane CAPEX highlights a trade-off between membrane unit cost and required membrane area. Ceramic MF membranes are characterized by a much higher cost per square meter, but their lower required area partly offsets this disadvantage. On the other hand, polymeric MF membranes benefit from a lower unit cost, although the larger membrane area required reduces part of the initial economic benefit. NF and RO membranes show intermediate unit costs, but their total capital cost remains strongly affected by the required membrane area.

Since membrane modules represent only a portion of the total investment, additional costs associated with auxiliary equipment were also considered. Based on typical cost distributions reported for membrane filtration systems, the total installed capital cost of the membrane unit was estimated to be approximately 2–3 times the cost of the membrane modules. The specific capital cost was then obtained by annualizing this investment using the CRF (i=10%, n=10) and dividing by the annual treated volume of OMW (3000 m³).

The main operating costs associated with membrane processes include membrane replacement and maintenance, cleaning operation, energy consumption of the feed pump and labour requirements. Membrane replacement costs were estimated based on the expected membrane lifetime according to:

$$C_{repl} = \frac{C_{mem}}{L \cdot V_{annual}} \quad (4)$$

where C_{repl} is the specific membrane replacement cost (€/m³), C_{mem} is the membrane cost (€), L is the expected membrane lifetime (years), and V_{annual} is the annual treated volume (m³/year). Typical

lifetimes of approximately 4–5 years for spiral-wound polymeric membranes, about 2 years for polymeric hollow-fiber MF membranes, and up to 10 years for ceramic MF modules were assumed.

Cleaning operations and routine maintenance were estimated as an additional fraction of membrane cost according to:

$$C_{maint} = \frac{f \cdot C_{membrane}}{V_{annual}} \quad (5)$$

where f represents the annual fraction of membrane cost associated with cleaning operations and routine maintenance. Typical values of f reported in techno-economic analyses of membrane systems range between approximately 3–7% of membrane cost per year, depending on membrane type and operating conditions.

Energy consumption was estimated based on the useful pump power required to overcome the membrane pressure drop. The pump power was calculated as [20]:

$$P = \frac{\Delta P \cdot Q}{\eta} \quad (6)$$

where P is the pump power, ΔP is the operating pressure, Q is the feed flow rate and η pump efficiency (assumed equal to 0.7).

The corresponding specific electricity cost was calculated as:

$$C_{energy} = \frac{P \cdot t_{annual} \cdot C_{el}}{V_{annual}} \quad (7)$$

where t_{annual} is the annual operating time (150 days/year and 8 h/day), C_{el} is the electricity price (0.20 €/kWh), and V_{annual} is the annual treated volume (3000 m³ OMW/year).

Labour costs were not explicitly included in the present analysis. In small olive mills the treatment unit would likely be operated by the mill owner or existing personnel during the milling season. Moreover, the expected high level of automation of the system would further decrease the labour cost, therefore no additional dedicated labour cost was assumed.

The resulting OPEX and CAPEX values for the investigated membrane systems are summarized in Table 8.4. The table reports the individual operating cost contributions (electricity consumption, membrane replacement, and cleaning/maintenance), the total specific OPEX, and the specific CAPEX expressed per cubic meter of treated wastewater (€/m³).

Table 8.4: OPEX and CAPEX, expressed as specific costs per cubic meter of treated OMW (€/m³) for the different membrane systems.

Membrane	C_{energy} [€/m ³]	C_{repl} [€/m ³]	C_{maint} [€/m ³]	OPEX [€/m ³]	CAPEX [€/m ³]
Ceramic MF ^a	0.020	0.69	0.21	0.92	2.82
Polymeric MF ^b	0.007	1.35	0.19	1.55	1.10
NF/DF ^c	0.24	0.72	0.14	1.11	1.47
RO ^d	0.16	1.52	0.30	1.99	3.10

^a L = 10, f = 0.03; ^b L = 2, f = 0.07; ^{c,d} L = 5, f = 0.04

A comparison of the results highlights the different economic profiles of the investigated membrane processes. In general, the specific capital costs remain relatively moderate for all configurations, ranging between approximately 1 and 3 €/m³ depending on the membrane type. This reflects the relatively small treatment capacity considered in this analysis and the limited investment required for the membrane modules and auxiliary equipment.

Regarding operating costs, membrane replacement and maintenance represent the main contribution to the total OPEX, while the energy cost associated with pumping remains comparatively low for MF systems and becomes more significant for NF and RO processes due to the higher operating pressures required.

The comparison between polymeric and ceramic MF membranes highlights a typical trade-off between capital and operating costs. Polymeric membranes involve a lower initial investment due to their lower unit cost, resulting in a smaller specific CAPEX. However, their shorter lifetime leads to higher replacement costs, which increases the overall operating expenditure. In contrast, ceramic membranes require a higher initial investment but benefit from longer operational lifetimes and greater resistance to fouling and aggressive cleaning conditions, resulting in lower replacement frequency. These findings are in line with Jarvis et al. [21], who emphasize that lower OPEX for ceramic is driven not only by reduced replacement frequency but also by a substantial decrease in integrity-related labor and maintenance.

For NF and RO systems, the operating costs are influenced both by membrane replacement and by the higher energy consumption required to overcome the operating pressure of these processes. In particular, RO shows the highest overall cost among the investigated membrane processes, mainly due to the combined effect of higher replacement costs and higher energy demand. This trend is consistent with previous studies on membrane treatment of olive mill wastewater, where RO is typically associated with higher operational costs compared with NF due to its higher operating pressures and energy requirements [22,23].

An additional aspect that may influence the economic performance of the membrane systems is the presence of the preliminary acidification–sedimentation step. This pretreatment removes a significant fraction of suspended solids and colloidal material from the OMW, producing a partially clarified stream prior to membrane filtration. As a consequence, the fouling tendency of the feed stream may be reduced, potentially improving membrane permeability stability and decreasing the frequency of cleaning operations. This effect could be particularly relevant for polymeric MF membranes, which are generally more sensitive to fouling and mechanical degradation than ceramic membranes.

Therefore, under the proposed treatment configuration, the effective membrane lifetime assumed in the present analysis could represent a conservative estimate, and longer operational lifetimes might be achievable in practice if the pretreatment effectively limits cake formation and pore blocking.

8.4.3. Polyphenol Recovery Performance

From a separation perspective, the investigated membrane stages play different roles in the recovery of the target phenolic compounds. MF showed very limited retention for all three compounds (3,4-dihydroxyphenilglycol, hydroxytyrosol and tyrosol), confirming that its main function is clarification rather than selective phenol recovery. NF provided a first selective fractionation step, with a marked reduction of glycol and hydroxytyrosol concentration in the permeate, while tyrosol showed a higher tendency to pass through the membrane. DF, through the addition of water and subsequent filtration, promotes the transfer of part of the retained phenolic compounds and enables the recovery of lower-molecular-weight molecules still present in the NF concentrate. In contrast, RO mainly acts as a concentration step, showing very high retention for all three target molecules and producing phenol-enriched final streams. Therefore, the membrane cascade should not be interpreted only as a wastewater treatment train, but also as a sequential recovery strategy in which each stage contributes differently to the final distribution of the target phenols.

To better evaluate the economic relevance of each membrane stage in terms of resource recovery, the treatment cost was related to the mass of recovered phenolic compounds [24]. The cost per kilogram of recovered compound was calculated according to:

$$C_{rec} = \frac{C_{treat} \cdot V_{treat}}{m_{rec}} \quad (8)$$

where C_{rec} is the cost per kilogram of recovered compound (€/kg), C_{treat} is the specific treatment cost of the membrane stage (€/m³), V_{treat} is the treated volume referred to 1 m³ of initial OMW (m³), and m_{rec} is the mass of recovered phenolic compounds in the corresponding concentrate stream (kg).

The estimated recovery costs are reported in Table 8.5, expressed as € over kg of recovered hydroxytyrosol or target polyphenols.

Table 8.5: Estimated cost of phenolic compound recovery for the investigated membrane stages.

Membrane	H recovered [€/kg]	(G+H+T) recovered [€/kg]
Ceramic MF	252	112
NF/DF	23	10.5
RO	41	18

From a techno-economic perspective, MF shows the highest cost per kilogram of recovered phenols, confirming that it should primarily be interpreted as a pretreatment step rather than a recovery process. NF and DF exhibit the lowest recovery costs, indicating that these stages play the most relevant role in the selective recovery of the target phenolic compounds. The RO stages, although associated with higher costs per kilogram recovered, mainly contribute to increasing the concentration of the phenolic streams rather than to their selective separation.

These values represent the cost associated with the recovery of the target phenolic compounds and do not include the potential market value of the recovered fractions. However, considering the growing interest in phenolic extracts for cosmetic, nutraceutical and agricultural applications, the

recovery of these compounds may contribute to partially offset the treatment costs and improve the overall sustainability of the process.

Further downstream improvement of these streams could be achieved by integrating additional purification technologies. A noteworthy option is electrodialysis, which, despite requiring a non-negligible energy input (although typically lower than RO), allows the removal of high salt concentrations while preserving a significant fraction of the phenolic compounds present in the stream.

8.5. Implementation Scenarios in the Ligurian Context

Based on the technical and economic considerations discussed in the previous sections, two possible implementation scenarios can be proposed for the management and valorization of olive mill wastewater within the Ligurian context.

The first scenario considers a relatively simple treatment configuration that could be installed directly at the olive mill level. In this case, the process would consist of an initial pretreatment step based on acidification and sedimentation, aimed at removing as much suspended solids as possible from the wastewater. This step is particularly important in order to reduce fouling potential and allow the subsequent application of a reverse osmosis (RO) stage. The RO unit would primarily act as a stabilization and concentration step, producing a phenol-rich retentate stream with a significantly reduced volume. This concentrated fraction could be stored more easily and transported to dedicated facilities where further valorization processes may take place. In principle, a microfiltration (MF) step could also be considered prior to RO to further improve feed clarification; however, the limited space typically available in Ligurian olive mills may represent a constraint for the installation of additional treatment units.

The second scenario involves the creation of a centralized treatment facility operating at a consortium level. In this case, the different membrane processes investigated in this techno-economic analysis could be integrated into a more complex treatment train, similar to the membrane cascade described in Chapters 2 and 3. Such a facility could collect OMW from several nearby olive mills and process it through a combination of MF, NF, DF and RO stages. Additional purification technologies, such as electrodialysis or ion-exchange resins, could also be implemented in order to further improve the quality of the recovered phenolic fractions.

A centralized approach could offer several advantages. First, it would reduce the operational burden on individual olive mill owners, who are often primarily focused on olive processing activities during the milling season. Second, it would allow the use of more advanced and complex treatment technologies that may be difficult to operate at small decentralized plants. Finally, the establishment of a consortium involving olive mills, research institutions and universities could promote technological innovation and facilitate the development of different valorization pathways for OMW-derived products. These may include the production of fertilizers, soil amendments, biopesticides,

animal feed ingredients, or cosmetic and nutraceutical products based on recovered phenolic compounds.

From a sustainability perspective, the proposed treatment configurations also present several advantages. The investigated membrane processes operate without the need for large quantities of organic solvents and mainly rely on physical separation mechanisms, making them relatively green technologies compared with conventional extraction processes. In addition, the recovery of phenolic compounds contributes to closing the production cycle of the olive oil sector by transforming a problematic wastewater into a potential source of valuable bioactive products. Another relevant aspect concerns the possibility of integrating renewable energy sources in the operation of the treatment system. Since the main energy demand of membrane processes is associated with pumping and pressure generation, the use of photovoltaic systems or other renewable energy sources could significantly reduce the operational energy costs. This aspect is particularly relevant in Mediterranean regions such as Liguria, where solar energy availability could support the implementation of more sustainable and energy-efficient OMW treatment systems.

Overall, these implementation scenarios highlight that the management of OMW in the Ligurian context can move beyond conventional wastewater treatment and evolve toward a resource recovery strategy. Depending on the organizational structure of the sector, both decentralized systems installed directly at olive mills and centralized consortium-based facilities may represent viable solutions. In both cases, membrane-based technologies provide a flexible framework for reducing the environmental impact of OMW while enabling the recovery of valuable phenolic compounds. Such approaches may therefore contribute to the development of more sustainable and circular management strategies within the regional olive oil production chain.

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CHAPTER 9: CONCLUSION AND FUTURE PERSPECTIVES

Olive mill wastewater represents one of the most problematic effluents generated by the agro-food sector, particularly in Mediterranean regions where olive oil production is highly concentrated and seasonal. The large volumes produced within short processing periods, together with the high organic load, phenolic content, acidity and salinity, make OMW difficult to manage using conventional wastewater treatment strategies. At the same time, this effluent contains a wide range of bioactive compounds, which have attracted increasing scientific interest due to their antioxidant, antimicrobial and functional properties. The main scientific challenge addressed in this thesis lies in reconciling these two aspects: mitigating the environmental burden associated with OMW while simultaneously enabling the recovery and valorization of its most valuable constituents. The present work addressed this challenge by developing and investigating an integrated process chain for the valorization of olive mill wastewater as a source of bio-derived active ingredients. The proposed strategy combines sequential pretreatment, membrane-based fractionation, and downstream refining operations to progressively reduce the complexity of the matrix and selectively recover phenolic-rich fractions suitable for further applications.

The acidification pretreatment proved to be a key step for reducing the complexity of the OMW matrix. The addition of sulfuric acid promoted the rapid sedimentation of suspended solids, generating a distinct sludge fraction while producing a clarified supernatant suitable for membrane processing. The sludge was then characterized through gravimetric, thermogravimetric and Soxhlet analyses, which revealed a low inorganic residue content (mainly oxides and potassium and calcium sulfates) and a high organic load mostly soluble in water (25%) and in ethanol (50%), suggesting two possible valorization pathways. Based on higher heating value measurements and kinetic studies, the sludge could potentially be exploited for energy-related applications, such as pyrolysis for biofuel production or gasification. Feeding trials performed on black soldier fly larvae demonstrated that inclusion levels up to 20% in the diet do not compromise larval growth. At the same time, acidification significantly improved the filterability of the clarified stream. This reduced the concentration of suspended solids (from 7 to 1.4 g/L) and enabled stable microfiltration. It also allowed the ceramic membrane system to achieve high volume concentration ratios (VCR up to 28). Importantly, comparable results were obtained with polymeric membranes, suggesting their possible use as lower-cost alternatives in large-scale implementations. An exploratory investigation of submerged hollow-fiber systems also provided preliminary insights into critical-flux behaviour in alternative filtration configurations.

Following pretreatment, nanofiltration was investigated as a selective fractionation step for phenolic compounds. Screening experiments conducted with recirculating flat-sheet membranes (NF90, NF270, TS40, NP010, NFX) identified loose nanofiltration membranes as the optimal solution for the selective permeation of low-molecular-weight phenolic compounds. These membranes were

subsequently implemented in spiral wound configuration, confirming their ability to separate phenolic compounds according to their molecular size, with low- MW phenolics preferentially enriched in the permeate (tyrosol- rejection below 20%), while larger and more structurally complex compounds remained in the retentate. A diafiltration step further enhanced this molecular weight- -based separation, producing a dark concentrate whose composition, although only partially resolved in this study, shows promise for future valorization. A subsequent reverse osmosis operation, performed on the NF permeate, allowed treatment of a stream already partially purified, thereby improving overall concentration efficiency and achieving a VCR of 17—significantly higher than traditional values typically reported for raw OMW. Overall, starting from a raw effluent containing 0.64 g/L of total target phenolics, the integrated membrane cascade concentrated these compounds up to 4.90 g/L in the RO(NF) retentate, corresponding a concentration factor of approximately 7.7.

To further improve the quality and usability of the recovered fractions, two desalination strategies were investigated. The first approach involved the use of anion exchange resins (Amberlyst A26). Initial batch experiments highlighted the strong influence of resin dosage on adsorption performance and, together with additional studies involving the adjustment of the feed pH (NF permeate) through NaOH addition, allowed the identification of the optimal conditions for scaling up the process to a packed-bed column configuration. The adsorption-desorption process enabled the recovery of a significant portion of phenolic compounds (up to 65% of target polyphenols) while simultaneously removing a substantial fraction of dissolved salts. Importantly, the study demonstrated that the recovery steps (desorption) can be performed without the use of organic solvents such as methanol, improving the environmental sustainability and potential economic feasibility of the process. Electrodialysis was investigated as an alternative desalination strategy. This study represents one of the first explorations of electrodialysis applied specifically to OMW-derived fractions. Different pairs of ion-exchange membranes were tested on streams previously processed by nanofiltration in order to evaluate salt removal efficiency and phenolic retention. The experiments demonstrated that standard heterogeneous ion-exchange membranes can significantly reduce the electrical conductivity of the streams while maintaining most of the phenolic content. Preliminary investigations also included energy consumption estimations and the operation of a semi-continuous configuration in order to simulate scale-up conditions and to assess early fouling phenomena within the electrodialysis stack. In particular, the influence of flow rate on polyphenol losses and salt removal rates was investigated. Higher flow rates reduced the contact times between neutral polyphenols and the membrane interface while simultaneously decreased the thickness of the diffusion boundary layer due to continuous renewal of the solution, thus facilitating salt transport.

In addition to separation processes, the study also explored the chemical modification of recovered phenolic compounds through enzymatic lipophilization with CAL B. Preliminary tests conducted on model compounds such as tyrosol and hydroxytyrosol showed high selectivity toward ester formation (90–100%) with relatively moderate conversion yields (60–27%). When applied to real OMW-derived phenolic mixtures obtained after resin treatment and lyophilization, the reaction allowed the

isolation of palmitate esters of phenolic compounds in solid form. These lipophilized derivatives were identified by NMR and quantified by HPLC calibration curves, obtaining interesting results with moderate apparent yields (23–35%). These products represent promising candidates for future applications in lipid-based formulations. In addition to the feeding trials performed on the sludge for insect diets, the sludge extract, the NF permeate, and the RO and DF concentrates were also tested for phytotoxicity using *Lepidium sativum* seeds germination assays. The liquid extracted from the sludge showed strong phytostimulant activity even at relatively high concentrations (25% v/v), followed by the NF permeate (which became phytotoxic above 5%), while the two concentrates exhibited strong herbicidal activity. Cytotoxicity tests performed on human keratinocyte model cells (HaCaT) indicated that selected fractions remain non-toxic within specific concentration ranges (0.005–0.02 mg GAE/mL), suggesting their potential safe use in agricultural formulations and topical products. Finally, selected phenolic-rich concentrates were incorporated into preliminary cosmetic formulations. Two prototype products were developed: an antioxidant hydrogel obtained by incorporating RO concentrate treated by electrodialysis into a hydroxyethyl cellulose (2%) matrix, resulting in a viscous system with a non-Newtonian pseudoplastic behavior, and a serum formulation obtained from lyophilized RO concentrate combined with hyaluronic acid and propylene glycol, exhibiting a Newtonian rheological behavior. Both formulations maintained acceptable pH values and preserved the antioxidant activity of the starting phenolic extracts.

These experimental results were further supported by a preliminary techno-economic assessment aimed at evaluating possible implementation strategies for the proposed valorization chain. Two potential scenarios were identified: a decentralized treatment units located directly at olive mills, focusing on simplified pretreatment operations (acidification and sedimentation) and stabilization steps (a reverse osmosis plant, possibly preceded by microfiltration), and a centralized processing area capable of carry out the full cascade of membrane and refining operations in order to produce saleable bio-based products.

9.1 Future perspectives

The results obtained in this work open several research directions aimed at further improving the technological maturity and scientific understanding of the proposed OMW valorization strategy.

A first direction concerns the further characterization and valorization of the sludge fraction generated during the acidification pretreatment. Although preliminary analyses provided useful information on its organic and inorganic composition, additional investigations should be performed to better characterize its mineral fraction. In particular, X-ray fluorescence (XRF) analyses could be used to identify and quantify the inorganic salts present in the sludge. In parallel, feeding trials with *Hermetia illucens* larvae should be expanded by testing lower inclusion levels (1–10%) and larger rearing scales in order to better evaluate the scalability of this valorization pathway.

Further work is required to optimize the membrane pretreatment step, particularly regarding the use of polymeric microfiltration membranes. Longer-term filtration experiments and a larger number of

tests below the critical flux should be carried out for both cross-flow and submerged hollow-fiber configurations in order to better evaluate membrane stability, fouling behavior, and sustainable operating conditions. Another important perspective concerns the improved chemical characterization of the fractions obtained through the NF–DF–RO membrane cascade. While the present study mainly relied on HPLC quantification of selected polyphenols, additional analytical techniques should be applied to identify the broader range of compounds present in these streams, particularly the dark-colored concentrates obtained after diafiltration.

With regard to downstream refining, the adsorption–desorption process based on ion-exchange resins should be further investigated through multiple adsorption–desorption cycles to assess resin stability and regeneration efficiency, to evaluate alternative resin materials, investigate different feed streams, and enable scale-up to larger processing volumes. Similarly, the exploratory work on electrodialysis should be expanded to include more complex matrices and additional operating conditions in order to better understand salt removal efficiency, phenolic retention, and fouling phenomena. Particular attention should be devoted to the physicochemical and electrochemical characterization of fouled membranes in order to distinguish between organic fouling and inorganic scaling and to assess their impact on long-term membrane stability and process performance.

Another promising research direction concerns the enzymatic lipophilization of OMW-derived phenolic compounds. Further investigations should focus on processing larger quantities of material and on testing additional feed streams such as reverse osmosis concentrates. This would help evaluate the potential scalability of the lipophilization process and expand the range of modified phenolic derivatives available for formulation studies. Finally, the application-oriented investigations performed in this work should be further validated through agronomic trials, larger-scale insect feeding experiments, and more comprehensive dermatological compatibility tests for cosmetic formulations. From a process engineering perspective, the preliminary techno-economic assessment could also be expanded to include the other technologies investigated in this thesis, together with complementary analyses such as life cycle assessment (LCA) and life cycle costing (LCC).

Overall, the results and perspectives presented in this work contribute to strengthening both the scientific understanding and the technological readiness of integrated OMW biorefinery systems, supporting the transition from laboratory-scale demonstration toward practical industrial implementation.

CHAPTER 10: SUPPORTING MATERIAL

HPLC ANALYSIS CONDITIONS – standard target polyphenols

Synergi Hydro-RP(2) Column 150x3 mm, 4 micron + pre-column

Injection volume: 5 μ L

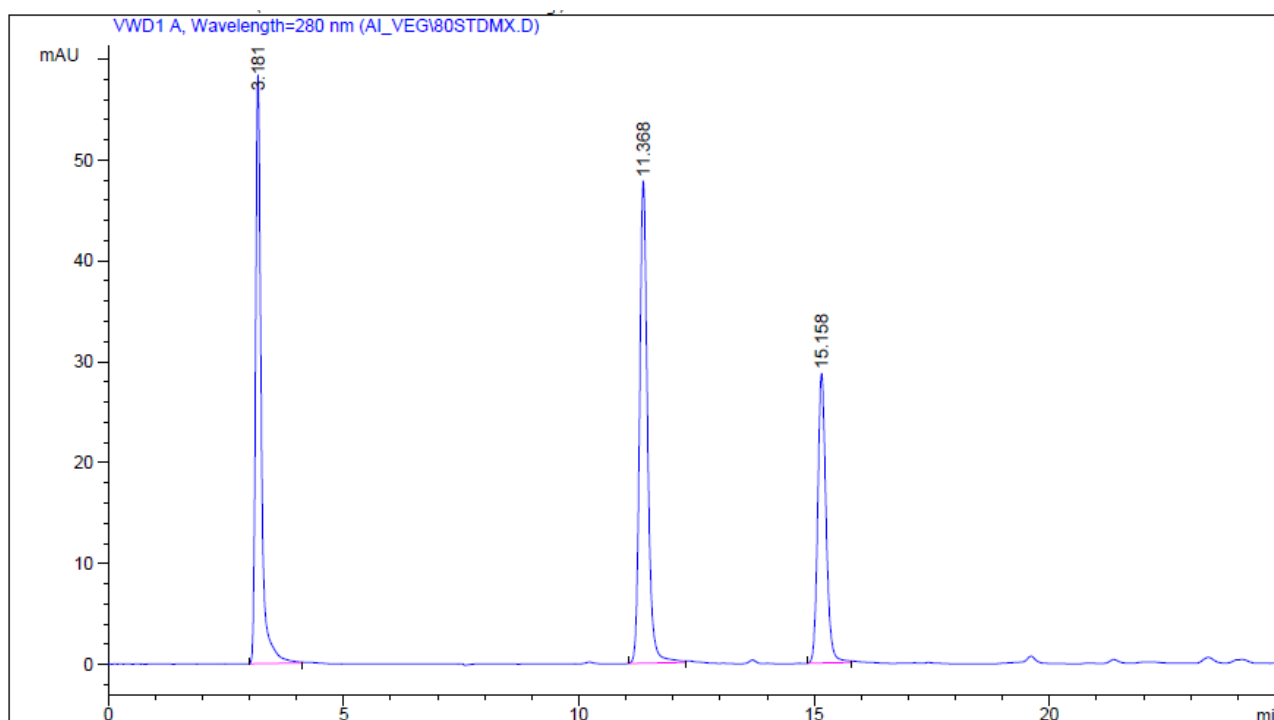
Temperature of the analyses: 23-25 $^{\circ}$ C

Wavelength: 280 nm;

Eluent gradient: A=H₂O-TFA 0.1%; B=ACN, 0-3'-B=0%, 3-26'-B=17%, 26-35'-B=100%

Dilution solvent: H₂O-TFA 0.1%

Target Polyphenols Chromatogram

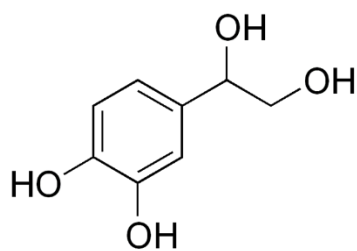


Legenda:

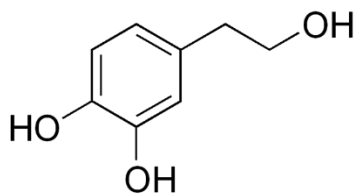
3,4-dihydroxyphenylglycol: 3.181 min

Hydroxytyrosol: 11.368 min

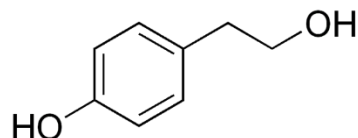
Tyrosol: 15.158 min



3,4-dihydroxyphenylglycol

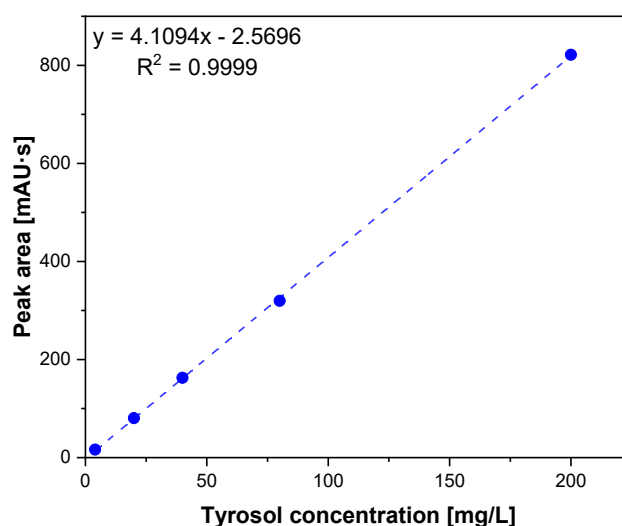
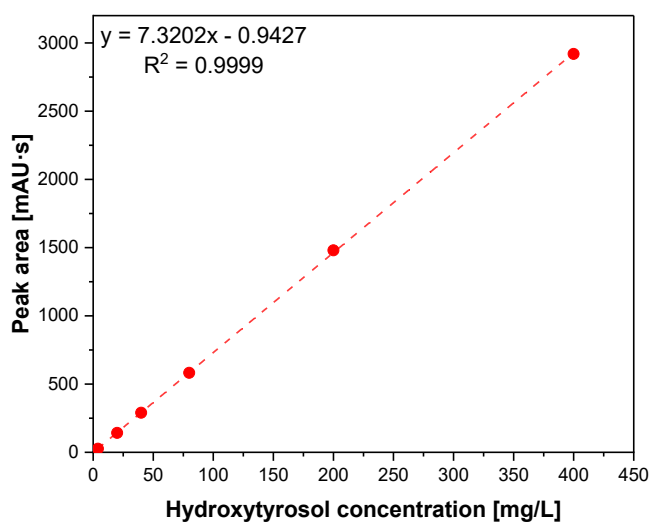
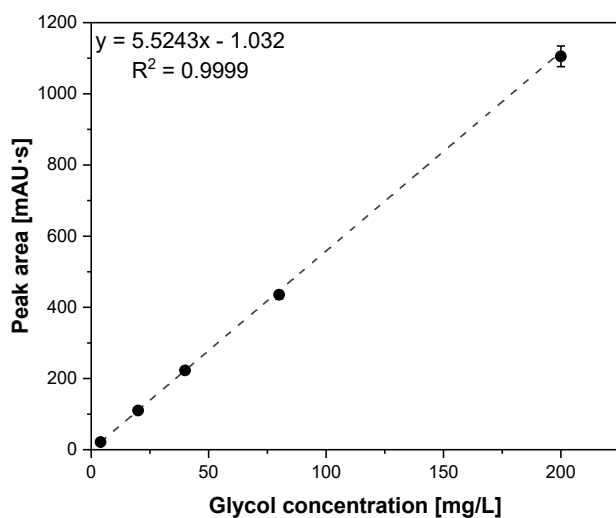


Hydroxytyrosol



Tyrosol

Calibration curves



Polyphenol target	Equation	sy/x	Mean RSD %
Glycol	$y = 5.5246x - 1.032$	7.1	5
Hydroxytyrosol	$y = 7.3202x - 0.9427$	4.2	2.5
Tyrosol	$y = 4.1094x - 2.5696$	3.8	3

sy/x: standard error of regression

$$\frac{sy}{x} = \sqrt{\frac{\sum (y_i - \hat{y}_i)^2}{n - 2}}$$

(distance from regression line, $n = 5/6$)
 y_i = observed peak area, $\hat{y}_i$ = predicted peak area
 n = number of concentration points

RSD % mean: instrument precision

$$RSD\% = \frac{1}{n} \sum \left(\frac{SD_i}{\bar{y}_i} \times 100 \right)$$

(replicate variability, 2 replicates per point)
 SD_i = std. deviation of replicates,
 $\bar{y}_i$ = mean of replicates at point i

Concentrations of each polyphenol in OMW samples were determined from the corresponding calibration curves and reported as value $\pm$ SD, where $SD = \text{concentration} \times \text{mean RSD\%}$ of the calibration curve.

HPLC ANALYSIS CONDITIONS – acylated target polyphenols

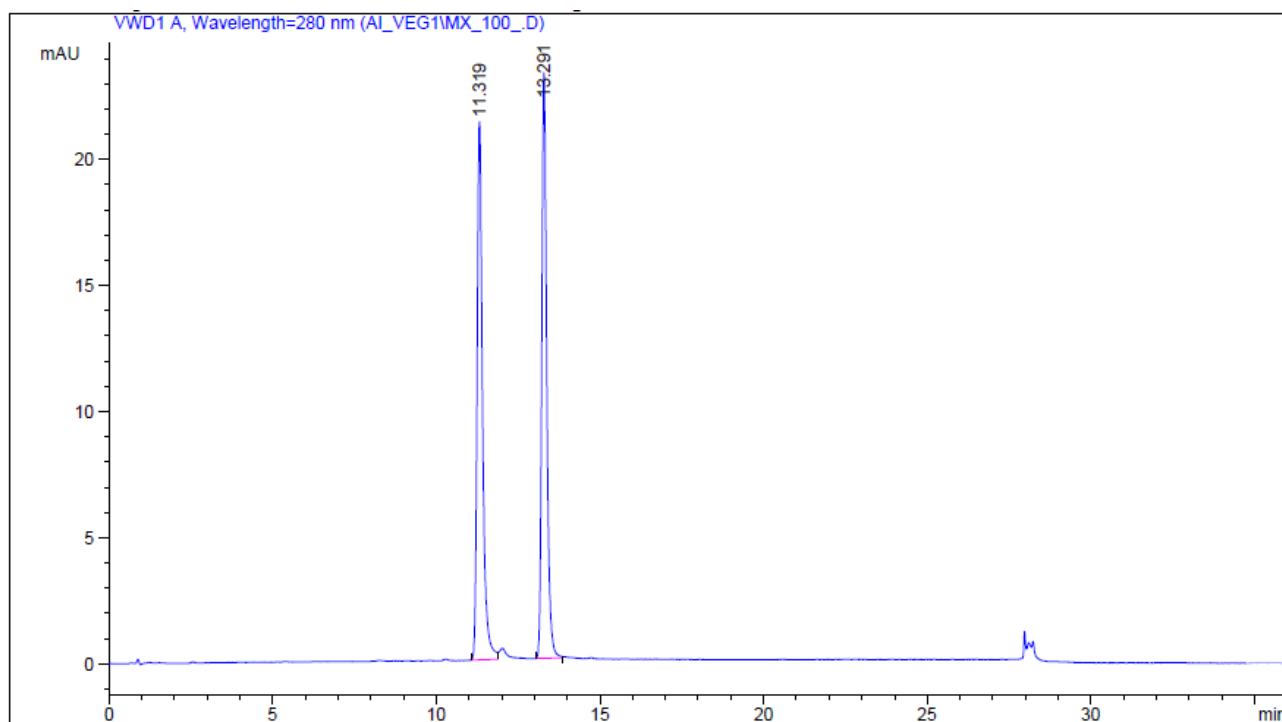
Synergi Hydro-RP(2) Column 150x3 mm, 4 micron + pre-column

Injection volume: 5 μ L

Temperature of the analyses: 23-25 $^{\circ}$ C

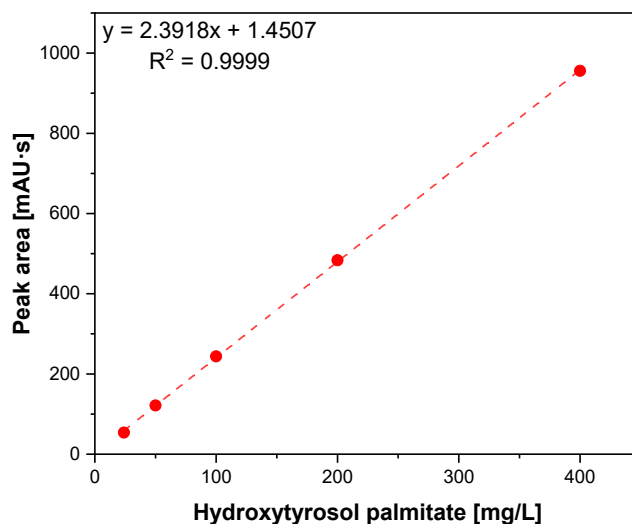
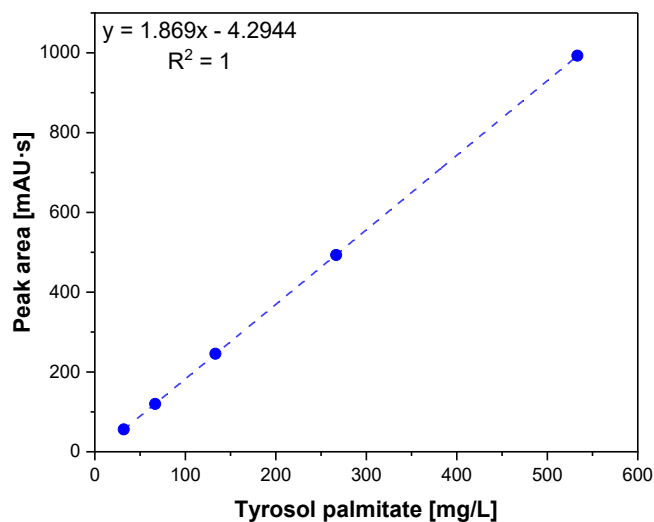
Wavelength: 280 nm;

Eluent gradient: A=H₂O; B=ACN, 0'-B=70%, 15-25'-B=100%, 25-26-B=70% 35'-B=100%

Acylated Polyphenols Chromatogram**Legenda:**

Tyrosol Palmitate: 11.319 min

Hydroxytyrosol Palmitate: 13.291 min

Calibration curves

Polyphenol target	Equation	sy/x	RSD% regression
Tyrosol palmitate	$y = 1.869x - 4.2944$	5.7	1.5
Hydroxytyrosol palmitate	$y = 2.3918x + 1.4507$	4.8	1.3

$\text{RSD\% regression} = \text{sy/x} / \text{mean calibration area}$

Concentrations of each palmitate polyphenol in OMW samples were determined from the corresponding calibration curves and reported as value $\pm$ SD, where SD = concentration $\times$ RSD% regression of the calibration curve.

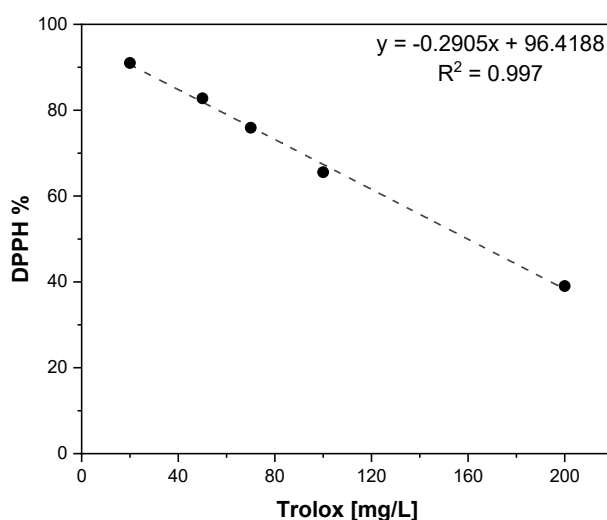
Nuclear Magnetic Resonance (NMR)

NMR spectra were taken in CDCl_3 at 400 MHz (^1H), and 100 MHz (^{13}C), using, as internal standard, TMS (^1H NMR: 0.000 ppm) or the central peak of CDCl_3 (^{13}C : 77.02 ppm). Chemical shifts are reported in ppm (delta scale).

DPPH Radical-Scavenging Activity

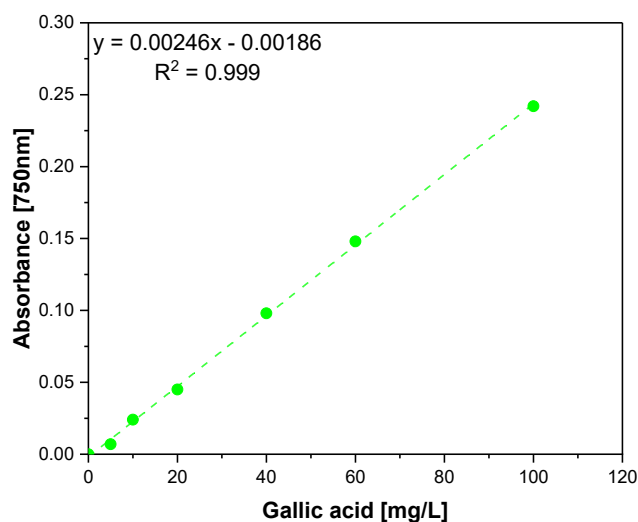
The antioxidant activity of the OMW was measured using the DPPH antioxidant assay. The DPPH assay is based on the bleaching rate of the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). A total of 0.1 mL of OMW was mixed with 3.9 mL of DPPH methanol solution (65 μM). Absorbance was measured at 517 nm after reacting for 30 min in the dark. The linear calibration curve was obtained using Trolox standards (ranging between 20 and 200 mg/L, $R^2 = 0.997$). The result was calculated as Trolox equivalent in mg/L, and the percentage of antioxidant activity (AA%) was calculated from the ratio of decreasing absorbance of sample solution ($A_0 - A_s$) to absorbance of blank DPPH solution (A_0), as follow:

$$\text{AA\%} = \frac{A_0 - A_s}{A_0} \times 100$$



Folin–Ciocalteu Spectrophotometric Determination

The total polyphenol (TP) contents in OMW were determined using the Folin–Ciocalteu (FC) spectrophotometric method. Absorbance was measured at 750 nm. TPs were quantified from a calibration curve prepared with gallic acid standard solutions in concentrations ranging from 5 to 100 mg/L ($R^2 = 0.999$) and expressed as g of gallic acid equivalent for OMW liter (g GAE/L).



UV-absorbing compound 280nm Spectrophotometric Determination

The concentration of UV-absorbing compounds was estimated from absorbance measurements at 280 nm and expressed as gallic acid equivalents (GAE). Calibration curves with gallic acid (5–100 mg/L) yielded R^2 values of 0.999.

