

UNIVERSITÀ DELLA CALABRIA



UNIVERSITA' DELLA CALABRIA

Dipartimento di Fisica

Dottorato di Ricerca in

Scienze e Tecnologie Fisiche, Chimiche e dei Materiali
Convenzione Università della Calabria-Consiglio Nazionale delle Ricerche

CICLO

XXIX

TITOLO TESI

DEVELOPMENT OF INTEGRATED MEMBRANE SYSTEMS FOR THE
TREATMENT OF OLIVE MILL WASTEWATER AND VALORIZATION OF HIGH-
ADDED VALUE BIOPRODUCTS

Settore Scientifico Disciplinare CHIM/03 - Chimica Generale e Inorganica

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SOMMARIO

Oggigiorno, è noto che tecnologie avanzate, pulite, in grado di lavorare in condizioni operative blande e a basso consumo energetico sono richieste per affrontare nuove sfide in materia di tutela dell'ambiente, uso razionale dell'acqua e produzione di composti bioattivi stabili di origine naturale. In questo contesto, la tecnologia a membrana consente di soddisfare questi requisiti. Tuttavia continui studi si rendono necessari per mettere a punto nuovi materiali e processi per specifiche applicazioni. Il trattamento delle acque reflue provenienti dalla produzione dell'olio d'oliva è tra le criticità del settore agro-alimentare. Il presente lavoro ha promosso avanzamenti nello sviluppo di sistemi a membrana innovativi per il trattamento delle acque di vegetazione. Questo tipo di refluo rappresenta un grave problema ambientale a causa dell'elevato carico organico e per le proprietà fitotossiche e antibatteriche dei composti fenolici, i quali resistono alla degradazione biologica. In aggiunta, la stagionalità e i grandi volumi prodotti in un breve arco di tempo, aumentano l'importanza per lo smaltimento di questo tipo di refluo. D'altra parte, le acque di vegetazione rappresentano un'importante risorsa di polifenoli per le proprietà benefiche per la salute, pertanto i composti fenolici possono essere valorizzati e utilizzati per scopi medici o agro-alimentare. Inoltre, essi rappresentano anche nuova formulazione a basso impatto ambientale per la produzione chimica. Lo sviluppo di nuove strategie per lo smaltimento di questi sottoprodotti appare estremamente utile dal punto di vista ambientale ed economico. Una soluzione vantaggiosa è quella di trasformare quello che fino a oggi è considerato "spazzatura da smaltire" in risorsa da sfruttare e da cui trarre profitto, attraverso il recupero di prodotti naturali ad alto valore aggiunto (bioprodotti) e acqua. In tale prospettiva, i sistemi integrati a membrana consentono il recupero selettivo di composti bioattivi, come i polifenoli così come la purificazione delle acque di vegetazione. La tecnologia a membrana è considerata un valido strumento per lo sviluppo industriale sostenibile, essendo in grado di rispondere bene all'obiettivo di un "processo intensificato" in termini di riduzione delle dimensioni dell'impianto, aumento dell'efficienza del processo e riduzione del consumo energetico. Tuttavia, un inconveniente delle operazioni a membrana per il trattamento delle acque di vegetazione, è legato allo sporco delle membrane, il quale riduce drasticamente l'efficienza del processo. Pertanto, uno stadio di pretrattamento delle acque di vegetazione a monte del processo a membrana si rende necessario al fine di limitare i fenomeni di sporco e quindi di aumentare l'efficienza

del processo. In questa tesi, è stato condotto uno studio mirato al recupero dei biofenoli e alla depurazione delle acque di vegetazione mediante un processo integrato a membrana. Inizialmente, l'attività di ricerca è stata focalizzata sul pretrattamento delle acque di vegetazione al fine di ridurre il contenuto dei solidi sospesi. A tale scopo, è stata identificata una nuova strategia per il pretrattamento delle acque di vegetazione, la quale ha consentito una totale eliminazione dei solidi sospesi, attraverso l'aggregazione e flocculazione delle particelle, mantenendo il pH delle acque di vegetazione al punto isoelettrico. In secondo luogo, la ricerca è stata focalizzata sullo studio delle potenzialità di operazioni di microfiltrazione e ultrafiltrazione su scala di laboratorio per il trattamento delle acque di vegetazione. A tale scopo, sono state utilizzate membrane di diversi materiali, organici e inorganici. Le prestazioni delle varie membrane sono state valutate in termini di flusso di permeazione, reiezione dei composti fenolici e riduzione percentuale del carbonio organico totale. Successivamente in base ai risultati del suddetto studio, è stato sviluppato un processo integrato a membrana mirato al recupero, alla concentrazione dei composti fenolici e inoltre alla formulazione di emulsioni contenenti biofenoli, nonché alla purificazione delle acque di vegetazione. A tale scopo, sono state investigate operazioni a membrana convenzionali quali microfiltrazione (MF) e nanofiltrazione (NF) e un'operazione relativamente nuova rappresentata dalla distillazione osmotica (OD) in scala prototipale per concentrare e ottenere frazioni arricchite in polifenoli; in aggiunta il processo di emulsificazione a membrana (ME) è stato studiato per l'incapsulamento delle frazioni concentrate. Per lo stadio di microfiltrazione, è stata valutata l'efficienza del controlavaggio con aria con lo scopo di mantenere costante il flusso del permeato durante il processamento delle acque di vegetazione. Nel complesso, il sistema integrato a membrana ha permesso la produzione di una frazione arricchita di polifenoli e una frazione acquosa che può essere utilizzata per l'irrigazione o per il lavaggio delle membrane. La frazione fenolica altamente concentrata prodotta mediante distillazione osmotica è stata usata come ingrediente funzionale per la formulazione di emulsioni acqua-in-olio (W/O) mediante emulsificazione a membrana. Il processo di emulsificazione a membrana a flusso pulsato è stato selezionato e studiato per l'incapsulamento dei composti fenolici in emulsioni, allo scopo di ottenere particelle uniformi e altamente concentrate in condizioni di basso stress meccanico. Lo studio ha riguardato, l'ottimizzazione della formulazione e dei parametri operativi di processo (pressione transmembrana, stress meccanico) per la produzione di emulsioni acqua-in-olio monodisperse e con un'elevata efficienza di incapsulamento della miscela dei composti fenolici. In aggiunta, nel presente lavoro è stata

studiata una nuova procedura per raggiungere un'alta efficienza di incapsulamento dei composti fenolici all'interno di particelle lipidiche solide, preparate con un metodo tradizionale (rotore-statore omogeneizzatore) e mediante la tecnica di emulsificazione a membrana. Sulla base dei risultati ottenuti con il prototipo su scala laboratorio per il trattamento delle acque di vegetazione, è stato realizzato un sistema integrato a membrana su scala produttiva presso un frantoio. L'impianto sviluppato prevede uno stadio di pre-filtrazione, operazioni di microfiltrazione, nanofiltrazione e un ulteriore stadio costituito dall'operazione di osmosi inversa. Questo stadio è stato utilizzato su scala produttiva invece dell'operazione di distillazione osmotica, in virtù del maggiore livello di maturità della tecnologia dell'osmosi inversa rispetto alla distillazione osmotica a membrana. Complessivamente, l'impianto su scala produttiva si è dimostrato efficiente per il completo recupero dei composti fenolici nel retentato e inoltre per il recupero di acqua purificata.

OBIETTIVI DELLA RICERCA

L'obiettivo principale del presente lavoro è stato quello di investigare e avanzare le potenzialità di un sistema integrato a membrana per il trattamento sostenibile delle acque di vegetazione. In particolare, questo lavoro di tesi è stato focalizzato alla valorizzazione delle acque di vegetazione attraverso il recupero e l'uso di composti fenolici riducendo allo stesso tempo la tossicità complessiva delle acque di vegetazione. Specifici obiettivi mirati a raggiungere questo scopo sono:

- Pre-trattamento delle acque di vegetazione per ridurre l'elevato contenuto di solidi sospesi e di conseguenza limitare i fenomeni di sporcamento durante il processo a membrana;
- Selezione dei materiali di membrana con le migliori prestazioni in termini di flusso di permeato e selettività verso i composti fenolici;
- Miglioramento del flusso allo stato stazionario durante il processamento delle acque di vegetazione mediante l'operazione di microfiltrazione;
- Sviluppo di un prototipo di un sistema integrato a membrana su scala laboratorio;
- Sviluppo di un sistema integrato a membrana su scala produttiva finalizzato al recupero di composti fenolici e alla depurazione delle acque di vegetazione;
- Studio del processo di emulsificazione a membrana per l'incapsulamento di polifenoli all'interno di emulsioni acqua-in-olio (W/O) e in particelle lipidiche solide con una distribuzione uniforme delle particelle.

CONTENUTI DELLA TESI

La tesi è organizzata in quattro capitoli. Il primo capitolo ha riguardato l'analisi dello stato dell'arte nella valorizzazione e trattamento delle acque di vegetazione e inoltre comprende una breve introduzione sulla composizione delle acque di vegetazione. La parte principale di questo capitolo è stata incentrata sulle attuali strategie utilizzate per il trattamento delle acque di vegetazione mediante la tecnologia a membrana evidenziando le limitazioni che riguardano questa tecnologia e le relative strategie messe in atto per superarle. Inoltre, è stata illustrata una panoramica delle formulazioni impiegate per la valorizzazione delle acque di vegetazione per il settore nutraceutico, cosmetico e farmaceutico (**Capitolo 1**). I capitoli successivi riguardano l'attività di ricerca sperimentale, come di seguito riportato:

- Studio di un metodo di pretrattamento delle acque di vegetazione. Studio dell'influenza dei materiali e della configurazione dei moduli a membrana (tubulare e spiralata) sulle prestazioni delle operazioni a membrana (microfiltrazione e ultrafiltrazione). Queste sono state valutate in termini di flusso di permeato, reiezione ai polifenoli e riduzione del carbonio organico totale (**Capitolo 2**) [1];
- Sulla base dei risultati illustrati nel capitolo 2, è stato progettato e realizzato un prototipo di un sistema integrato a membrana su scala laboratorio e successivamente su scala produttiva presso un frantoio.
Il sistema integrato a membrana è stato sviluppato per il recupero e la concentrazione dei composti fenolici e inoltre per la formulazione di emulsioni contenenti biofenoli.
Studio dell'efficienza del controlavaggio con aria compressa al fine di limitare lo sporcamento delle membrane e quindi di incrementare le prestazioni del processo di microfiltrazione.
Per quanto riguarda le membrane per nanofiltrazione, è stata studiata l'influenza dei materiali, configurazione, taglio molecolare o dimensione dei pori sul flusso di permeato, reiezione ai composti fenolici, riduzione del carbonio organico totale e sull'indice di sporcamento delle membrane.
Sviluppo di un sistema integrato a membrana su scala produttiva impiegando uno stadio di pre-filtrazione e operazioni di microfiltrazione, nanofiltrazione e osmosi

inversa per il recupero dei composti fenolici e la purificazione delle acque di vegetazione (**Capitolo 3**) [2];

- Formulazione di emulsioni acqua-in-olio e particelle lipidiche solide per l'incapsulamento di composti fenolici mediante emulsificazione a membrana. Studio dell'influenza del contenuto di fase dispersa e dei parametri di processo e chimici sulla dimensione e distribuzione delle particelle. Studio del rilascio dei biofenoli da emulsioni acqua-in-olio (W/O).

Studio dell'influenza di due metodi di emulsificazione per la produzione di particelle lipidiche solide in termini di dimensione e distribuzione delle particelle ed efficienza di incapsulamento di biofenoli (**Capitolo 4**) [3].

Note e referenze

[1] Una parte di questo capitolo è stato pubblicato: Bazzarelli F., Poerio T., Mazzei R., D'Agostino N., Giorno L., "Study of OMWWs suspended solids destabilization to improve membrane processes performance", *Separation and Purification Technology*, 149 (2015) 183-189.

[2] Una parte di questo capitolo è stato pubblicato: Bazzarelli F., Piacentini E., Poerio T., Mazzei R., Cassano A., Giorno L., "Advances in membrane operations for water purification and biophenols recovery/valorization from OMWWs", *Journal of Membrane Science*. 1 (2016) 402-409.

[3] Una parte di questo capitolo è stato pubblicato: Piacentini E., Poerio T., Bazzarelli F., Giorno L., "Microencapsulation by Membrane Emulsification of Biophenols Recovered from Olive Mill Wastewaters", *Membranes*, (2016) 6, 25.

SUMMARY

Nowadays, it is well recognized that advanced clean technologies, able to work in mild conditions and with low energy input are necessary to face challenges in environment protection, rational use of water, production of naturally derived stable bioactive compounds. Membrane technologies fulfill these requirements. Studies are necessary to tune materials and processes for specific applications. The treatment of wastewaters coming from olive oil production is among the critical issues in agro-food industry. The present work promoted advances in the development of novel membrane systems for the treatment of olive mill wastewater (OMWW). These waters represent a severe environmental problem due to their high organic load and phytotoxic and antibacterial phenolic compounds, which resist to biological degradation. Additionally, the large volume of OMWW produced in combination with the short discarding time, increases the importance for disposal of this waste. On the other hand, OMWW represents a significant source of polyphenols for health benefits, which can be revalorized and used for medical or agro-alimentary purposes. They also represent novel environmentally friendly formulation for chemical manufacturing.

The development of new strategies for the disposal of these by-products appears to be extremely useful from an environmental and economic point of view. An advantageous solution is to transform what until now was considered *junk* to be disposed of in *resource* to be exploited and from which to draw profit, through the recovery of high-added value natural products (bioproducts) and water. In this context, integrated membrane systems can permit the selective recovery of bioactive compounds, such as polyphenols as well as water recovering and purification. Moreover, membrane technology is considered a powerful tool for the sustainable industrial development, being able to well respond to the goal of the “process intensification strategy” in terms of reduction of the plant size, increase of the plant efficiency, reduction of energy consumption and environmental impact. Nevertheless, one drawback of membrane filtration of OMWW is the membrane fouling that drastically reduces the process performance. Therefore, OMWW pretreatment upstream of membrane process is necessary to limit fouling phenomena and to increase filtration efficiency. In this thesis, a comprehensive study from OMWW treatment to biophenols recovery and valorization and water purification by means of integrated membrane process was carried out. Initially, studies focused on the decrease the fouling phenomena. For this purpose, a

novel strategy for a suitable pretreatment of OMWW was identified that permitted to obtain the total removal of suspended solids, through the aggregation and flocculation of particles by maintaining the pH of OMWW at isoelectric point. Secondly, the research focused on the assessment of the potentiality of OMWW treatment by microfiltration and ultrafiltration process at the laboratory scale. Different organic and inorganic membrane materials were investigated, evaluating the permeation flux and the performance in terms of TOC (Total organic carbon) and polyphenols rejection. Afterwards, processes for OMWW purification aimed at obtaining of biologically active fractions at high concentration as well as their encapsulation were developed. For this purpose pressure-driven membrane processes such as microfiltration (MF) and nanofiltration (NF) and a relatively new membrane operation such as osmotic distillation (OD) were developed on lab scale prototype to obtain and concentrate fractions; membrane emulsification (ME) was studied for the encapsulation of concentrated fractions.

For MF operation, the efficiency of an air-backflushing cycle was evaluated to keep constant the permeate flux during the OMWW processing. The overall integrated membrane system produced an enriched fraction of polyphenols, as well as a water stream that can be reused for irrigation or membrane cleaning. The highly concentrated polyphenols produced by osmotic distillation, is used as functional ingredients for formulation of water-in-oil (W/O) emulsions by membrane emulsification.

The pulsed back-and-forward ME has been selected as low shear encapsulation method because it is particularly attractive for the production highly concentrated microemulsions without causing coalescence. The best operative conditions (transmembrane pressure, wall shear stress) to control particle size and size distribution and obtain high productivity (dispersed phase flux) have been investigated. Water-in-oil emulsions with a narrow size distribution and high encapsulation efficiency were obtained.

Furthermore, in the present work a novel procedure for encapsulation of olive polyphenols with high load into solid lipid particles using traditional method (rotor-stator homogenizer) and membrane emulsification was studied. Finally, a productive scale plant of the integrated membrane system was developed and installed at olive mill. The plant included the pre-filtration unit, microfiltration, nanofiltration and a further step represented by reverse osmosis.

The reverse osmosis has been used at large scale instead of membrane distillation due to its higher technology readiness level.

Overall, this productive scale plant system proved efficient for fully recovery of biophenols in the retentate stream as well as reclamation of purified water.

RESEARCH OBJECTIVES

The main objective of the present work was to investigate and advance the potentiality of integrated membrane system for sustainable olive mill wastewater (OMWW) treatment. The valorization of OMWW by recovery and use of phenolic compounds whilst reducing the overall toxicity of the aqueous stream is an attractive approach followed in this work. Specific objectives to reach this aim include:

- OMWW pre-treatment to reduce high suspended solids content and consequently to limit fouling phenomena during membrane process;
- Selection of membrane materials with best performance in terms of permeate flux and selectivity towards polyphenols;
- Improvement of steady-state flux of microfiltration in the processing of olive mill wastewater;
- Development of an integrated membrane system on lab scale prototype;
- Development of integrated membrane system on productive scale aimed at polyphenols recovery and water purification from real olive mill wastewater;
- Study of emulsification processes for polyphenols encapsulation within water-in-oil emulsions or solid lipid particles with narrow size distribution.

DISSERTATION OUTLINE

The thesis is organized into four chapters. The first one provides a comprehensive review of the state-of-the-art of olive mill wastewater (OMWW) treatment and valorization including a brief introduction of OMWW composition. The main part of this chapter analyzed the current strategies used for OMWW treatment by membrane technology and identified bottlenecks and novel strategies to overcome them. Furthermore, an overview of OMWW valorization for nutraceutical, cosmetic and pharmaceutical interest is reported (**Chapter 1**). The subsequent chapters cover the experimental research activity, as reported below:

- Study of the pretreatment of OMWW and evaluation of the performance of different microfiltration and ultrafiltration membrane materials and configurations for the treatment of OMWW. Membrane performance was evaluated in terms of permeate flux and selectivity towards polyphenols and capacity to reduce total organic carbon (**Chapter 2**) [1];
- On the basis of results illustrated in chapter 2, a scale up to lab-scale prototype integrated membrane system and then to productive scale was promoted. Development of lab-scale prototype integrated membrane for OMWW treatment. The research activity aimed at polyphenols recovery, concentration and emulsion formulations containing biophenols. Initially, the performance of selected microfiltration ceramic membranes in terms of permeate flux were investigated. The efficacy of air backflushing cycles in order to improve the permeate flux and limit the fouling phenomena for microfiltration unit was evaluated. For what concerns nanofiltration membranes, the influence of materials, configurations, molecular weight cut-off or pore size on permeate flux, TOC removal, polyphenols rejection and fouling index were investigated. Finally, the integrated membrane process on productive scale based on pre-filtration, microfiltration, nanofiltration and reverse osmosis for water and polyphenols recovery was developed (**Chapter 3**)[2];
- Formulations of W/O emulsion and solid lipid particles loaded with phenolic compounds by membrane emulsification. The influence of process and chemical parameters, dispersed phase fraction on particles size and size distribution has been

investigated. A biophenols release from W/O emulsions was also studied.

A comparative study of the two methods for production of solid lipid particles in terms of their influence on particles size, size distribution as well as biophenols encapsulation efficiency was studied (**Chapter 4**) [3].

Note and references

[1] *Part of this chapter has been published: Bazzarelli F., Poerio T., Mazzei R., D'Agostino N., Giorno L., "Study of OMWWs suspended solids destabilization to improve membrane processes performance", *Separation and Purification Technology*, 149 (2015) 183-189.*

[2] *Part of this chapter has been published: Bazzarelli F., Piacentini E., Poerio T., Mazzei R., Cassano A., Giorno L., "Advances in membrane operations for water purification and biophenols recovery/valorization from OMWWs", *Journal of Membrane Science*. 1 (2016) 402-409.*

[3] *Part of this chapter has been published: Piacentini E., Poerio T., Bazzarelli F., Giorno L., "Microencapsulation by Membrane Emulsification of Biophenols Recovered from Olive Mill Wastewaters", *Membranes*, (2016) 6, 25.*

Olive mill wastewaters treatment and their valorization

Abstract

Olive mill wastewater (OMWW) is the main waste that is generated during the olive oil extraction process. This by-product stream is characterised by a high polluting organic load, due to a high content of organic substances, including sugars, tannins, polyphenols, polyalcohols, pectins and lipids. The management, treatment and safe disposal is necessary to avoid serious environmental problems.

The main difficulty of OMWW treatment is associated with the seasonal operation, huge amount of OMWW produced in the framework of few months, high organic loading, and the presence of hardly biodegradable organic compounds, such as phenolic compounds and long chain fatty acids. The disposal of OMWW is a significant environmental problem because of the presence of organic substances and the phytotoxic activity of phenolic compounds. However, OMWW is an inexpensive source of natural antioxidants because of its high content in polyphenolic compounds. Furthermore, the OMWW can be used for the energy and biopolymers production. A large number of studies on various treatment methods have been published worldwide. Most of them deal with conventional treatment technologies, including anaerobic digestion, physico-chemical pre-treatments or advanced oxidation processes. The efficiency, complexity, and cost-effectiveness of these methods regarding the OMWW treatment can vary significantly. Great attention is given to membrane technology for recovery, purification and concentration of phenolic compounds from OMWW with regard to their specific molecular weight cut-off, reducing the polluting load of OMWW. The recovery of phenolic compounds from OMWW allows the detoxification of the OMWW and also the potential utilization of polyphenols as functional ingredients in foods, cosmetics or for pharmacological applications given their excellent antioxidant properties.

1.1. Introduction

Olive oil production is one of the main agricultural activities in the countries of the Mediterranean River Basin. Olive farming and the olive oil industry carry both economic and social importance, especially in these countries. The leading olive oil producers are Spain, Italy, Greece and Portugal (Figure 1.1). Olive oil has excellent nutritional properties, and its consumption, traditionally restricted to the Mediterranean area, is increasing worldwide, prompting countries such as Argentina, Australia, the US and South Africa to emerge as producers by promoting intensive olive tree cultivation. In the last decade, olive oil production has increased by approximately 40% worldwide. This phenomenon can be explained by health benefits of the Mediterranean diet, of which olive oil is a major component [1]. The rising popularity of olive oil is predominantly attributed to its oleic acid and phenolic compound contents.

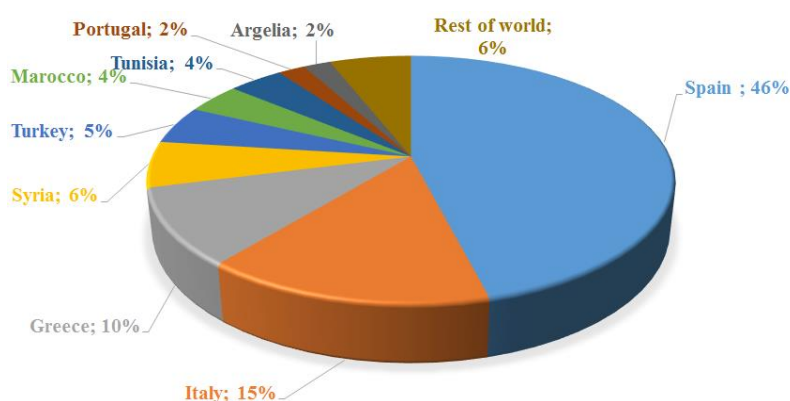


Figure 1.1. Olive oil production worldwide (International Olive Oil Council, IOOC 2014).

Olive oil extraction involves different processes such as olive washing, grinding, beating and the extraction itself, that constitutes the basic stage of the whole process. The amount and physico-chemical properties of the wastes produced will depend on the method used for the extraction [2]. Olive oil is extracted from the pulp of olive fruit typically by three operational steps [3]: (a) olive crushing, where fruit cells are broken down and the oil released; (b) mixing, where the remaining paste is slowly mixed to increase the oil yield; and (c) oil separation from the remaining wastes. This latter step could be conducted according to one of the following processes: traditional discontinuous press process, carried out by means of olive pulp pressing and filtrate centrifugation and continuous process, carried out by means of direct centrifugation (two-phase or three-phase centrifugal

extraction) of olive pulp diluted with water (Figure 1.2). During olive oil extraction processes, water is added in some of these steps to squeeze out most of the oil from the olive. The mix between olive vegetation water and this under-process added water is called olive mill wastewater (OMWW). Among the three processes of olive oil production, pressure and 3-phases centrifugation systems generate solid cake-like and huge amounts (up to 30 million m³ per year) of OMWW [4]. In particular, it has been estimated that for every ton of olive fruits processed, approximately 1.2-1.8 m³ of wastewater is generated by three-phase centrifugation systems [5]. While two-phase systems involve a reduction of OMWW volumes but an increased concentration in organic matter. The OMWW is one of the most harmful effluent produced by agro-food industries because of its high polluting load and high toxicity to the whole ecosystem (plants, bacteria, aquatic organisms and air) owing to its acidic pH and to its content in organic substances such as phenols, which are phytotoxic [4]. The production of large OMWW volumes during a very short discarding time, which is usually defined from October to February, increases the importance of this waste from both an environmental and economical point of view. In terms of pollution effect, 1 m³ of OMWW is reported to be equivalent to 200 m³ of domestic sewage. Thus, OMWW treatment or valorization is a major environmental issue.

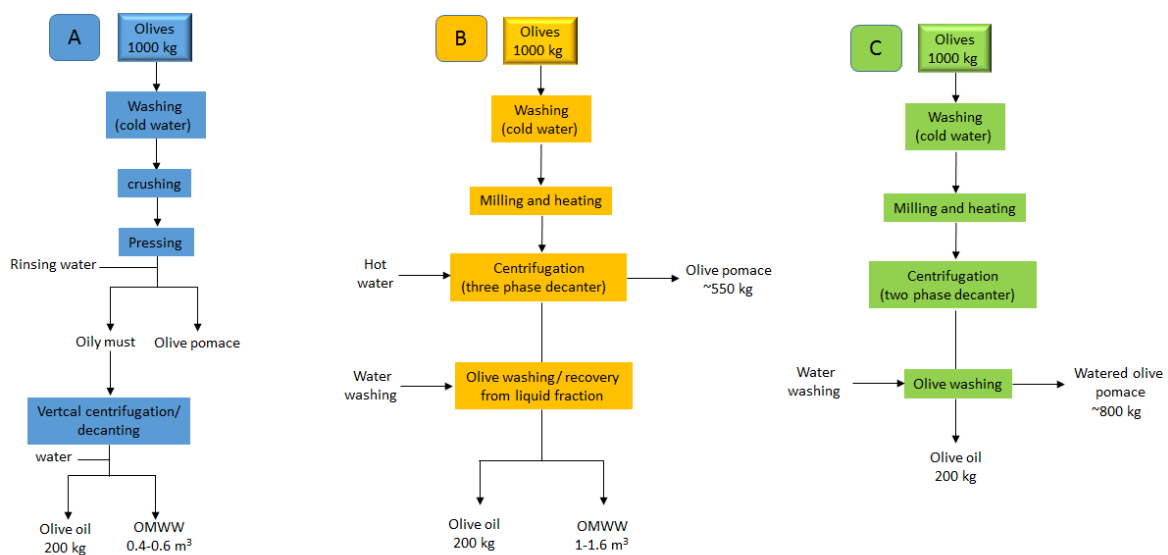


Figure 1.2. Olive oil extraction processes. (a) traditional process; (b) two-phase centrifugation system; and (c) three-phase centrifugation system.

In this context, many studies on the treatment approaches and valorization options of olive mill waste residues, predominantly those allowing for the recovery of valuable natural components such as phenolic compounds, dietary fibers, bioethanol, biohydrogen, biogas, enzymes, polymers and other were carried out (Figure 1.3).

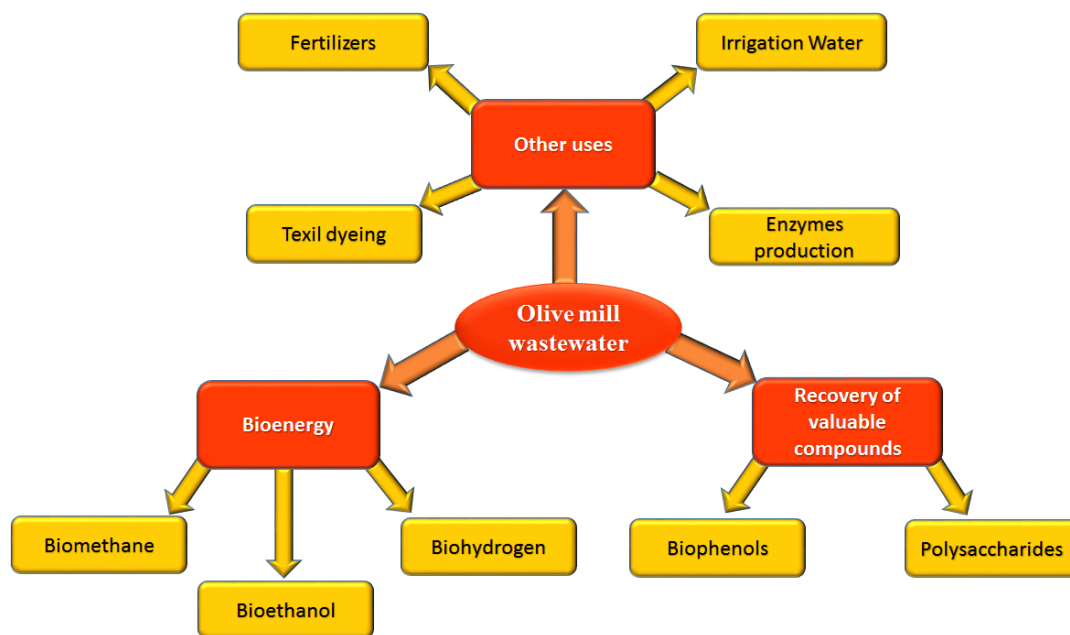


Figure 1.3. Valorization opportunities for oil mill wastewater.

1.2. Characteristics of olive mill wastewater

The chemical composition of the OMWW is highly variable (Table 1.1), depending on many factors such as olive variety, ground type, climatic conditions, cultivation system, degree of ripeness and storage time of the fruit, as well as the olive oil extraction method. Typical OMWW composition is between 83% and 94% of water, between 4% and 16% of organic matter and between 0.4% and 2.5% of mineral salts. Its organic matter content consists of fats, sugars, nitrogenous substances, organic acids, polyalcohols, pectins, polyphenols, mucilages and tannins. OMWW also contains valuable resources such as mineral nutrients, especially potassium calcium and sodium. The yeast, mold and bacteria were the main microorganisms found in OMWWs. Most of them are originated from soil and freshwater environments. The organic load of OMWW, measured by its Biological Oxygen Demand (BOD) or its Chemical Oxygen Demand (COD), is much higher than domestic wastewater. In particular, chemical oxygen demand (COD) is ranging between 40

and 220 g/L, while biochemical oxygen demand (BOD) is also very high, ranging between 35 and 110 g/L. The OMWWs are characterized by a dark-color caused by lignin polymerized with phenolic compounds and high electrical conductivity and pH between 4.8 and 5.7 (slightly acid). Thanks to the presence of large amounts of proteins, polysaccharides, mineral salts, and other useful substances for agriculture, such as humic acids, OMWW has a high fertilizing power. Therefore, OMWW might be used as natural, low-cost fertilizer available in large amounts. Unfortunately, besides these useful substances for agriculture, OMWWs also contains phytotoxic and biotoxic substances, which prevent disposal. The phytotoxic and antibacterial effects of OMWWs have been attributed to its high phenolic content. OMWWs can contain up to 25 g of phenols per liter while in the European Union, the accepted maximum phenol concentration in wastewaters is 1 mg/L [6] On the other hand, polyphenols are compounds with potential health benefits. In light of these findings, the OMWWs are recognized as a potential low-cost material rich in bioactive compounds that can be extracted and applied as natural antioxidants. They are able to donate a hydrogen atom to the lipid radical formed during the propagation phase of lipid oxidation, therefore, they could be added to fatty foods to prevent the formation of flavors and toxic compounds resulting from lipid oxidation.

Table 1.1. Composition of olive mill wastewater [7-8-9].

Physicochemical characteristics			Mineral composition		
Parameter	Unit	Range	Element	Unit	range
pH	-	2.24-5.9	K	g/L	0.73-8.6
Conductivity	mS/cm	5.5-12	Na	g/L	0.05-0.8
TOC	g/L	20.2-45	Ca	g/L	0.03-0.9
TSS	g/L	25-128.4	Cl	g/L	0.76-1
COD	g/L	40-220	Mg	g/L	0.03-0.19
BOD	g/L	35-110	Mn	mg/L	0.9-20
Sugars	g/L	1.3-4.3	Cu	mg/L	2.4-2.96
Polyphenols	g/L	0.5-25	Zn	mg/L	2.94-4.98
Total nitrogen	g/L	0.06-2.1	Fe	mg/L	6.5-20
Dry residue	g/L	11.5-90	Pb	µg/L	6.7-10
Lipids	g/L	1.64-12.2	Cd	µg/L	0.03-1

One of the valorization objectives regarding wastes, effluents and food processing by-products, is the recovery of fine chemicals [10]. Efficient sustainable treatments should guarantee not only a significant reduction of BOD and COD values, but also the possibility of selectively recovering some phenolic compounds. OMWW may be regarded as an inexpensive useful resource of compounds to be recovered because of their potential economic interest to be used for biogas and biopolymers production, fertilizers for agriculture. Moreover, natural phenols from olive and its by-products are now recognized as potential targets for food, cosmetic and pharmaceutical industries.

1.3. Production of fertilizers

The antimicrobial and phytotoxic properties of OMWW indicate that it should not be directly applied to soils and crops. However, its high water, organic matter and plant nutrient content make it a candidate for bioconversion as a valuable fertilizer and soil conditioner. N₂-fixing bacteria of the gen *Azotobacter* utilize phenols, sugars, organic acids, etc. of OMWW as a carbon source, i.e., they degrade them and convert atmospheric nitrogen into several organic nitrogenous compounds. So, the process of treating OMWW with an enriched aerobic microbial population of this kind results in no phytotoxic thick liquid that could be characterized as an organic soil-conditioner biofertilizer [11]. In this way the waste is fully recycled to land through an environmentally “clean” process while energy and water savings are also achieved at the same time. In addition, the antimicrobial activity of OMWW is harnessed for soil sanitation against certain plant pathogens [12].

1.4. Production of biopolymers

The high COD content can be a hidden source of value, because all the chemicals can be separated from the waste stream and residual organic content used as raw material for biopolymers production. Olive oil mill effluents represent a potential feedstock for production of polyhydroxyalkanoates (PHAs). Indeed, the acidogenic fermentation step is not inhibited to a great extent by lipidic and phenolic substances, which, on the other hand, inhibit the further step of methanogenesis. In particular, fermentation of OMWWs produces volatile fatty acids (VFAs), which are the most highly used as substrate for aerobic bacteria to produce PHAs [13]. They are promising microbial biopolymers, mainly because they show physical properties similar to those of some petroleum-derived plastics and therefore can replace polypropylene in a wide range of applications, they are completely biodegradable and biocompatible [14].

1.5. Production of biogas

Anaerobic fermentation of olive mill wastes has drawn considerable attention because of the conversion of organic materials into biogas, which contains mainly methane and CO₂. Biogas has traditionally been used either for thermal or electric power purposes, or even for injection into the natural gas grid after cleaned of impurities. The high organic load of OMWW makes anaerobic treatment a very attractive treatment option for such waste. In fact, almost 80% of organic compounds of OMWW are biomethanizable. Theoretically, a yield of 37 m³ of methane per m³ of OMWW could be achieved [11]. However, obtaining high anaerobic treatment efficiency of OMWW has been hampered by very high organic loading, high acidity, low nitrogen content and the presence of compounds difficult to biodegrade, including phenolic compounds, lipids and long chain fatty acids, most of which are inhibitory for the sludge bacteria (methanogens primarily) responsible for the biodegradation process. Several approaches have been suggested to overcome these difficulties [15]:

- Dilution of OMWW to reduce organic loading rates and keep feed constituents that inhibit the process at low and safe levels;
- Correction of alkalinity with the addition of NaHCO₃, NaOH or Ca(OH)₂;
- Pretreatment to remove phenols and lipids before aerobic treatment;
- Addition of an easily biodegradable substrate, to facilitate degradation of inhibitory substances;
- Co-digestion with other waste to succeed co-metabolism and alleviate the effect of inhibitory factors.

The co-digestion of olive mill wastewater with other organic residue streams is reported in several works. Azaizeh et al., [16] evaluated the efficiency of the co-digestion strategy for treatment of swine wastewater (SW) and OMWW (olive mill wastewater) mixtures. The SW is characterized by low organic load, but exhibits a high concentration of nitrogen compounds and high alkalinity. Therefore, mixing OMWW with SW permitted a dilution of the inhibitory compounds in OMWW, but also the adjustment of pH to a neutral value and compensates for nitrogen shortage, resulting eventually in increased biodegradability and methane production. The co-digestion of OMWW with SW has advantages over anaerobic digestion of OMWW alone, particularly in introducing bacteria capable of phenol bio-degradation through the addition of SW. Mesophilic batch reactors fed with mixtures of SW and OMWW showed that the highest COD removal of 85-95% and biogas

production of $0.55 \text{ L} \cdot \text{g}^{-1}$ COD were obtained at a mixture consisting of 33% OMWW and 67% SW. In another study, the anaerobic co-digestion of olive mill wastewater (OMWW) with olive mill solid waste (OMSW) which is a substrate rich in nitrogen was investigated by Boubaker et al. [17]. The experiments were carried out using tubular digesters operated at mesophilic temperatures. The amount of OMSW used as a co-substrate was 56 g of dry OMSW per liter of OMWW, besides $\text{Ca}(\text{OH})_2$ was added to OMWW to ensure a neutral medium (pH: 7.0-8.0) for the methanogenic bacteria growth and to avoid the cessation of methane production. The results showed that the best methane yield from the co-digestion of OMWW (Total Chemical Oxygen Demand, TCOD = 56 g COD/l) with OMSW was 0.95 l/l/d. while it was 0.7 l/l/d when OMWW was digested alone. Azbar et al. [18] investigated a chemical pretreatment to improve the anaerobic biological degradation of olive mill effluent. For this purpose, chemical pretreatment of the wastewater generated from olive oil production process, namely olive mill effluent (OME), was conducted by means of acid cracking followed by coagulation–flocculation process. The experimental results obtained in this work suggest that pretreatment significantly enhances the biodegradability of OME, which was much lower if it was digested alone (without pretreatment). Over 80% increase in biogas production was obtained when digesting OME after chemical pretreatment. Dhouib et al. [19] investigated an integrated technology for the treatment of the recalcitrant contaminants of olive mill wastewaters (OMWW). The process based on fungal (*Phanerochaete chrysosporium*) pretreatment followed by anaerobic digestion and ultrafiltration process as a post-treatment for complete detoxification and color removal allowing water recovery and reuse for agricultural purposes. A multi-tubular membranes having 2, 25, and 100 kDa and a surface of 0.85 m^2 were used. The pre-treated OMWW was well converted into methane by anaerobic digestion. In particular, the methane yield (CH_4 per COD) in (L g^{-1}) was 0.15 for untreated OMW and 0.34 for pre-treated OMW.

1.6. Bioactive compounds properties and recovery

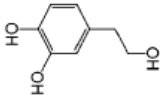
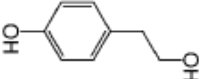
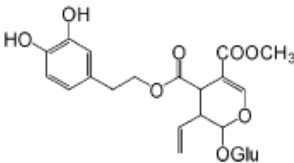
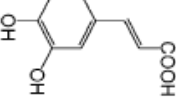
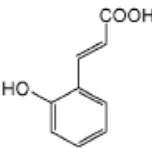
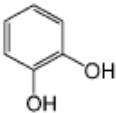
Olives are rich in simple and complex phenolic compounds, but during the olive oil extraction process, due to their low partition coefficients (partition coefficients oil/water ranging from $6 \cdot 10^{-4}$ to 1.5) only 2% of the total phenolic content of the olive fruit passes into oil phase while the remaining amount is lost in the OMWW (approximately 53%) and in the pomace (approximately 45%) [20]. The high concentrations of natural phenolic compounds, which are known as powerful natural antioxidants, anti-inflammatory, antibacterial, and antiviral activities, make these wastewaters a potential and a cheap interesting natural source for these bioactive substances. These compounds are usually synthesized by chemical methods that are responsible of their high price. Therefore, the recovery of the polyphenolic content of OMWW not only makes OMWW less toxic and easier to treat, thus promoting the overall sustainability of the OMW management, but it also provides economic benefits. Generally, phenolic compounds include a great number of organic substances, which have the common characteristic of having an aromatic ring with one or more substitute hydroxyl group and a functional chain. Up to now, more than 50 phenolic compounds have been detected in OMWW. Two major categories are observed [11]:

1. Phenolic monomers, flavonoids, not autoxidized tannins, and other compounds with $MW < 10 \text{ kDa}$.
2. Medium and high MW ($MW > 10 \text{ kDa}$) dark colored polymers resulting from the polymerization and autoxidation of phenolic compounds of the first group.

Typical biophenols occurring in OMWW are derived from cinnamic acid such as cinnamic acid, p-coumaric acid, caffeic acid, and ferulic acid. Another group of phenolic compounds present in OMWW is derived from benzoic acid: p-hydroxybenzoic acid, protocatechuic acid, vanillic acid, veratric acid, gallic acid, syringic acid and β -3,4-dihydroxyphenyl ethanol derivatives, such as tyrosol, hydroxytyrosol and 3,4-dihydroxyphenylglycol. In addition to phenolic acids, OMWW contains flavonoids which are mainly represented by apigenin, luteolin, quercetin and rutin as well as secoiridoids (oleuropein and oleuropein aglycone). Other important compounds present in olive fruit and its by-products are pigments, organic acids and pectins [21]. These compounds can be reused in food, cosmetics or even in pharmaceutical formulation. Many studies have found that the most antioxidant compounds from olive mill residue were oleuropein, verbascoside,

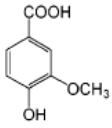
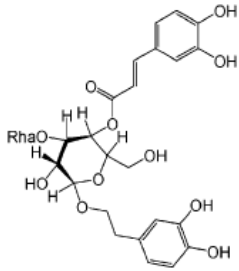
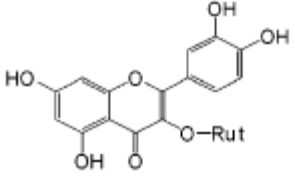
hydroxytyrosol glucoside and oleuropein aglycone. An overview of phenols bioactivities are reported in Table 1.2.

Table 1.2. Major biophenols in OMWW and their activities [20-22].

Biophenols	Bioactivities
 <p data-bbox="355 629 560 663">Hydroxytyrosol</p>	<p data-bbox="719 524 1386 667">Antioxidant, cardioprotective and antiatherogenic, chemopreventive, antimicrobial, anti-inflammatory, skin bleaching, anti-osteoporosis</p>
 <p data-bbox="408 831 515 864">Tyrosol</p>	<p data-bbox="847 748 1259 824">Antioxidant, anti-inflammatory, cardioactive, antiatherogenic</p>
 <p data-bbox="384 1099 536 1133">Oleuropein</p>	<p data-bbox="751 891 1355 1144">Antioxidant, antiatherogenic, cardioprotective, hypoglycemic, antihypertensive, antimicrobial, antiviral, anti-inflammatory, cytostatic, enzyme modulation, endocrinal activity</p>
 <p data-bbox="384 1341 536 1375">caffeic acid</p>	<p data-bbox="831 1220 1270 1335">Antioxidant, anti-inflammatory, chemoprotective, antiatherogenic, antimicrobial</p>
 <p data-bbox="352 1592 563 1626">p-coumaric acid</p>	<p data-bbox="842 1496 1262 1529">Chemoprevention, antimicrobial</p>
 <p data-bbox="400 1812 520 1845">Catechol</p>	<p data-bbox="895 1682 1206 1823">Antioxidant, anticancer, carcinogenic, phytotoxic</p>

Continued

Table 1.2. Continued

Biophenol	Bioactivity
 <p data-bbox="352 533 515 566">vanillic acid</p>	<p data-bbox="898 421 1177 510">Antioxidant, antimicrobial activity</p>
 <p data-bbox="347 913 523 947">Verbascoside</p>	<p data-bbox="914 645 1161 1014">Antioxidant, chemoprevention, cardioactive, antihypertensive, antiatherogenic, anti-inflammatory, sedative</p>
 <p data-bbox="379 1283 451 1317">Rutin</p>	<p data-bbox="914 1093 1161 1294">Antioxidant, antiatherogenic, anti-inflammatory, chemopreventive</p>

Various techniques have been proposed, individually or combined, to recover phenolic compounds from OMWWs, such as solvent extraction, chromatographic separations and membrane processes.

Many studies on the recovery of polyphenols from OMWWs have pointed to the use of liquid-liquid extraction [23-24]. This method is more expensive than aqueous processing; the presence of residual solvents in the extract and the coextraction of undesirable compounds are additional drawbacks. The use of supercritical fluids overcomes some disadvantages of organic solvents (such as flammability and toxicity); however, in this case, expensive high-pressure equipment is needed. Methods for obtaining hydroxytyrosol from the by-products of olive oil extraction by means of two-step chromatographic treatment have been also proposed by Fernandez-Bolanos et al. [25]. An effective extraction of polyphenols from different organic residues was obtained through solid phase

extraction (SPE) approaches. A selective phenol-recovery approach via a solid phase extraction (SPE) procedure was also developed by Bertin et al. [26]. Four most promising resins employed in the previous work, namely Amberlite XAD7, XAD16, IRA96 and Isolute ENV+, were tested and compared for their adsorption and desorption features towards the natural phenols occurring in the two real OMWWs. Among the applied commercial resins, ENV+ gave rise to the best overall recovery productivities. The elution of the adsorbed OMWW total phenols was successfully carried out especially through the employment of acidified ethanol, whereas that of more polar low molecular weight phenols (including HT) was better performed with ethanol. Similarly, Scoma et al. [27] proposed an integrated physicochemical-biotechnological approach for multipurpose valorization of OMWWs, in which more than 60% of natural polyphenols were recovered through an SPE procedure by employing resin (Amberlite XAD16) as adsorbent and ethanol as the biocompatible desorbing phase. SPE technology provides cleaner extracts, a simpler protocol, shorter processing times, easier automation, and cheap procedures over conventional technologies. Common disadvantages are related to the incomplete removal of interferences, low recovery of analytes, and high variability in results. An alternative approach for separating, purifying and concentrating bioactive phenolic compound from OMWW is to use membrane technology. The recovery of antioxidants from OMWW by using membrane operations are of interest due to their several advantages mainly low temperature, low energy consumption, no additive requirements and no phase change. Comparing different technologies, Takac et al. [28] concluded “the future direction of the processes for the recovery of antioxidants from OMWWs is presumably toward the utilization of membranes in a sequential design.”

1.7. Olive mill wastewater treatment

Several waste management approaches, including chemical and physical treatments, advanced oxidation processes, disposal methods, biological treatments, electrocoagulation method, as well as on various combinations of them, have been proposed to reduce the polluting load and, consequently, the final waste disposal [29]. Most of depolluting treatments of OMWWs aim at destroying their organic matter not to be used as a raw material for the production of high-added value products. Intensive research in the field of OMWWs management suggests that, OMWWs can be considered a precious resource of potentially useful substances after either direct recovery or chemical transformation.

Therefore, research is focused toward flexible and efficient treatment solutions that could ensure the detoxification of the waste with the possibility of recovering and recycling some valuable components. In the recent years, more environmentally friendly technique represented by membrane technology have been investigated and used for OMWWs treatment. Membrane technologies, attempt to utilize the OMWW for production of valuable antioxidant and clean water, according to the EU principle of total discharge of the waste materials, the reduction in operational costs brought about by the advances in membrane technology has the attraction of membrane technologies. The basic properties of membrane operations make them competitive with conventional methodologies, which offer several advantages as reported above when compared with conventional technologies for concentrating and/or fractionating bioactive phenolic compounds from different vegetable sources. Zagklis et al. [30] carried out an analysis on different methods of olive mill wastewater treatment (membrane filtration, coagulation/flocculation, anaerobic digestion and Fenton oxidation) in terms of their effectiveness and cost. Effectiveness of each method was estimated in terms of COD and phenolic compounds reduction, while for the economic impact the operational costs were taken into account. The analysis showed that the most effective processes in terms of organics reduction are membrane filtration, electrolysis, supercritical water oxidation and photo-Fenton. The lowest cost category involves biocomposting and membrane filtration. Regarding membrane filtration, it is characterized by the lowest economic impact because the cost of treatment can be covered by exploiting recovered compounds [31].

1.7.1. Chemical processes

Chemical processes such coagulation, flocculation, oxidation processes have been employed in OMW treatment. Additional chemicals are used in these processes in order to destabilize the suspended and colloidal matter of OMW and form an insoluble solid that can be removed easily from the waste [32]. Oil, suspended solids, COD, and BOD are decreased in this way. Destabilization of these colloids can be achieved either by reducing or increasing pH (neutralization), or by the addition of a precipitate-inducing agent (precipitation/flocculation). The most important inorganic flocculants that have been used for OMWW treatment are ferric and ferrous chloride, ferric sulphate, and aluminum sulphate. Another coagulation agent is lime ($\text{Ca}(\text{OH})_2$). By treating OMWW with lime, oil, and COD reduction, decolorization, and important reduction in odor emissions are achieved. The liquid obtained after treatment contains no phytotoxic substances and it can

be treated further more easily. Furthermore, after lime treatment the liquid phase is evaporated more easily than the raw wastewater, which is in the ponds. Since a large quantity of fatty components, which do not permit the evaporation of water by forming an impermeable film on the surface of the wastewater in the ponds, have been removed by lime [33] Although lime is an inexpensive and readily available material, lime coagulation generally has several drawbacks, such as a low efficiency in COD removal, an increase in water stiffness, an increase in pH up to 11-12, and the production of a large amount of slop. The chemical method, although simple, are more suitable as pre-treatment to reduce the total suspended solids because the treated liquid still has a high polluting load.

1.7.2. Electrocoagulation

Electrocoagulation (EC) technology is an alternative method to classic chemical coagulation. EC is based on the fact that the stability of colloids and suspensions is influenced by electric charges. Therefore, if additional electric charges are supplied to the charged particles via appropriate electrodes, the surface charge of the particles is neutralized and particles combine into larger and separable agglomerates. In addition to the precipitation process, electrocoagulation is also effective in the removal of soluble species as oxidation processes also take place [34]. This process has proven very effective in removing contaminants from water and is characterized by reduced sludge production, no requirement for chemical use, and ease operation and lower equipment costs. Adhoum et al. [35]. Investigated the treatment of OMW by EC using aluminum electrodes. The removal efficiency of the treatment was determined by monitoring the decrease of total phenol, COD and dark color intensity. They concluded that application of electrocoagulation procedure permitted removal capacity of 76% of COD, 91% of polyphenols and 95% of dark color present in fresh and stored OMW.

1.7.3. Advanced oxidation processes

Advanced oxidation processes (AOPs) are known for their capability to mineralize a wide range of organic compounds. They are characterized by the production of the highly oxidative HO radical at ambient temperature via a number of photochemical or non-photochemical pathways. This powerful radical is able to completely transform organic compounds to CO₂. The principal AOPs used for OMWW treatment are Fenton's reagent reaction (H₂O₂ plus a ferrous salt), O₃ plus UV radiation, H₂O₂ plus UV radiation, H₂O₂/O₃ UV radiation and photocatalysis, where solar energy also may be used [36]. The Fenton reagent is a homogeneous catalytic oxidation process involving the reaction of hydrogen peroxide with ferrous ions. This reaction generates hydroxyl radicals that have a high oxidation potential. The presence of organic substrates generates a series of reactions that can lead ultimately to the decomposition of organic substrate in carbon dioxide and water. As it is not an electrically driven method, it has low energy consumption, but the main drawback of these processes lies on the cost of the reactants, H₂O₂ and Fe²⁺ ions. For this reason, Nieto et al. [37] introduced the cheaper Fe³⁺ salt rather Fe²⁺ for the degradation of organic matter present in olive-oil mill wastewater from two-phase olive-oil extraction process. It was shown that organic matter is efficiently degraded through a Fenton-like reaction using FeCl₃ as catalyst in the presence of hydrogen peroxide. This approach allows achieving a removal of 92.6% of chemical oxygen demand and 99.8% of total phenols. Photo-Fenton is similar to Fenton as it uses the same reagents, with the difference that UV radiation is applied to the solution. Supercritical water oxidation is the oxidation of the waste on catalysts such as Pt/γ-Al₂O₃ or without the presence of a catalyst, above the critical temperature of water and at high pressure (25–35 MPa). This method is very effective for reduction of the organic content but because of the high temperatures and pressures employed, the treatment cost and energy consumption are high [30]. Ozonation uses O₃ as an oxidant for oxidation of the waste. This method is usually adopted for water disinfection, but it also has a high potential as pretreatment method. The characteristic of ozone is that it is rather selective towards double bonds. Theoretically, it would leave intact the proteins and the sugars of OMWW, which are biodegradable anyway, and attack selectively the double bonds of unsaturated fatty acids and phenols. Ozonation process, one should consider two possible trajectories of oxidation. A direct trajectory through the reaction between ozone and the dissolved compounds, and an indirect one through the reaction of radicals generated from ozone decomposition (hydroxyl radical) and dissolved

compounds. The combination of both trajectories for the elimination of compounds depends on the chemical nature of those compounds. AOPs manage a great COD reduction but their operating costs are considerably high. It could be said that chemical oxidation emerges as a suitable alternative, when biological degradation is not applicable.

1.7.4. Physical processes

Simple physical processes such as evaporation, sedimentation, lagooning, filtration and centrifugation have been employed to treat OMW. None of these processes alone is able to reduce the organic load and toxicity of OMW to acceptable limits. The drying of OMW in open evaporation ponds with retention time of several months is a common, low-cost practice in rural areas of the Mediterranean. However, this method requires large areas and causes significant nuisance due to the emitted odors and the possible contamination of aquifers. Evaporation and sedimentation can concentrate OMW to the extent of 70–75%, this being mainly attributable to phase separation/dehydration and not as much to subsequent organic matter degradation [38]. The remaining concentrate and the supernatant, in the case of sedimentation, need further treatment. Similarly, in evaporation processes the remaining ‘paste’ and the distillate need further treatment. The solar distillation can be employed for the dewatering of olive mill wastewater. An efficient separation of solids and liquid from the olive mill wastewater was achieved. Application of solar distillation to OMW showed that the distillate produced was free from solids and 80% lower in terms of COD [39]. Irreversible thermal treatment such combustion and pyrolysis are used as radical and destructive techniques that eliminate any possibility of further use of OMWW. They have the advantages of reducing the volume of waste and providing the possibility of energy recovery, but they require expensive facilities and involve possible emission into the atmosphere of toxic substances. Usually a combination of physical processes or often a combination of physical processes coupled with coagulation/flocculation method leads to more efficient organic matter removal.

1.7.5. Biological treatment

Biological degradation of a chemical refers to the elimination of the pollutant by the metabolic activity of living organisms, usually microorganisms and in particular bacteria and fungi. The biological treatment includes aerobic and anaerobic processes according to the type of the microflora used. A stage of aerobic pretreatment can degrade a fraction of the pollutants and reduce effluent toxicity reducing amount of phenols, making the effluent blackberries susceptible to subsequent treatment [40]. Aerobic processes, such as activated sludge and trickling filters, are usually exploited to remove dissolved or colloidal pollutants from wastewaters at low concentration. Although aerobic biological treatments succeed higher efficiencies, these processes are limited by the unbearable cost of the continuously provided mechanical aeration. Anaerobic digestion is carried out in airtight vessels by bacteria, which do not require oxygen to decompose organic compounds. Anaerobic treatment can be useful for dealing with such wastes, with advantages including low costs for energy and chemicals and also low sludge production. Compared to aerobic process, anaerobic process allows removing organic matter in higher concentration streams. A major limitation of anaerobic digestion of OMWs is inhibition of methanogenic bacteria by simple phenolic compounds and certain organic acids [41]. The inhibition associated with polyphenols has been extensively studied. However, it is clear that the effect of the toxicity is related to the molecular structure of polyphenol, the inhibitory effect is found to increase as the number of hydroxyl group decreases. Therefore, pretreatment methods that modify or remove these natural inhibitors can improve digestion of OMW.

1.7.6. Disposal method

The most common practice for the management of olive oil mill wastes (OMWs) involves disposal in evaporation ponds or direct disposal on soil. Traditional disposal on the soil is still the typical solution adopted in Italy. The Italian law in force (L. 574/96) allows discharge of a maximum of $50 \text{ m}^3\text{ha}^{-1}\text{year}^{-1}$ when OMWs come from a traditional mill and $80 \text{ m}^3\text{ha}^{-1}\text{year}^{-1}$ when OMWs come from a continuous mill [42]. The main disadvantages of this method are bad odors, growth of mosquitoes, the need for land very far from residential areas, and transfer costs. Besides, multiple effects on soil environment, including changes in the potential of the soil to interact with organic compounds may occur, also is phytotoxic to the crops and prohibits seed germination. Alternatively,

OMWW can be transported to olive tree fields and spread with certain limitations, providing soil enrichment with nutritious compounds. Magdich et al. [43] investigated the long-term effect of spreading OMW on olive tree plantations and the appropriate quantities of this wastewater that would have a positive effect on the physiological parameters such as net photosynthesis, stomatal conductance and transpiration and biochemical properties such as photosynthetic pigments, soluble sugars, total phenols, antioxidant activity and leaf mineral nutrient of these plants. After eight successive years in an olive field, the olive tree responses were dependent on OMW applied dose. Thus, an increase in chlorophyll and carotenoid contents, photosynthetic activity, leaf mineral nutrient and productivity were evidenced for olive plants amended by 50 and 100 m³ha⁻¹year⁻¹. However, at a dose-treatment 200 m³ha⁻¹, OMW agronomic application influenced negatively the olive performances revealed by leaf gas exchange characteristics and leaf nutrient content reductions. Effects caused by OMW land disposal may have variable persistence times. For example, after 3 months since OMW soil application, the induced soil phytotoxicity was found insignificant. Sierra et al. [44] were studied a location used for 10 years as an uncontrolled olive oil mill wastewater disposal site. The results show that the wastewater infiltration in the soil has caused carbonate dissolution, redistribution, and modifications in pH values, electrical conductivity, nutrient contents, phenolic compounds and biological activity of the horizons.

1.7.7. Membrane technology

Pressure-driven membrane operations such as microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO) are based on the principle of selective permeation of solutes through polymeric or inorganic semi-permeable membranes; the driving force for mass transfer of solutes across the membrane is a hydrostatic pressure difference. Generally, MF and UF are used mainly for primary treatment purposes while NF and RO are used for final treatment for fractionate and concentrate the recovered polyphenols. In processing OMWWs using the combinations of MF/UF and NF/RO operations, MF and UF retentates are characterized by an organic fraction that can be used as fertilizers or in the production of biogas in anaerobic reactors, whereas the NF and RO retentates containing enriched and purified low molecular weight polyphenols has been proposed for the food, pharmaceutical, and cosmetic industries. In particular, MF membrane allows the separation of suspended solids, certain bacteria and some colloids. In contrast, UF is found to be useful in the removal of macromolecules and biological species

such as bacteria. Separation in both MF and UF processes is done mainly based on size exclusion. The different fluxes are described by simple linear relations between the flux and the driving force. The transport mechanisms of microfiltration and ultrafiltration are governed by Darcy's law [45].

$$Jv = -Lp \frac{dp}{dz}$$

Here V_p is volume flux, p is the pressure, L_p is a coefficient expressing the permeability of the membrane and dp/dz is the driving force.

The NF has separation abilities to remove species with very low molecular weight such as sugars, dyes, organic micropollutants, etc. They are also able to retain ions and exhibit a high selectivity between mono and multivalent ions. Ion exclusion mechanisms are affected not only by steric effects, which are due to the size differences between pores and solutes, but also by electrostatic interactions involving the Donnan and dielectric effects. Instead, in RO process essentially all dissolved constituents are rejected while water is in principle the only material passing through the membrane. This rejection is due to size exclusion, charge exclusion and physical-chemical interactions between solute, solvent and membrane. The transport mechanism is based on the sorption-diffusion mechanism. According to this model, the separation is caused by different solubility and diffusion rates of permeating component in the membrane material. The RO process are higher operating pressures than NF, subsequently higher investment and maintenance costs are achieved through this system. RO process uses 21% more energy as compared to NF. The Fick's law describes the transport mechanism of NF and RO process.

$$J_i = -D_i \frac{dC_i}{dz}$$

Here J is flux, C_i is the concentration and D_i diffusion coefficient of component i , while dC/dz is the driving force.

In addition to pressure driven membrane operations, forward osmosis (FO), osmotic distillation (OD) and membrane distillation (MD), have emerged as new technologies with great potential in OMWW treatment (Table 1.3).

Table 1.3. Main membrane processes used for olive mill wastewater treatment [45].

Process	Type of membrane	Applied driving force	Separation mechanism
Microfiltration	Macroporous 0.1-10 micron	Hydrostatic pressure (1-5 bar)	Size exclusion, convection
Ultrafiltration	Macroporous or mesoporous 0.02-0.1 micron	Hydrostatic pressure (1-8 bar)	Size exclusion, convection
Nanofiltration	Mesoporous or microporous 0.002- 0.02micron	Hydrostatic pressure (3-30 bar)	Size exclusion, electrostatic interactions, solution/diffusion
Reverse osmosis	Dense	Hydrostatic pressure (10-100 bar)	Solution/diffusion
Osmotic distillation	Porous hydrophobic	Concentration gradient	Diffusion
Membrane distillation	Porous hydrophobic	Temperature difference	Diffusion
Forward osmosis	Dense	Osmotic pressure difference	Solution/diffusion

Membrane distillation (MD) and osmotic distillation (OD) can be used to selectively extract water from aqueous solutions under atmospheric pressure. Both processes are based on the use of a macroporous hydrophobic membrane separating two aqueous solutions. The hydrophobic nature of the membrane prevents liquid from entering its pores due to the surface tension forces. Therefore, liquid/vapor interfaces are formed at the entrances of the membrane pores. The driving force for the water vapor transport through the membrane is the vapor pressure difference between the two solution-membrane interfaces that is caused by the existing temperature gradient in MD and concentration gradient in OD [5]. Instead, the FO is a membrane process that uses an osmotic pressure gradient as a driving force to transport water across an ideally semipermeable membrane. FO has shown great potential in treating OMWW due to its low fouling sensitivity and high rejection capacity. Combination of emerging membrane technologies (FO, MD, OD) with the pressure driven membrane processes can improve recovery and concentration of high value-added co-products with remediation of good quality water from a large a recalcitrant vegetable water.

1.7.7.1. Strategies used to reduce the fouling during MF and UF of OMWWs

Regarding the OMWWs treatment by membrane operations, the main drawback for the implementation of membrane technologies is fouling phenomena that drastically reduces the flux, the efficiency of membrane separation and also alters the selectivity of the membrane. The first phenomenon that triggers over the membrane surface is concentration polarization. It is a reversible phenomenon caused by increasing concentration of solutes near the membrane boundary region. Concentration polarization may also reach high values to enable the formation of gel layers. If the concentration within the gel layer exceeds solubility concentration values, a solid starts to precipitate on the membrane surface. [46]. In order to minimize feed side polarization of concentration, appropriate fluid dynamic conditions have to be used notably through the development of membrane modules with an improved design. On the other hand, membrane fouling is an irreversible phenomenon and may involve membrane pore blocking, clogging, cake formation on the membrane surface. More specifically, the fouling mechanism can be internal fouling when the pollutant size is lower than the membrane pore size, pore blocking if pollutant and membrane pore have the same size or it could be cake formed when pollutant size is larger than membrane pore size and adhesion of particles on the membrane [47].

In the olive processing wastewaters, the MF and UF are used to remove suspended solids from raw wastewater. Considering that OMWWs are characterized by high suspended solids content (between 25-128.4 g/L) [42] and by the gelling substances, like pectins, that give rise to fouling phenomena strongly reducing the membrane efficiency. These processes represent a critical step for the selective separation of phenolic compounds from OMWWs owing to the rapid decrease in permeate flux and irreversible fouling of the membranes. Thus, pretreatment of raw OMWW are key factors for improving the productivity of membranes and avoid high fouling rates, which would rapidly lead to zero flux conditions if no pretreatment is conducted on the raw effluent upstream the membrane process. Moreover, an optimization of process parameters such as feed flow rate, temperature, transmembrane pressure and in addition, the type of membrane materials is also fundamental for high membrane process efficiency. Hence, different approaches have been developed and applied as pre-treatment steps based on chemical, physical and biological methods or combination of them, to mitigate the problem of fouling during OMWW processing. In Tables 1.4 and 1.5 are reported the most significant pre-treatment of OMWWs prior of membrane process.

Table 1.4. Different types pre-treatments used before MF and operating parameters during process.

Pre-treatment	Aim	Membranes	Flux (L/hm ²)	TMP (bar)	Feed flow rate/axial velocity	Ref.
pH adjustment, enzymatic treatment, centrifugation	pH treatment to avoid phenols oxidation. Enzyme treatment and centrifugation to reduce clogging effect on membrane	Sunflower-chaped ceramic block membrane, 0.1 to 1.4 μm	45 after 4.3 h	1.7	6 m/s	[48]
Acid treatment	Acidification to prevent phenols oxidation	Ceramic ZrO ₂ , 0.45 μm	50 after VRF 3	1.5	4.7 m/s	[49]
Coagulation and aerobic biodigestion	Critical flux evaluation as a function of particle size	PVDF, 0.3 μm	8.48	3	-	[50]
Filtration with cotton fabric filter	Remove coarse material	Ceramic Al ₂ O ₃ , 0.2 μm	42 after 6.5 h	0.7	760 L/h	[51]
Pectinase loaded membranes	Self-Cleansing of MF Membrane	PE, 0.4 μm	30 after 8 h	0.1	0.36 m/s	[52]

Table 1.5. Different types pre-treatments used before UF and operating parameters during process.

Pre-treatment	Aim	Membranes	Flux (L/hm ²)	TMP (bar)	Feed flow rate/axial velocity	Ref.
pH adjustment and filtration (cartridge filter)	Effect of pH on fouling	PAN, 100 kDa	30 for 2h	1	100 L/h	[53]
Electro-Fenton reaction and anaerobic digestion	Removal of COD and phenolic compounds	Multi-tubular membranes, 100 kDa	59 after 1h	1.75	-	[54]
Three steps of pre-microfiltration (pore size of 50, 5 and 0.2 μ m)	Remove large particles, suspended solids, oil and grease.	PS, 100 kDa	38.9 after (VRF 4)	2	-	[55]
Coagulation-flocculation process followed by photocatalysis	Fouling control	PA, 8 kDa	9.6	9	550 L/h	[56]
pH adjustment	Effect of feed pH on the removal of color, phenolic compounds, total solids and chemical oxygen demand and on permeate fluxes	PES, 50 kDa	49 (highest value not constant during the time)	3	-	[57]
Filtration (50 micron filter) followed by a flocculation	Critical flux evaluation	PA, 8 kDa	9.21	-	-	[58]

The following studies gave examples of pretreatments applied before to carry out the membrane operations. Borsani et al. [59] developed a pilot plant allowing the treatment up to 300 m³/d of OMWW corresponding to about a production of 500 t/d of processed olives. The proposed process comprised three step of pre-treatment of OMWW prior to membrane treatment: dirty water storage (30 days), oil removal (97% oil removal capacity) and the settling of suspended solids. After pre-treatments, the OMWW was processed by a tangential filtration through 30 kDa polysulphone membranes. The plant was operated continuously for 24 h per day at 10 bar and 35°C and 11.25 m³/h of permeate flow. The UF process together with a subsequent biological treatment succeeded treating 150 m³/d effluents (11 m³/h of UF permeate and 180 m³/h of sewage) for one year keeping the outlet stream BOD (40 ppm) and COD (160 ppm), below the Italian environmental discharge limit. Turano et al. [60] proposed a physical pretreatment consisting in centrifugation step, in which the suspended solids are removed before ultrafiltration (UF) unit. The

combination of centrifugation and ultrafiltration allows a COD reduction of about 90%. Moreover, a complete membrane rejection of fats, with respect to salts, sugars and polyphenols, is attained. Pizzichini et al. [48] also used centrifugation with pH adjustment and enzymatic treatment before the membrane operations. Akdemir et al. [53] proposed a pretreatment of OMWWs based on pH adjustment and cartridge filter filtration (20 micron). The authors compared the performance of two different polymeric UF membranes, one of polyvinylidene-difluoride (with a MWCO of 30 kDa) and the other an ultrafiltration membrane with a MWCO of 100 kDa using pretreated OMWW. Besides, the effects of main operating parameters (transmembrane pressure, feed flow rate, pH and membrane type) on the permeate flux and membrane fouling were examined. The highest permeate flux (25.9 L/m² h) was obtained using ultrafiltration membrane under operational conditions of Q_f=200 L/h flow rate and TMP=4 bar, while the highest removals were obtained at Q_f=100 L/h flow rate and TMP=1 bar. Several works report pH modifications of OMWWs with the aim of preserving polyphenols from undesirable oxidation processes before MF process [49-53].

Garcia-Castello et al. [51] carried out a filtration with a cotton fabric filter before MF operation. MF flux of about 42 L/hm² using a transmembrane pressure of 0.7 bar and an axial velocity of 5.5 m/s was obtained with pre-treated OMWWs. Paraskeva et al [38] tested different types of filtration to remove suspended solids before UF process. They used a filter-press with paper filters of 80 micron, filtration through sand bed filters, vacuum pressure paper filters, polypropylene screen. They reported that 80 micron polypropylene filter was the simplest equipment and the pre-filtration procedure was faster than the other methods. Electro-Fenton (EF) was used by Khoufi et al. [54] as first stage of a three-step method for the OMWWs treatment to oxidize toxic and recalcitrant organic compounds including polyphenols. This approach consists in the addition of a mixture of ferrous salts and hydrogen peroxide, able to generate hydroxyl radicals. The second and third step consisted of an anaerobic biological treatment and an ultrafiltration post-treatment, respectively. Drouiche et al. [61] applied UF process in combining with advanced oxidation process using UV/H₂O₂ to remove a large part of total solids and organic carbon. Stoller et al. [58] reported different pretreatment of OMWW such as coagulation (with aluminum hydroxide and aluminum sulphate), aerobic biodigestion (by means of fungi) and photocatalytic organic matter reduction (by means of nanometric titanium dioxide anatase powders irradiated by UV light). After the pretreatment, the clarified wastewater was withdrawn from the reactor, filtered by a 75 micron sieve and

sent to the feed tank of the pilot plant based on MF, UF, NF and RO membrane system in series. All pretreatment processes successfully tested down to RO gave rise to final permeate volume recoveries equal to 62% of the beginning feedstock, with a COD value equal to 456 mg l⁻¹, 242 mg l⁻¹ and 385 mg l⁻¹ for aluminum sulfate, biological treatment+aluminum sulfate and photocatalysis, respectively. The authors report that desired target requirements, which is COD values below 500 mg l⁻¹ was reached (The Italian allowance for the municipal sewer system discharge of wastewaters). Ochando-Pulido et al., [62] investigated the reclamation of two-phase OMW by RO on a bench-scale, to achieve the quality to recirculate the final effluent to the olives washing machines in the manufacture process to finally close the loop. The raw OMW was previously subjected to an advanced oxidation process (AOP) based on homogeneous Fenton-like reaction. The authors reported that high and stable permeate fluxes were yielded by the RO membrane unit upon recirculation of a fraction of the permeate stream to the bulk tank. Under those conditions, 100% suspended solids, phenols and iron removal was achieved, in addition to 99.4% and 98.2% overall COD and conductivity rejection efficiencies were obtained, respectively. In another work, Ochando-Pulido et al., [63] pretreated wastewater derived from the washing of the olives (OWW) and from the olive oil washing during the vertical centrifugation (OMW) in ratio 1:1 by pH-temperature flocculation stand-alone or followed by photocatalysis with lab-made TiO₂ nanoparticles under UV irradiation. The wastewater pretreated was sent to integrated batch UF and NF membranes-in series process. Significant and stable fluxes were observed on both UF and NF membranes, 15.5 and 22.2 L h⁻¹ m⁻², respectively. Finally, the treatment line just comprising UF preceded by pH-T flocculation and UV/TiO₂ photocatalysis provided an effluent compatible for irrigation and permitted reducing considerably the membrane plant dimension. In another study Ochando-Pulido et al. [64], carried out treatment for olive mill wastewater coming two-phase olive oil production process (OMW-2) on industrial scale. The OMW are pre-treated using Fenton-like oxidation followed by flocculation-sedimentation and filtration through olive stones and with a final step of reverse osmosis (RO). Operating pressure of 25 bar helped to achieve significant steady state permeate flux (21.1 L h⁻¹ m⁻²) and minimize membrane fouling, ensuring less than 14.7% flux drop and up to 90% feed recovery.

Dhaouadi et al., [65] in order to optimize the filtration and reduce the fouling very a short backwashing was adopted using external ceramic membrane bioreactor. The experimental study was carried out with various diluted OMW solutions continuously fed to the reactor

in order to permit the biomass to become gradually acclimated. The biomass was previously adapted to phenol, being highlighted by the authors the fact of its importance as it permits the microorganisms possess the enzymatic material necessary to degrade the phenolic compounds, thus producing a new population which is adapted to this toxic agent and which is able to consume it as a substrate. During a backpulse, pressurized air (2-4 bar) is used to force a known volume of permeate to go back through the membrane to the feed side. This approach allowed to stabilize permeate flux around $92 \text{ L h}^{-1}\text{m}^{-2}$, with zero suspended solid and no phenolic compounds. Alternative to conventional pre-treatments are biotechnological techniques integrated with membrane process that could play a key role in OMWW valorization as well as fouling mitigation. The removal of pectins integrated with an MF step to reduce the severe fouling that occurred during MF of OMWWs was investigated by Gebreyohannes et al. [52]. Pectinases were immobilized on the MF membrane surface and flux performance as a function of time was monitored and compared with inert membrane. Steady-state flux through the enzyme immobilized on the membrane surface was 50% higher than for the inert membrane. The mechanism for the better performance of the biocatalytically active on the surface is due to degradation of deposited pectins (in situ self-cleansing) in combination with the continuous removal of product inhibition from the reaction site. In another work Gebreyohannes et al. [47], developed biocatalytic membrane micro reactors (BMRs) using superparamagnetic nanoparticles both as enzyme carrier to form bionanocomposites and as nanofiller to form organic-inorganic (O/I) hybrid membrane. BMR is applied in an in-situ enzymatic membrane cleaning for a pectin/polygalacturonase system, as encountered in a typical vegetation wastewater.

1.7.7.2. Integrated membrane operation: state-of-the-art

After separating the organic load from the wastewater as reported above, the phenolic compounds can be recovered and concentrated using a combination of different membrane process in sequential design. The integration of membrane operation provides multiple effects including: environmental remediation thus comply with environmental regulations, pure water reclamation hence reduced fresh water consumption and recovery of costly biophenols. The most common flow sheet used to treat OMWW is shown in Figure 1.4. In particular, different fractions were derived from integrated membrane system: a fraction of MF and UF concentrated containing the larger organic components, which can be

submitted to anaerobic digestion for the production of biogas, a fraction of NF and RO concentrated containing enriched and purified phenolic compounds of potential interest for food, nutraceutical, pharmaceutical, or cosmetic applications. Besides, a fraction of RO permeate almost pure water for recycling/irrigation or for free disposal to aqueous acceptors (lakes, rivers, or sea).

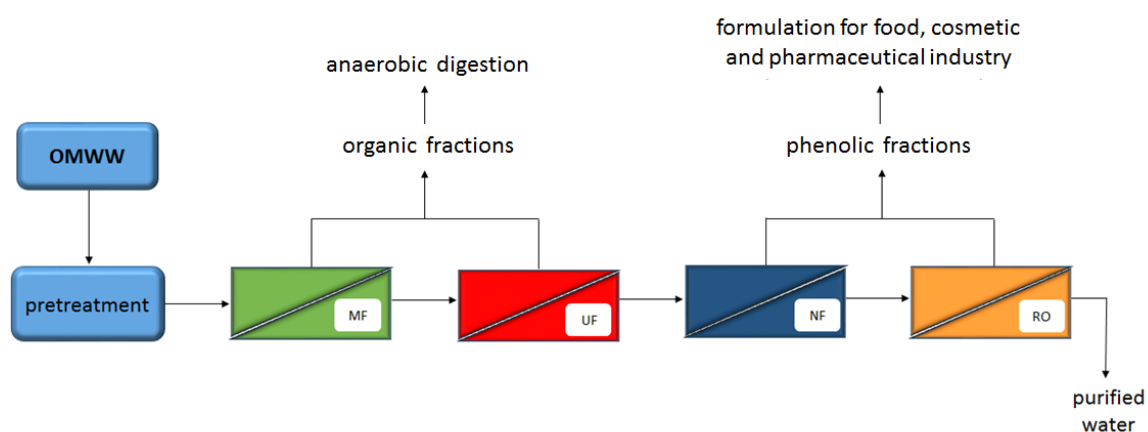


Figure 1.4. Flow sheet of integrated membrane process for OMWW treatment.

Depending on the purpose of whether a high degree of purification is needed, or recovery of biophenols is the target, the membrane selection and their arrangements might vary. Paraskeva et al. [66] proposed a combination of UF and NF or RO membranes for the complete fractionation of OMWWs into by-products. The OMWW samples, prefiltered with a polypropylene screen (80 μm), were submitted to UF membrane made of zirconium oxide with pore sizes of 100 nm and a surface area of 0.24 m^2 . The following NF treatment of the UF permeate with spiral-wound polymeric membranes (MWCO of 200 Da) produced a concentrated stream containing more than 95% of phenolic compounds of the initial value. Further and better separation of the various fractions may be achieved through RO membranes. This step permitted a decrease of up to 98.95% of the raw water conductivity, with a recovery between 75-80% of the initial OMWW volume. The content of polyphenols in the RO concentrate was determined as 6.782 g L^{-1} with rejection polyphenols close to 100%. The combination of ultrafiltration, nanofiltration and/or reverse osmosis the OMW produced permeate fraction with quality characteristics that make it possible to be discharged in aquatic systems according to national or EU regulations or to be used for irrigation.

Canepa et al. [67], reported the first application of membrane technology for OMWWs treatment. This work proposed a treatment OMW based on membranes and adsorption process at pilot scale. The treatment process comprised UF with polysulfone membranes, after which the UF permeate stream was treated with adsorbing polymers. The resins eluate was finally conducted to treatment with two RO polypiperazine-amide membranes in series with nominal rejection to NaCl of 97% and 95% respectively for first and second RO unit. The COD and polyphenols rejection of about 93% were obtained in the final RO treated effluent. The proposed integrated membrane process, without adding any chemicals or thermal energy, permits the treatment of OMWW, obtaining about 70% of fresh water with good characteristics for both recycling and irrigation. Coskun et al. [68] investigated the treatment of three-phase OMW previously centrifuged, then filtered via UF membranes followed by NF, and finally RO membranes. The membrane fluxes reached values up to 28.3 L/m² h for the NF membranes, while the RO process showed a flux up to 15.5 L/m². The maximum COD removal efficiencies obtained at 10 bar ranged from 59.4-79.2% for the NF membranes, whereas between 96.2% and 96.3% for the RO membranes, respectively. For the selected RO membranes, the conductivity removal efficiencies obtained at 25 bar ranged between 93.2% and 94.8%. An integrated membrane process for producing clean water from OMW was developed by Zirehpour et al. [5]. The treatment process is based on three stages; MF pretreatments to eliminate organic load, suspended solids as well as oil and grease, an UF stage and NF as final treatment stage (NF270, NF90 and self-made NF). The NF-270 had resulted higher permeate flux than other examined NF membranes, while NF-90 showed high removal efficiency for all pollution (COD, UV254, total phenols, conductivity, color indices studied by more than 90%. A combination of UF and NF processes for OMW treatment was investigated by Stoller [69]. First, the critical fluxes were determined and the appropriate operating conditions for the UF and the NF process were calculated. The result showed that, the UF step permitted rejection values of 50% for COD and 47% for polyphenols. The NF observed a rejection value of 77% for COD and 97% for polyphenols. The NF residue contained a polyphenolic content of 16.0 mg L⁻¹ with concentration factor of 9.8. Hamza et al. [70], studied the performance of pilot-scale process in terms of its ability to remove the pollutant load and recover fine chemicals from OMW. The process was based on a combination between enzymatic hydrolysis by a β -glucosidase in order to release of hydroxytyrosol and membrane filtration technology. The membrane separation was integrated with concentration procedures through the vacuum evaporation of the ultrafiltration permeate at 45 °C for 2 h.

The concentration of UF permeate has increased the hydroxytyrosol concentration up to 7.2 g L⁻¹.

In another work [71], the β -glucosidase was employed for enzymatic hydrolysis of the NF retentate that is highly concentrated with polyphenols using integrated membrane systems (MF-UF-NF). An integrated membrane system for the valorization of OMWs through the selective recovery of valuable biophenols was investigated by Conidi et al. [72] In this work, pressure-driven membrane processes such as MF and UF were used as pretreatment steps to produce a permeate stream containing phenolic compounds, and then submitted to a bioconversion step using a biocatalytic membrane reactor. In this last system, the oleuropein is converted to oleuropein aglycone by β -glucosidase immobilized in a polymeric membrane. A multiphasic biocatalytic membrane reactor (MBMR) was also used to guide the transformation of biophenols to a specific valuable product (the isomer of oleuropein aglycone) and its simultaneous isolation in the organic phase. In this step, two different fractions were produced: an organic phase containing the isomer of oleuropein aglycone and an aqueous phase containing water-soluble biophenols. The maximum oleuropein conversion reached was about 45.7%. Russo et al. [49] studied the reclamation of OMW by preliminary MF followed by two UF step (6 kDa followed by 1 kDa membranes) and final RO operation. Best productivities were 50 L/hm² for VRF equal to 3 for the ceramic MF membrane, whereas 10–15 L/hm² for UF with polymeric membranes of the MF permeate and up to 35 L/hm² when ultrafiltering the UF permeate with 1 kDa ceramic membranes. Finally, 20-25 L/hm² were yielded by the RO membrane. The final product had a concentration of free low-molecular weight phenols at 0.5 g L⁻¹, with 80% being hydroxytyrosol. OMWs were fractionated on the laboratory scale using a sequence of different membrane operations by Cassano et al. [73]. In the process proposed, OMWs were pre-treated by UF using hollow fiber with pore size of 0.02 micron in order to remove suspended solids and reduce fouling phenomena in the subsequent membrane operations. The UF permeate was then submitted to a UF process using a flat-sheet membrane (MWCO of 1 kDa). The UF permeate was concentrated by using a spiral-wound NF membrane (NF90, Filmtec/Dow) in a concentrated phenolic solution. Analyses of total phenols in samples from the integrated membrane process revealed rejections of about 26% and 31% for both UF membranes and a rejection of 93% for the NF membrane. Basically three different fractions are produced: a concentrated solution containing organic substances at high molecular weight (retentate of both UF processes): this fraction, depleted of polyphenolic compounds by UF, could be submitted to an anaerobic digestion

for the production of biogas; a concentrated solution (NF retentate) enriched in polyphenolic compounds suitable for cosmetic, food and pharmaceutical industries as liquid, frozen, dried or lyophilized formulations; a water stream (NF permeate) which can be reused in the olive oil extraction process as process water or in the integrated membrane system as membrane cleaning solution or in the diafiltration step to increase yield of polyphenols in UF permeates. Fouling by OMWW is also highly dependent on type and characteristics of the membrane. For this purpose Cassano et al. [74] made a comparative study to evaluate the performance of UF membranes based regenerated cellulose (MWCO of 5 and 10 kDa) and PES (MWCO of 4 and 10 kDa). Results showed that regenerated cellulose membranes exhibited lower rejections toward phenolic compounds; higher permeate fluxes and lower fouling index compared to PES membranes. Garcia-Castello et al. [51] analyzed the potentialities of an integrated membrane system for the recovery, purification and concentration of polyphenols from olive mill wastewater (OMWW). The proposed system included some well-known membrane operations such as microfiltration (MF) and nanofiltration (NF), osmotic distillation (OD) and/or vacuum membrane distillation (VMD).

In this work, the vegetation water after pre-treatment was submitted to the MF step. The MF permeate was fed to a NF process and finally the NF permeate was concentrated by OD or VMD. Nanofiltration led to a solution of polyphenols susceptible to be concentrated and used in food, cosmetic or pharmaceutical sectors. A more concentrated solution, enriched in polyphenols (about 0.5 g/L), and particularly in hydroxytyrosol (0.28 g/L), was obtained by osmotic distillation.

Concerning the efficiency of the two concentration processes analyzed, the VMD has the advantage of higher transmembrane fluxes, and it is remarkable that the energy consumptions should be lower in OD. Others authors proposed the use of membrane distillation and osmotic distillation for the concentration of polyphenolic compounds phenols. For this purpose, El-Abbassi et al. [75] investigated by the performance microporous polytetrafluoroethylene (PTFE) membrane of different pore sizes. (0.2, 0.45 and 1 micron). They reported that the PTFE membranes of 0.2 micron exhibit reasonable permeate fluxes ($2.9-4.2 \text{ Lh}^{-1}\text{m}^{-2}$) and a high retention of the phenolic compounds in the feed membrane side. The obtained concentration factor of phenolic compounds was up to 1.2 after 280 min of crude OMW processing by OD. When using MD with a temperature difference of 20°C and a mean temperature of 30°C , this concentration factor reached a value up to 1.9 after 30 h of operating. Di Lecce et al. [76] made the fractionation of OMW

using a two-step MF (tubular polypropylene) and NF (polyamide thin-film composite spiral wound) membrane process at pilot scale. Previously, the OMW samples were filtered through a cotton fabric filter to reduce the suspended solid concentration. Results revealed a rejection of the NF membrane towards COD, dry matter, phenolic compounds and antioxidant activity higher than 98%. The purified NF permeate stream obtained presented COD and phenolic contents values very close to those requested for discharge into surface waters. Konstantinos et al. [77] proposed a novel scheme of concentration of initially MF clarified OMWW, using a two-step tubular RO technology. The OMWW, prior to RO concentration, was clarified in two successive steps: passing through a coarse rotating finisher and by ceramic microfiltration, in order to remove suspended matter and facilitate the RO concentration. The clarified raw material was then concentrated using a two-step tubular RO technology, employing a low rejection (retention 60% as CaCl_2) membrane in the first stage and a high rejection (retention 99% as NaCl) in second step. The results of this study show that the proposed two-stage RO scheme may be potentially applied in a commercial scale and contribute towards processing of OMWW to produce liquid antioxidant and water suitable for disposal to natural resources. Regarding water reclamation, considering an annual discharge of 30 million m^3 OMWW, integration of the membrane can save up to 21 million m^3 of fresh water withdrawal per annum. In some cases, up to 90% volume reduction was reported. This leaves us with the management of sludge that only has 10–15% moisture content [47]. Coskun et al. [78] investigated the performance of RO membranes (GE osmonics Polyamide, NaCl rejection 99.5%) using OMWWs after pre-treatment with centrifugation and ultrafiltration step. The COD removal efficiencies and EC decrease were approximately 98% were obtained. In others studies, Forward Osmosis (FO) is applied to de-hydrate OMWW within the logic of zero liquid discharge and by-products valorization. Single-step FO operated with 3.7 m MgCl_2 draw solution and 6 cm/s crossflow velocity resulted in a volume reduction of 71%, complete decolorization of the permeate, and more than 98% rejection to OMWW components, including biophenols and ions. The concentrated stream leaving FO was further treated by UF (PSU UF 100 kDa flat sheet) or NF (tubular ceramic NF 10 nm) to purify and fractionate the biophenolic compounds. The use of NF and UF to recover polyphenols from a 50% dehydrated OMWW resulted in 92% and 83% retention, respectively [79].

1.8. Patent development on OMWW treatment by membrane technology

Several patents have been developed to recover biophenolic compounds from OMWW using membrane technology. An integrate membrane process based on MF, UF, NF, and RO operations for the recovery of bioactive compounds from OMWWs was proposed by Villanova et al. [80]. In this approach, OMWWs are previously pretreated by rough filtration and then submitted to a sequential treatment of membrane processes from MF to RO. The final step permitted concentrations of hydroxytyrosol of more than 1 g L^{-1} (initial OMW had 0.26 g L^{-1}) and tyrosol of more than 0.6 g L^{-1} (initial was 0.08 g L^{-1}). The process includes chromatographic separations of tyrosol, hydroxytyrosol, and other phenolic compounds from the concentrated RO. In addition, oxidization of tyrosol to hydroxytyrosol was performed. Method of obtaining a natural hydroxytyrosol rich concentrate from olive tree residues and subproducts using NF and RO processes was patented by De Magalhães Nunes da Ponte et al. [81]. In this work, an aqueous extract obtained from leaching of an olive cake with water at ambient temperature, was fed to nanofiltration unit using membrane with an MWCO of 250 Da. Recovery yield of hydroxytyrosol of 70% in the permeate stream was obtained. The obtained permeate was further processed by reverse osmosis using an SW30 membrane. The natural extract of hydroxytyrosol between 15 and 98% mass fraction is achieved, and its utilization in the form of solid particles, as an aqueous solution, as an emulsion or as lipidic based nanoparticles in the food, pharmaceutical and cosmetics industries is proposed.

Integrated membrane system based on MF, UF, NF and RO operations was patent by ENEA [48] for the recovery of bioactive compounds from OMWWs. Before to carry out the membrane process, the OMWWs are pre-treated as reported above and subsequently submitted to an MF step carried out with ceramic membranes (pore size of 0.1 and 1.4 μm) and the MF permeate is then fed to a UF unit equipped with spiral-wound polymeric membranes with an MWCO ranging between 1 and 20 kDa. Solid residues from the centrifugation step can be added to MF and UF retentates and used as a basis for compost production or submitted to aerobic or anaerobic treatments. The UF permeate is submitted to an NF treatment with spiral-wound polymeric membranes (polyamide or nylon, MWCO of 150-250 Da). A final RO treatment by using spiral-wound composite polyamide membranes produces a concentrated liquid enriched in purified polyphenols of interest for pharmaceutical applications and ultrapure water suitable for beverage formulations. In particular, the RO operation produced a retentate containing 464.870 ppm free low MW

polyphenols composed of 390.449 ppm hydroxytyrosol, 27.400 ppm tyrosol, 14.968 ppm oleuropein, 14.329 ppm caffeic acid and 17.724 ppm protocatechuic acid.

In another patent published by Romani [82], is illustrated an integrated process for recovery of a polyphenol fraction and anaerobic digestion of OMWW. More specifically, the invention provides OMWW pre-dilution, centrifugation, selective fractionation for the recovery of polyphenolic compounds by means microfiltration (MF) of the surnatant, followed by a ultrafiltration (UF) treatment carried out on the microfiltration permeate; the retentate coming from the MF partly undergoes again the pre- dilution and partly, mixed with the UF retentate, is reprocessed in centrifuge in order to undergo further cycles of extraction of polyphenols from the moist husks. UF permeate is in turn fed to the treatment with polymeric resins to obtain purified fractions enriched in polyphenols by means of an operation of adsorption-desorption on inert polymeric resin.

Furthermore, treatments of the dephenolised olive mill wastes on aerobic and anaerobic columns, as a preliminary step for the treatment thereof for biogas and high quality fertilizers having a good bioavailability in the soil.

1.9. Polyphenols-based formulations

Polyphenols are object of growing interest in pharmaceutical and food industries as potential therapeutic agents against serious diseases, like cancer, diabetes and cardiovascular disorders, acting against reactive oxygen species and preventing cell damages caused by oxidative stress. Nevertheless, polyphenols, containing unsaturated bonds in their molecular structure, are not chemically stable as they are susceptible to oxidative deterioration, when exposed to oxygen, light, moisture, and temperature. Oxidation of polyphenols leads to unpleasant flavor taste, affecting thus the quality and limiting the shelf life of the products in which they are incorporated [83]. For this purpose, polyphenols needs to be masked before incorporation into food products and encapsulation is considered as a relevant technique. It consists of the preparation of an emulsion or dispersion that contains the encapsulating agent and the core material (bioactive compound). The utilization of encapsulated polyphenols in micro/nanoscale systems instead of free compounds can overcome the drawbacks of their instability, alleviate unpleasant tastes or flavors, as well as improve the bioavailability and half-life of the compound in vivo and in vitro. In recent years, encapsulation technology has increased in importance in the food industry, particularly in the development of functional and healthy

foods. Studies on polyphenol encapsulation have mainly focused on protection from environmental conditions (light, oxygen, temperature and water), avoiding oxidation and increasing the shelf life of active compounds [83]. Moreover, encapsulation in a nanoengineered carrier can be used in order to achieve targeted delivery of the molecules into the diseased tissue with a controlled release profile. Wide variety systems such as emulsions, liposomes and polymer particles have been used as components of food, cosmetics, and drugs enhancing the bioavailability of polyphenols. Conventional techniques for preparing particles include rotor stator systems, high-pressure homogenizers, ultrasonic homogenizers and magnetic stirrers. The principle of these methods is as follows: droplets are broken and re-broken to make final expected droplet sizes. With each successive breaking and re-breaking of the droplets, the energy required increases because the smaller the droplet the more it resists deformation. Consequently, these methods have in common some disadvantages such as high-energy requirement, high shear and stress forces created, difficult to control droplet sizes and polydisperse distribution [84]. Relatively newer emulsification technique, such as membrane emulsification described following (section 1.10), offers great potentials in manufacturing emulsions with tailored microstructure in terms of droplet size and size distribution.

For polyphenols encapsulation, a number of approaches have been introduced to formulate such systems, which consists of polysaccharides (maltodextrins), polymers, proteins (gelatin, casein and soy) and vegetable oil. An overview of polyphenols-based formulations are reported in Table 1.6, which different techniques are used. Navarro et al. [85] used ultrafiltration and nanofiltration of OMWWs and successive spray drying with maltodextrin and acacia fiber as antiglycative ingredients for foods or pharmacological preparations. Petrotos et al. [86] clarified OMWWs by using membrane technology and the recovered biophenols, after been processed by RO membrane technique followed by freeze dry, were encapsulated to be used for enrichment of yogurt and dairy products. Troisi et al. [87] evaluated the ability of biophenols obtained from OMWWS through ultrafiltration and successive spray drying in controlling the ultrahigh-temperature milk treatment thermal processing. Mourtzinis et al. [88] used olive leaf extract, rich in oleuropein, to form an inclusion complex with β -cyclodextrin mixing of the components in aqueous media and subsequent freeze-drying. The encapsulation can protect compound interest from oxidation, besides used phase solubility studies showed that encapsulation of olive leaf extract in cyclodextrin increased the aqueous solubility of the polyphenolic residue from olive leaf by more than 150%. In another works, β -cyclodextrins were used to complexing

tyrosol [89], acid gallic [90], hydroxytyrosol [91]. Hydroxypropyl- β -cyclodextrins (CD) were used to embed caffeic acid (CA) inside their cavity with increasing water solubility of CA [92]. The authors suggest that HP-CD is powerful pharmaceutical tools for the encapsulation or releases of this potent antioxidant. Kosaraju et al. [93] reported encapsulation of olive leaf extract (OLE) in chitosan microspheres by a spray-drying process. The loading percent of polyphenolic compounds through encapsulation of OLE achieved was 27%. Gonçalves et al. [94] evaluated the precipitation and encapsulation of hydroxytyrosol-rich extract into a lipophilic solid matrix through supercritical fluid technology (SCF). By using glycerol monostearate as lipid carrier to encapsulate the extract, it was possible to produce solid lipid particles rich on hydroxytyrosol.

Caporaso et al., [95] produced emulsion formulated with phenolic extracts from olive mill wastewater (OMW) in model olive oil-in-water (O/W) emulsions to study their effect on their physical and chemical stability. Spray dried OMW polyphenols were added to a model 20% olive O/W emulsion stabilized with whey protein isolate (WPI) and xanthan gum. The authors showed that addition of encapsulated OMW phenolic did not result in a significant improvement of physical stability. The formation of oxidation products was higher when higher concentrations of encapsulated polyphenols were used, indicating a possible binding with the WPI added in the system as a natural emulsifier. Robert et al. [96] performed experiments to assess the effect of acetylation of starch or inulin on the encapsulation efficiency of these agents with gallic acid (GA) and to evaluate the release behavior of GA from microparticles in water. The results of this work showed that GA encapsulation efficiency was higher for native inulin (83%) than for native starch (47%). Instead, the addition of an acetyl group to starch improved the GA-polymer interaction, with consequent improvement of encapsulation efficiency. However, the opposite effect was observed for acetylated inulin. The acetylation of inulin only slightly controlled the release of GA from microparticles in water (hydrophilic system). However, the GA release was fast from all systems evaluated, which suggest that the microparticles would be best suited to dry mixes or instant foods, to maintain the nutritional value of GA. Katuwavila et al. [97], presented an study aimed to improve the skin penetration of caffeic acid, a very effective free radical scavenger, by encapsulating in liposomes. Caffeic acid loaded liposomes prepared using the reverse phase evaporation technique showed an encapsulation efficiency of 70%. In vitro diffusion through a dialysis membrane enabled 70% release of encapsulated caffeic acid within 7 h, whereas 95% of free caffeic acid diffused within 4 h in buffer solution (pH 7.4). The complexes formed by two polyphenols,

trans-Ferulic acid (FA) and Gallic acid (GA) with 2-Hydroxypropyl- β -Cyclodextrin (HP- β CD), by the spray-drying method, were studied by Olga et al. [98]. Estimated encapsulation efficiency measurements revealed an antagonistic effect between the two phenols, arising from the fact that the encapsulation of FA was found to be favored against GA in the coencapsulated complex. Polyphenol mixtures, composed of four different molecules (tyrosol, caffeic acid, vanillic acid and p-coumaric acid), were encapsulated chitosan/dextran multilayer microcapsules by the electrostatic layer-by-layer self-assembly technique. The loading efficiency of $42\pm 0.6\%$, was obtained with an ethanol with a total release of 11.5 ± 0.7 mg of total polyphenols per $11.3 \mu\text{L}$ of microcapsules after 240 min of incubation in acidic environment. The results suggest that polysaccharide-based capsules can be successfully used to encapsulate and release low water-soluble molecules, such as polyphenols [99]. Chatzidaki et al. [100] carried out a study of two different W/O dispersions, an emulsion and a microemulsion, containing medium chain triglycerides (MCT) as the continuous phase and used as reservoirs of hydroxytyrosol (HT). The proposed systems were studied for the scavenging activity of the encapsulated antioxidant toward galvinoxyl stable free radical showing a high scavenging activity of HT in both systems. Water-in-oil-in-water (W/O/W) emulsions loaded with a bioactive oleuropein were prepared via high-pressure homogenization and subsequent microchannel (MC) emulsification. The formulation W/O was prepared using as internal aqueous phase sodium phosphate buffer containing D(+)-glucose and oleuropein (0.1-0.7 wt. %). The oil phase consisted of soybean oil and tetraglycerin monolaurate condensed ricinoleic acid esters. The external aqueous phase for W/O/W emulsion was sodium phosphate buffer containing D(+)-glucose and decaglycerol monolaurate [101]. In another work, O/W emulsion and an emulsion gel containing oleuropein were prepared, applied and evaluated on healthy volunteers who had undergone UVB irradiation to investigate its protective and/or lenitive activity. O/W emulsion and emulsion gel prepared using silicon oil and paraffin oil respectively showed that oleuropein formulations highlighted lenitive efficacy by reducing erythema, transepidermal water loss and blood flow of about 22, 35 and 30% respectively [102].

Mohammadi et al. [103], produced a W/O/W formulation in order to protect and control the release of phenolic compounds of olive leaf. Emulsions W/O/W, were formulated by W/O micro-emulsions as primary emulsions and applying a complex of biopolymers as hydrophilic emulsifiers in the external aqueous phase. Multiple emulsions were made in a two-step procedure. First, W/O micro-emulsion was produced using soybean oil and span

80 (organic phase) and aqueous phase containing olive leaf extract as dispersed phase. The pre-double emulsions were formed by gradually adding the primary W/O microemulsions into the continuous aqueous phase containing biopolymers during homogenization with rotor-stator homogenizer (8.000 rpm for 5 min). Two types of biopolymer solutions were applied: (1) single layer whey protein concentrate (WPC), and (2) two layer whey protein and pectin (WPC-P). These W/O/W emulsions were then further emulsified using the mentioned homogenizer (15.000 rpm for 8 min). The encapsulation efficiency of phenolic compounds for stabilized W/O/W emulsions with WPC and WPC-P were 93.34% and 96.64%, respectively, which was decreased to 72.73% and 88.81% at 20th storage day. These results suggest that nano-encapsulation of olive leaf extract within inner aqueous phase of W/O/W emulsions was successful, and there could be a high potential for the application of olive leaf extract in fortification of food products. Acosta et al. [104], designed chitosan-based hydrophilic system containing an olive leaf extract. Encapsulation of olive leaf extract in chitosan microspheres was carried out by a spray-drying process. The resulting data showed that the developed formulations were stable up to three months. The encapsulation efficiency was around 44% and the release properties of polyphenols from the microspheres were found to be pH dependent. At pH 7.4, polyphenols release was complete after 6 h; whereas the amount of polyphenols released was 40% after the same time at pH 5.5. Vashisth et al., [105] used electrospinning approach to encapsulate ferulic acid in polymeric nanofibrous matrix (poly (D-L lactide-co-glycolide)/polyethylene oxide) to combat the shortcomings such as physiochemical stability and premature degradation. These ferulic acid encapsulated could be potentially explored for therapeutic usage in biomedical sector. Di Mattia et al., [106] prepared olive oil-in-water (o/w) emulsions stabilized by β -lactoglobulin (BLG), enriched with olive phenolic compounds, namely oleuropein, tyrosol and hydroxytyrosol, and added with maltodextrins. Emulsions were prepared by two successive steps: a pre-emulsion was obtained with a high shear mixing at 20.000 rpm for 1 min high, pressure homogenization of the pre-emulsion was then carried by applying five homogenization cycles at 150 bar. Maltodextrins significantly affected both the properties and oxidative stability of the systems. The effect of MD on properties and stability was related to the higher viscosity of the systems, but also to complex interactions that might have taken place among emulsifier, phenolic compounds and maltodextrins. The effects of vesicular incorporation on verbascoside diffusion through skin were investigated using skin fragments excised from newborn pigs [107]. Liposomes load with verbascoside were produced from soy

phosphatidylcholine and cholesterol according to the film hydration method. Results showed that verbascoside can be incorporated in liposomes (encapsulation efficiency of 57–66%), preventing its degradation. Besides, liposomes promoted drug accumulation into the stratum corneum but they did not give rise to any significant transdermal verbascoside delivery. Rutin and naringenin were flavanone predominant in olive and grape fruit respectively. These compounds were encapsulated in phosphatidylcholine-based onion-type multilamellar vesicles (MLVs). Naringenin was shown to be poorly encapsulated (<10%) but highly adsorbed on MLVs surface (>60%). Conversely, rutin showed high encapsulation efficiency (>60%). In another formulation, rutin was encapsulated within the internal aqueous phase of W/O/W multiple emulsions with an encapsulation efficiency higher than 80% [108]. Rutin-loaded nanocapsules based in poly-(ε-caprolactone) and rutin-loaded nanoemulsion based in grape seed oil with encapsulation efficiency close to 100% were prepared by the interfacial deposition of preformed polymer method and spontaneous emulsification, respectively [109].

Tabelle 1.6. Summary of polyphenols-based formulations.

Phenolic compounds	Materials	Type of formulation	Technical production	Encapsulation efficiency (%)	Ref.
Olive oil polyphenol powders	Maltodextrin and acacia fiber	-	Spray dried	80.2	[86]
Olive leaf Extract rich in oleuropein	β -Cyclodextrin	Inclusion complex	Freeze-drying.	-	[89]
Olive leaf extract	Chitosan	Microsphere	Spray-drying	27	[95]
Polyphenols extracted	Maltodextrin	Microcapsule	Spray drying	76	[112]
Polyphenols from olive kernel and leaves	Maltodextrin	-	Freeze drying	Olive kernel: 82.39 to 92.12 for olive leaves: 87.98 to 91.06	[117]
Tyro sol	Cyclodextrins	Inclusion complex	Coevaporation and kneading	-	[91]
Gallic acid	Native starch (NS) Native inulin (NIn) Starch acetate (AS) Inulin acetate (Ain)	Microsphere	Spray-dryer	NS: 26.7-49.5 AS: 25.8-69.2 NIn: 67.5-96.4 AIn1: 42.6-85.8	[98]
Caffeic acid	Phosphatidylcholine cholesterol	Liposome	Reverse phase evaporation technique	70	[99]
Mixtures of tyrosol, caffeic acid, vanillic acid and p-coumaric acid	Chitosan/dextran	Microcapsule	Electrostatic layer by-layer self-assembly technique	42	[101]
Hydroxytyrosol	Medium Chain Triglycerides	W/O emulsion and microemulsion	Stirring	Hydroxytyrosol in emulsions and microemulsions were 500 ppm and 600 ppm	[102]
Hydroxytyrosol	Perilla oil Gelatine	Emulsion, simple and double Gelled double emulsion	High pressure homogenizer	55	[110]
Syringopicroside and hydroxytyrosol	mPEG-PLGA	nanoparticle	Nanoprecipitation	32.38	[111]
Verbascoside	Cholesterol	Liposome	Film hydration method	57-66	[107]
Rutin	Sunflower oil	W/O/W	Homogenizer for W/O spinning disc reactor for W/O/W	>80	[108]

Continued

Table 1.6. Continued

Rutin	Poly-(ε-caprolactone) Grape seed oil	Nanocapsule nanoemulsion	Interfacial deposition	Close to 100	[109]
Rutin	β-cyclodextrin	Inclusion complex	Coprecipitate	-	[113]
Rutin	Calcium pectinate beads	Gel formulation	Iontropic gelation	95	[114]
Rutin	Phosphatidylcholine	Multilamellar vesicle	Mixing	>60	[115]
Rutin	Triglyceride ester	Nanoemulsion	High pressure homogenization	82	[116]

1.10. Membrane emulsification principle

Membrane emulsification (ME) is an emerging technology of droplet generation in which a dispersed phase is forced through the pores of a microporous membrane in order to form droplets. To ensure a regular droplet detachment from the pore outlets, shear stress is generated at the membrane/continuous phase interface by recirculating the continuous phase using a pump or by agitation in a stirring vessel. The ME technology is characterized by accurate control of the droplet size and size distribution by selecting the membrane with specific pore size, reduced energy requirements, highly flexible manufacturing plant, use of mild operation conditions, and multiple possibilities for manufacturing simple and multiple emulsions and micro/nano particles, such as lipid nanoparticles, nanocapsules, gel microbeads, microcapsules and liposomes [118]. The shear stress is lower than classical emulsification system, because small droplets directly formed by permeation of dispersed phase through the pore of membrane instead of disruption of large droplets in zone of high energy density. In membrane emulsification, droplet formation from an individual pore involves the droplet growth and droplet detachment processes. In general, a linear relationship between membrane pore diameter (D_p) and droplet diameter (D_d) has been observed. It depending on the operating conditions and emulsion composition. A hydrophilic membrane is used in the production of O/W emulsion, while a hydrophobic membrane is used in the production of W/O emulsion.

The main factors influencing membrane emulsification in order to produce uniform particles include [119]:

- membrane parameters, including porosity, mean pore size, pore geometry, pore distance and wettability;
- phase parameters, including interfacial tension, emulsifier type and concentration, viscosity and density of dispersed and continuous phases, phase composition, pH and ionic strength;
- process parameters, including wall shear stress, trans-membrane pressure, membrane module configuration and temperature;

A new method of membrane emulsification method, used in the present thesis for biophenols encapsulation in W/O emulsion and solid lipid particles, is represented by pulsed back-and-forward cross-flow batch membrane emulsification (Figure 1.5) [120].

This technique allows to produce uniform particles using an axial back-and-forward shaking flow of the liquid continuous phase within the lumen of a tubular microporous membrane at fixed amplitude and frequency (single pass pulsed membrane emulsification). The dispersed is pressed through the membrane and the force that determines droplets detachment was exerted by pulsed flow of the continuous phase within the module tangentially to the membrane surface.

The main advantage of the pulsed back-and-forward cross-flow membrane emulsification is that it eliminates the shear stress outside of the membrane related to the recirculation of emulsion droplets along the pump and fitting circuit as it happens in conventional cross-flow membrane emulsification. Therefore, this method is suitable for the production of monodispersed particles containing shear sensitive bioactive molecule.

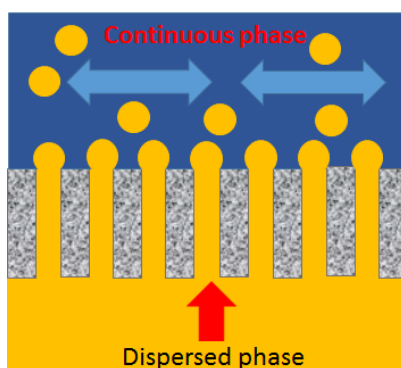


Figure 1.5. Schematic drawing illustrating the pulsed membrane emulsification.

1.11. Conclusions

In many olive-producing countries, olive oil production represents a considerable share of the economy. However, wastes from the olive oil activity are a serious environmental issue due to their heavy load of phenolic compounds, lipids and organic acids. Various treatment processes for the management and reclamation of OMWW have been proposed such as physicochemical, biological and advanced oxidation methods, but these treatments are not able to abate completely the high polluting load. On the other hand, several studies have proved that membrane processes are a sustainable approach for olive mill wastewater treatment.

The use of membranes operation in a sequential design offers a very interesting solution aimed at valorizing its by-products as well as to reduce the pollution load. Integrated membrane systems have demonstrated to be efficient for the recovery, purification, and concentration of phenolic compounds from OMWW, which are difficult to obtain by traditional methodologies.

The analysis carried out on the previous works reported about OMWW treatment by membrane integrated processes demonstrated the high potential of membrane technology on industrial scale in combination with pre-treatment step to achieve high operating fluxes by reducing membrane fouling. Several strategies have been proposed to minimize the fouling phenomena during OMWW processing. Centrifugation, coagulation-flocculation, pre-filtration and enzymatic treatments have been identified as successful strategy. About the valorization of OMWW by products, previous strategies were based on the use of bioconversion to produce different types of high-added value. An alternative strategy was to use the recovered polyphenols compounds as functional ingredients in products formulation for pharmaceutical, cosmetics and food applications. Several polyphenols encapsulation strategies have been proposed in order to preserve the biochemical functionalities of the bioactive components.

The critical analysis of studies reported in literature on the application of membrane technology for OMWW treatment, demonstrated various aspects and many findings that inspiring the advances promoted by the research activity carried out in this doctoral thesis. In particular, the need to separate the bioactive compounds of interest from enzymes or bacteria as soon as possible following the waste production in order to avoid degradation phenomena is an important issue for polyphenols valorization.

In the present work, innovative strategies to improve permeate flux, especially in the first step processes (MF), have been promoted. Furthermore, a systematic holistic investigation of all processes, from OMWW pre-treatment, to processing and potabilization of water as well as polyphenols purification, concentration, fractionation and emulsion based formulation, was carried out.

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CHAPTER 2

OMWWs pretreatment and their performance in MF/UF membrane operations

2.1. Introduction

Olive Mill wastewaters (OMWWs) are extremely polluting and need to be treated before being discharged or to provide opportunities for their reuse or recycling. Because of their high suspended solids content, a pre-treatment method able to abate the severe fouling occurring during the subsequent micro and ultrafiltration membrane processes is mandatory. Indeed, the major obstacle in the development and large-scale application of membrane processes for olive mill wastewater treatment is the loss of membrane flux due to fouling. The high amount of suspended solids contained in OMWWs makes mandatory a pre-treatment step to reduce fouling and increases micro- and ultra-filtration efficiency. Different approaches have been developed and applied as pre-treatment steps based on physical, physical– chemical and biological methods or combination of them. The coarse filtration is the most used physical approach to reduce solids content, applied alone or in combination with other methods. Filtration through three polypropylene membranes with pore size of 50, 5 and 0.2 micron used in series, was reported as a pre-treatment step before ultrafiltration (UF) and nanofiltration (NF) processes by Zirehpour et al. [1]. Filtration with a cotton fabric filter was reported by Garcia-Castello et al. [2] and Cassano et al. [3]. Pre-treatment approaches before UF and NF processes based on either filtration through a 50 μm filter, flocculation eventually combined with photocatalysis and aerobic treatment have been also reported by Stoller et al. [4–7]. Electro-Fenton (EF) was used by Khoufi et al. [8] as first stage of a three-step method for the OMWWs treatment to oxidize toxic and recalcitrant organic compounds including polyphenols. This approach consists in the addition of a mixture of ferrous salts and hydrogen peroxide, able to generate hydroxyl radicals. The second and third step consisted of an anaerobic biological treatment and an ultrafiltration post-treatment, respectively. A new approach to reduce fouling in the OMWWs pre-treatment during MF was used by Gebreyohannes et al. [9]. In this case, a pectinase-loaded membrane, able to reduce pectins as they accumulate on the membrane surface, permitted to obtain a significant reduction of membrane fouling. Different studies

highlighted the importance of pH variation during the pre-treatment processes, pH values being mainly adjusted with the aim of preserving polyphenols from undesired oxidation processes. Russo et al. [10] acidified OMWWs with hydrochloric acid and citric acid up to pH 3.5 to prevent phenols oxidation processes. Akademir et al. [11] used two pH values, 2 and 6, both followed by filtration with a cartridge filter. Ochando-Pulido et al. [12] performed pH flocculation process at pH 2.5 followed by two further treatments (photocatalysis and UF) before the nanofiltration step. El-Abbassi et al. [13] studied the effect of pH on the process performance at various pH values, ranging from pH 2 to pH 12. Stoller and Ochando-Pulido [14] in a recent paper reported a comprehensive critical analysis of all the OMWWs pre-treatments in relation with critical and threshold flux concepts to membrane process plant design. Nevertheless, none of these studies deeply investigated the influence of pH variation on the suspension stability as a way to promote aggregates formation, thus improving the effective removal of suspended solids.

This work focuses on treatment of OMWWs before performing membrane operations. For this purpose, a new approach of OMWWs pre-treatment process based on the study of suspension destabilization was reported. Sample stability determination is an important parameter to define in order to minimize aggregation phenomena facilitating the removal of small particles to filter out for wastewater treatment. The pre-treatment studied in this part of work consists in promoting the coagulation of the suspended particles and to process the coagulated solution with a metallic filter with pores diameter of 35 μm . The filtered solution was then processed with MF and UF membranes. In addition the performance of UF membranes were also evaluated using the permeate coming from MF process. It must be remarked that the solid flocculated fraction recovered after the acidic pre-treatment is not an additional waste to be treated, but it can be used as biomass to produce biogas [14]. The results of microfiltration and ultrafiltration processes after pre-treatment were compared with membrane processes that have used some of the pre-treatment approach above discussed in terms of permeate flux measured at the steady state. Besides, a characterization, in terms of carbon content, amount of phenols and pectins, was performed on the olive mill wastewater before and after the pre-treatment and the MF and UF processes.

2.2. Materials and methods

2.2.1. Chemicals

- Olive mill wastewaters (OMWWs), produced according to a 3-phase centrifugation process, were supplied by olive mill “Olearia San Giorgio, Fratelli Fazari company”. Olive mill wastewaters used were obtained in different milling period from September to February.
- Phenolic compounds, such as hydroxytyrosol, tyrosol, oleuropein, caffeic acid, p-cumaric acid, vanillic acid, were used as standard for HPLC calibrations. All the phenolic compounds were purchased from Sigma Aldrich, while oleuropein from Extrasynthese.
- Acetonitrile, acetic acid and methanol (Sigma Aldrich) were used for HPLC mobile phase preparation.
- Sulphuric acid and sodium hydroxide (Sigma Aldrich) were used for the pH changing of the OMWWs.
- Meta hydroxyphenyl and tetraborate acid (Sigma Aldrich) were used to evaluate the content of pectins in the OMWWs

2.2.2. Membranes

The membrane process was carried out using different membrane materials, configurations and pore size or molecular weight cut-off (MWCO) in order to evaluate the permeate flux and the rejection of the selected membranes towards polyphenolic compounds, pectins and organic carbon. The rejection (R) of the selected membranes towards specific compounds was determined according to the following equation:

$$R_j = \left(1 - \frac{C_p}{C_f} \right)$$

where C_p and C_f are the concentrations of a specific component in the permeate and feed, respectively.

The characteristics of commercial membranes investigated in this work are reported in Table 2.1 and 2.2.

Table 2.1. Summary of MF membrane characteristics tested in the membrane processes for OMWWs treatment.

Membrane configuration	Hollow fiber	Spiral wound	Tubular
Membrane materials	PVDF-PP Blend	PVDF	ZrO ₂
Nominal pore size (micron)	0.2	0.3	0.1
pH operating range	1-10	1-10	0-14
TMP (bar)	0.6	0.7	0.5
Axial flow rate/flow rate (m/s)/(L/h)	/18	/200	0.22/

Table 2.2. Summary of UF membrane characteristics tested in the membrane processes for OMWWs treatment.

Membrane configuration	Tubular Multichannel	Tubular	Spiral wound	Hollow fiber	Flat sheet	Flat sheet
Membrane materials	Al ₂ O ₃	ZrO ₂	PS	PES	PAN	PA
Nominal pore size (micron)	0.03	0.02	–	–	–	–
Nominal molecular weight (kDa)	–	–	20	80	30	3.5
pH operating range	0-14	0-14	1-14	0-14	2-10	1-10
TMP (bar)	2.5	2	1	0.6	1	6.5
Axial flow rate/flow rate (m/s)/(L/h)	1.46/	1.62/	/200	/18	/200	/200

2.2.3. OMWWs characterization

The OMWWs characterization was performed according to the following protocol:

a) *Light scattering measurements*

Particle size and particle size distribution were measured using a laser light scattering system (Malvern Mastersizer 2000, Malvern Instruments). The mean particle size was expressed as the volume weighted mean diameter, (or De Brouckere diameter), $D[4,3]$.

$$D[4,3] = \frac{\sum D_i^4 n_i}{\sum D_i^3 n_i}$$

The volume weighted mean diameter is most sensitive to the presence of large particles. The width of particle size distribution was expressed as a Span number, calculated by the following expression:

$$Span = \frac{D[90] - D[10]}{D[50]}$$

Where: $D[90]$ is the particle diameter corresponding to 90 vol. % on a relative cumulative particle size curve; $D[10]$ is the particle diameter corresponding to 10 vol. % on a relative cumulative particle size curve; $D[50]$ is the particle diameter corresponding to 50 vol. % on a relative cumulative particle size curve, this value is also known as the mass median diameter. The mean particle diameter and span values were calculated on the basis on at least two or three different experiments.

b) *Carbon content quantification*

Carbon content was evaluated by using a TOC-VCSN from Shimadzu. Total Organic Carbon (TOC) is calculated as difference between TC (Total Carbon) and TIC (Total Inorganic Carbon).

c) *Total solids quantification*

All the OMWWs used were characterized in terms of total, suspended and dissolved solids. Total solids are the sum of suspended and dissolved solids contained in a sample, while dissolved solids are solids that are not retained by a filter of 0.45 μm . Total solids were obtained by measuring the dry solids obtained by evaporating water in a thermobalance (Ohaus S.r.l) at 100 °C, while the amount of suspended solids is obtained from the mass balance between total and dissolved solids.

d) *Phenolic compounds determination*

Analysis of phenolic compounds was carried out by using an HPLC system (Agilent 1200 Series, USA) equipped with an UV detector. A C18 column (Luna, 250×4.6 mm, 5 μm) supplied by Phenomenex (Torrance, CA) was used; the operating conditions were as follows: flux, 1 mL/min; temperature, 25°C; pressure, 100 bar; wavelength, 280 nm. The mobile phase was a mixture of 99:1 water/acetic acid (v/v) (solvent A) and a mixture of 90:9:1 methanol/acetonitrile/acetic acid (v/v/v) (solvent B). The gradient elution used was divided in six steps for a total run time of 60 min as follows: starting from 90% solvent A and 10% solvent B, increase to 30% solvent B over 10 min and then isocratic for 5 min, increase to 40% solvent B over 10 min, to 50% over 15 min and to 100% solvent B over 10 min, and finally isocratic for 10 min. The system was equilibrated between different runs for 20 min using the starting mobile phase composition. All measurements were performed in triplicate. A maximum error of ±2% was registered. The total amount of phenols was determined by Folin-Ciocalteu method [15].

The method is based on the reduction of tungstate and/or molybdate in the Folin–Ciocalteu reagent by phenols in alkaline medium resulting in a blue colored product. Gallic acid was used as standard to obtain calibration curve.

e) *Pectins determination*

A sample of OMWWs was treated with ethanol at 85°C for 10 min to induce pectins precipitation, then the solution was centrifuged at 7000 rpm for 10 min in order to recover the precipitate. After that, it was washed 4 times with water (2.5 mL). NaOH 1M was added to solution for the pectins quantification, 0.6 mL of sample was mixed with 3.6 mL of sulfuric acid tetraborate (0.25% w/v). The obtained solution is refrigerated in crushed ice. The mixture was shaken in a vortex mixer and then heated in an oil bath at 100 °C for 5 min. After cooling in a water-ice bath, 60 μL of 0.15% (w/v) solution of m-hydroxydiphenyl was added to mixture. The final solution was shaken, and, within 5 min, the absorbance of a pink complex of galacturonic acid was read at 520 nm in a Perkin-Elmer spectrophotometer. The galacturonic acid is formed from the hydrolysis reaction of pectins with sulfuric acid. A standard solution of galacturonic acid was used for the preparation of the calibration curve.

2.2.4. Pre-treatment of OMWW protocol

The pre-treatment step consisted in pH adjustment to destabilize the suspensions with the aim of achieving the coagulation and precipitation of undesired solids according to Stokes law:

$$v = \frac{g(\rho_s - \rho_f)D^2}{18\mu}$$

where v is the sedimentation rate (cm/sec), d the diameter of the suspended particles (cm), ρ_1 its density and ρ_2 is the density of the medium (g/cm^3), g is the acceleration of gravity (980.7 cm/sec^2) and η is the viscosity of the external phase in poises (g/cm sec).

The pH adjustment was carried out using sulfuric acid based on the possibility to give 2 mol of hydrogen ions for 1 mol of used acid, that implies minor amount of acid to reach the same pH value. Besides, the presence of ions sulfate in the treated stream could permit, by adding an equimolar amount of copper hydroxide, copper sulfate formation that can be used as fertilizer. The coagulation phenomenon was studied as a function of pH, by subsequently adjusting the pH values at 1.5, 4.5, 6, and 9. The pre-treatment effect on microfiltration and ultrafiltration fluxes, polyphenols content, suspended solids was investigated. The pH of OMWW received from the olive oil factory was 4.5 and pH of 1.5 was obtained adding 3.2 (± 0.2) mL of sulfuric acid (95–98% purity) to 1L of OMWW, while pH 6 and 9 were obtained by adding 7 mL and 13.5 mL of NaOH 1 M, respectively.

2.2.5. MF/UF equipment and membrane characteristics

MF and UF processes were performed in a bench scale cross flow system. The plant is formed by a feed and permeate tank, a peristaltic pump (Masterflex L/S) to circulate the feed along the hollow fiber and MF tubular membranes and a magnetic drive gear pump (Techma GPM S.r.l) for spiral wound and flat sheet membranes. A stainless steel module to accommodate the membranes. Two manometers were in addition located before and after the module in order to measure the inlet and outlet pressure and consequently the transmembrane pressure (TMP). The TMP value was regulated by a pressure control valve on the retentate side. A cooling system, fed with tap water, was used to keep constant the temperature of the feed solution at 25°C . For each experimental test the optimized operative conditions in terms of TMP and axial velocity or pump flow rate were used. In Figure 2.1, a schematic representation of the MF/UF system used is showed.

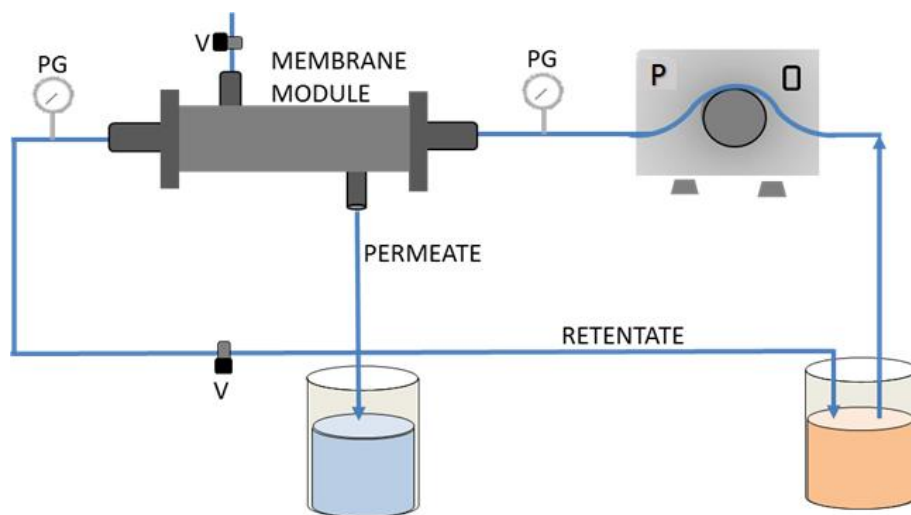


Figure 2.1. Schematic representation of microfiltration/ultrafiltration system. PG: pressure gauges, P: pump, V: valve.

The experimental tests were carried out at constant feed concentration. Besides, in order to achieve higher filtration efficiency, a specific arrangement of the integrated membrane system with MF membrane followed by UF membranes in series mode have been also proposed. In Figure 2.2 flow sheet illustration membrane operations used for OMWWs treatment is reported.

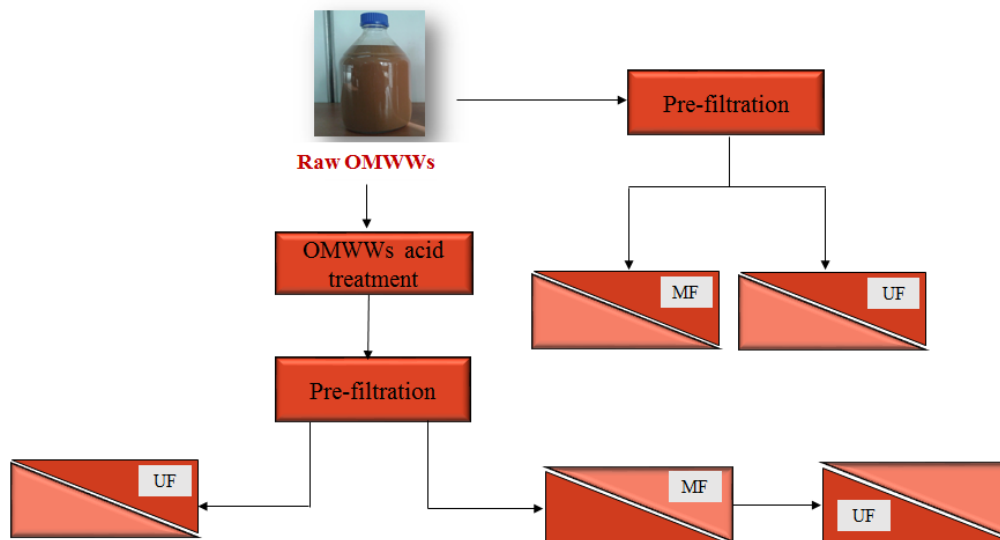


Figure 2.2. Schematic representation of membrane operations used to treat OMWWs.

During the filtration experiments the permeate flux was continuously monitored by measuring the permeate volume collected in a certain time according to Darcy's Equation:

$$Jv = \frac{dV_p}{A_m dt}$$

where Jv is the permeate flux, A_m is the effective membrane area, V_p is the total volume of permeate, and t is the filtration time.

After the treatment with OMWWs, the membranes were cleaned with an 1% (w/w) alkaline detergent (Ultrasil 50 Germany) and 0.1% (w/w) NaOH at a temperature of 40 and 60°C for polymeric and ceramic membranes respectively.

2.3. Results and discussion

2.3.1. Light scattering measurements

Light scattering measurements of OMWWs solution were carried out at different pH. The results obtained in terms of particle size and span are reported in Figure 2.3.

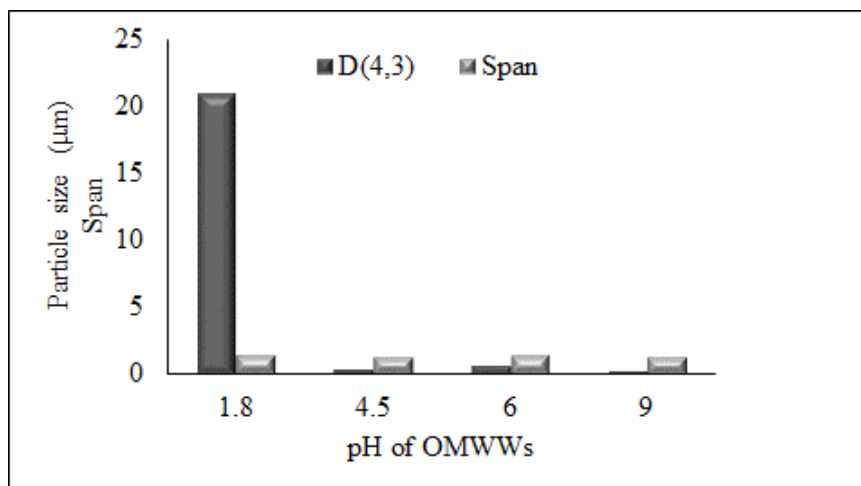


Figure 2.3. Particle size aggregates of OMWW as pH function.

All the analysed solutions have polydisperse distribution ($\text{Span} > 0.8$) and except for the acid pH the size of the more represented particles are in the range of 0.3-0.65 μm , the same as in the received OMWW (pH 4.5). The obtained results indicated the formation of large agglomerates at pH 1.8 with a particle size equal to 21 micron after 1 hour of treatment. At this pH value the attraction forces of particles prevail over repulsion, resulting in aggregation of the solids, which they may be removed through filter of 35 microns. In Figure 2.4 some pictures of OMWW appearance at different pH is shown. In particular the images of OMWWs as received (pH 4.5) at pH of 1.8 and at pH 9 are shown in Fig. 2.4a, b, c, respectively. At pH 4.5 the OMWW suspensions is completely stable, instead at pH of 1.8 it is possible to note a great amount of precipitate. At pH 9 a changing in the colour indicated a degradation of phenolic compounds confirmed by HPLC analyses. Indeed the acid pH of 1.8 allows obtaining different advantages such as avoiding: phenols oxidation, mildew growth and smell formation.

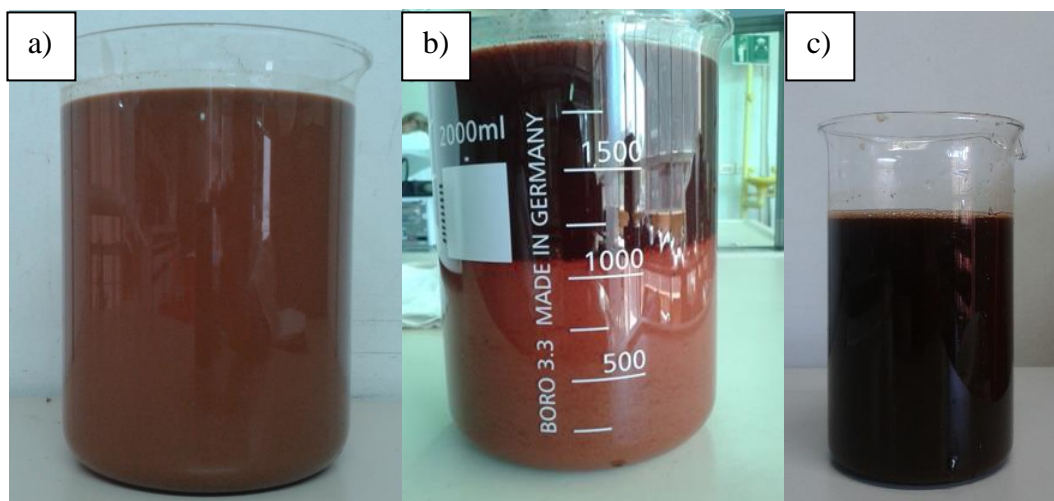


Figure 2.4. OMWWs appearance: a) received from olive mill (pH 4.5); b) after acid pre-treatment (pH 1.8); c) after basic pre-treatment (pH: 9).

2.3.2. Microfiltration of OMWWs

The OMWWs pre-treated until the isoelectric point and filtered through stainless steel filter were then processed by MF processes using the best operating conditions reported in Table 2.1. The collected fractions were then analysed in terms of total solids amount, polyphenols, pectins, carbon content as well as microfiltration and ultrafiltration flux. In order to understand the effect that the acid treatment can have on flux, membrane process was carried out by using different membrane materials with and without pre-treatment before it. In Figure 2.5, the comparison between fluxes measured through three different MF membranes is shown. In all the cases, a significant flux improvement was obtained when using OMWWs after acid pre-treatment. The best performance, after acid pre-treatment at the isoelectric point, was achieved using the inorganic zirconia membranes; where the flux reached a steady state of 60 L/hm² after about 1 h, remaining constant for 3 h.

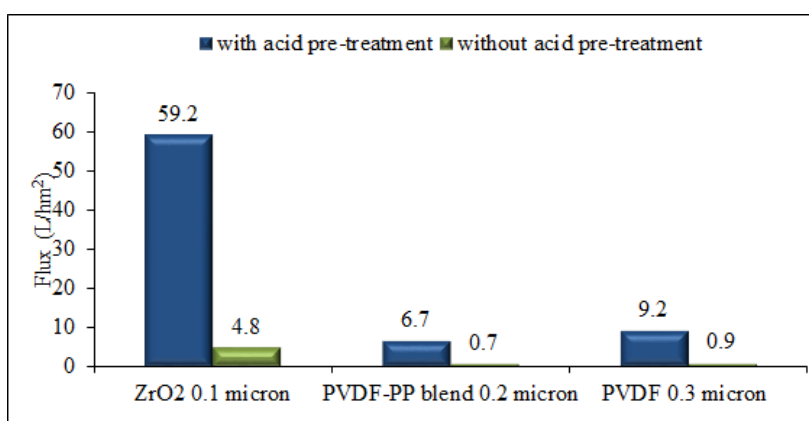


Figure 2.5. Steady state flux of various MF membranes using pre-treated and raw OMWWs.

2.3.3. Ultrafiltration of OMWWs

The behavior of UF membranes is reported in Figure 2.6. For all membrane materials used, the effectiveness of the acid pre-treatment on the flux improvements is also observed. The best performance, after acid pre-treatment, was achieved also in this case using the zirconia inorganic membranes, where the flux reached a steady state of about 23 L/hm². Regarding polymeric materials, the PAN material had resulted higher flux than other examined polymeric membranes (16.2 L/hm²). Indicating that the PAN material has lower interaction between membrane and foulants. It can be due to the more hydrophilic nature of the membrane. Zirehpour et al. [1], reported that membrane surface hydrophilicity is important property which affects upon the behaviour of flux, because the amounts of free water is absorbed by highly hydrophilic groups of membranes surface to form a thin water film, which prevents the deposition OMWWs of components on the surface or membrane pore walls. In this study, independently of membrane materials used, an improvement of fluxes values of approximately 10 times was obtained using the pre-treated OMWWs.

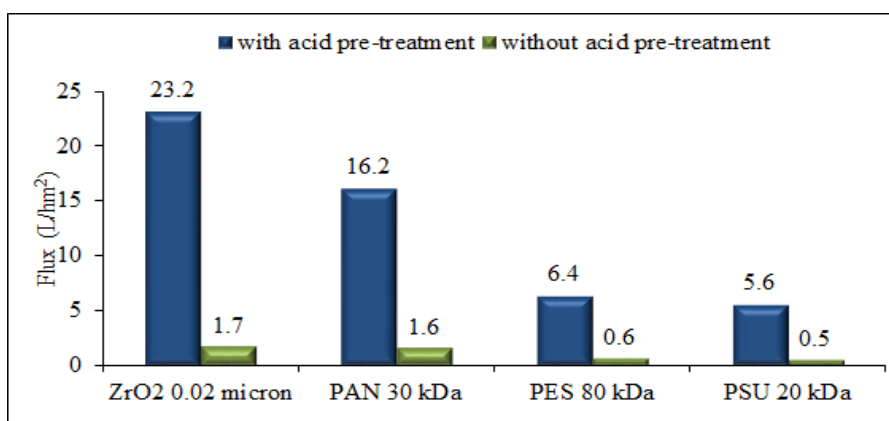


Figure 2.6. Steady state flux of various UF membranes using pre-treated and raw OMWWs.

Considering UF membranes (ZrO₂ and PAN membranes) that showed the highest permeate flux, a significant improvement of the productivity was obtained using as feed the permeate coming from MF process. In particular, steady state permeate fluxes of 46 L/m²h and 31 L/m²h were obtained for ZrO₂ and PAN membranes, respectively. Regarding, the Al₂O₃ and PA membranes, these have produced steady-state flux of 57 L/m²h, while for PA membrane the flux was of 28 L/m²h (Figure 2.7).

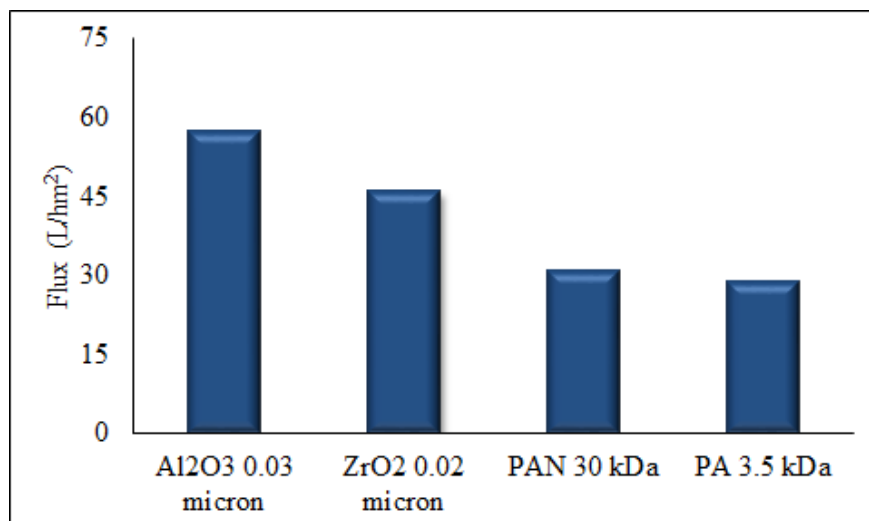


Figure 2.7. Ultrafiltration of pre-treated OMWWs with MF membrane. Steady state flux at different operating pressures (transmembrane pressure of 2.5, 2.0, 0.6 and 6.5 bar for Al₂O₃, ZrO₂, PAN and PA membranes, respectively).

2.3.4. Phenols quantification

To confirm that the pre-treatment does not destroy the polyphenols, which can be recovered using the suitable membrane processes, phenols measurements were carried out in the OMWWs as received and after pre-treatment. Besides, to verify the possible interaction with the membrane materials they were also measured after each MF and UF membrane processes. HPLC profiles of the OMWWs as received and pre-treated are shown in Figure 2.8, showing that no significant changes occurred in term of identified polyphenols. Indeed, the main phenols detected in both cases are: catechol, hydroxytyrosol, tyrosol, vanillic acid and caffeic acid.

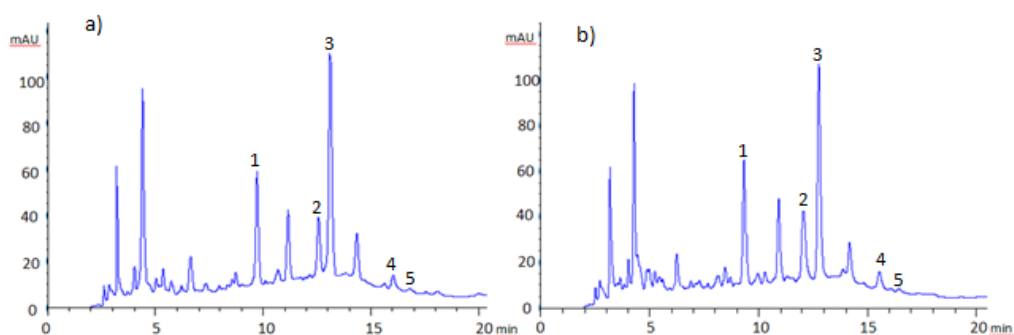


Figure 2.8. HPLC chromatograms of polyphenols from OMWWs as received (a) and after pre-treatment (b). 1: catechol; 2: hydroxytyrosol, 3: tyrosol, 4: caffeic acid, 5: vanillic acid.

On the other hand, changes in the total phenol content were observed. The total polyphenols of OMWWs as received and after pre-treatment are 2811 and 1943 mg/L, respectively, the phenols amount in the treated OMWWs decreasing of about 30%. This reduction is due to the adsorption of polyphenols onto coagulated/precipitated particles as a result of the applied pre-treatment. They have been recovered through extraction with methanol at 45°C. In particular, in 1 gr of wet solids are recovered about 15 mg of polyphenols. Table 2.3 reports the total phenols amount and membranes phenols rejection for all the tested MF and UF membranes, while in Table 2.4 the phenols concentration and their rejection through MF and UF zirconia membranes are reported. Results reported in Table 2.4 evidenced higher phenols adsorption on polymeric membranes materials rather than inorganic ones. This behavior can also explain the higher fluxes obtained using zirconia membranes. Indeed, the higher phenol rejection (10.1%) of the PVDF-PP membrane (pore size 0.2 micron) with respect to 2% of ZrO₂ membrane (pore size 0.1 micron) is most likely due to interaction phenomena between phenols and membranes rather than a size exclusion mechanism. These results confirm that the membrane material has a significant bearing on membrane-solute interactions, rather than the membrane pore size. Regarding UF process, the membranes with MWCO of 3.5 kDa showed higher rejections toward total polyphenols compounds in comparison with others membrane, this result can be due to both the interaction phenomena between phenols and membranes and the nature of this membrane which has properties close to nanofiltration membranes. For this membrane weight cut-offs (MWCO) is in the region of 0.2-2 kDa [16].

Table 2.3. Total phenols content and membrane phenols rejection in the MF and UF processes.

Membrane process	Membrane (pore size)	Feed (mg/L)	Permeate (mg/L)	Rj (%)
MF	ZrO ₂ (0.1 micron)	1860	1823	2.0
	PVDF (0.3 micron)	1728	1610	6.8
	PVDF-PP (0.2 micron)	1897	1706	10.1
UF	ZrO ₂ (0.02 micron)	1797	1583	11.9
	PS (20 kDa)	1943	1668	14.2
	PAN (30kDa)	1843	1543	16.3
	PES (80 kDa)	1838	1536	16.4
	PA (3.5 kDa)	1910	1390	27.2

The HPLC analysis of the samples collected from the MF operation (ZrO₂ 0.1 micron) show that the rejection of the ZrO₂ membrane towards different polyphenols was between 1.9% (tyrosol) and 4.5% (hydroxytyrosol), while for the UF operation (ZrO₂ 0.02 micron), the polyphenols rejection was between 4.2% (catechol) and 11.3% (tyrosol). Besides, the analytical evaluation of free low molecular weight polyphenols in samples of OMWWs has detected that tyrosol is the most representative compound of the feed solution representing with a concentration up to 225.8 mg/L, followed by catechol (60-67 mg/L) and hydroxyrosol (36.2-40.3 mg/L). A low amount of vanillic and caffeic acid are detected.

Table 2.4. Amount of detected phenols and MF/UF membrane phenols rejection using ZrO₂ membranes.

Membrane process	Sample	Catechol	Hydroxytyrosol	Tyrosol	Vanillic acid	Caffeic acid
MF (0.1 micron)	Feed (mg/L)	60.0	40.3	225.8	0.40	6.50
	Permeate (mg/L)	58.7	38.5	221.5	0.39	6.40
	Rj (%)	2.2	4.5	1.9	2.5	1.5
UF (0.02 micron)	Feed (mg/L)	67	36.2	202.7	n.d.	3.2
	Permeate (mg/L)	64.2	33.9	179.8	n.d.	2.90
	Rj (%)	4.2	6.4	11.3	n.d.	9.4

n.d. = not detected

2.3.5. Pectins quantification

The pectins are one of the strong foulants present in the OMWWs that survives the traditional pretreatment, The acid treatment is shown to be useful to reduce the content of pectins in OMWWs. In particular, the acid treatment allowed to reduce the content of pectin of 57%. Regarding the membranes that showed the highest flux to the OMWWs, the reduction of the content of pectins was the almost similar. The MF and UF processes carried out with ceramic materials allowed the reduction of the pectin content of 57 and 60%, respectively. The evaluation of pectin in samples of OMWWs before and after acid treatment and after membrane process is reported in Table 2.5.

Table 2.5. Pectins content in the OMWWs and after the MF and UF processes.

Sample		Pectins (g/L)	Pectins reduction (%)
OMWWs after filtration with filter of 35 micron		0.93	57
OMWWs after acid pre-treatment and filtration with filter of 35 micron		0.40	
MF ZrO ₂ (0.1 micron)	Feed	0.37	57
	Permeate	0.16	
UF ZrO ₂ (0.02 micron)	Feed	0.45	60
	Permeate	0.18	

2.3.6. Solids and TOC analyses

Results related to total and dissolved solids of the OMWWs, as received, after pre-treatment are reported in Table 2.6. Unlike of untreated OMWWs, which contain both suspended and dissolved solids, it is possible to note that in the pre-treated OMWWs at the isoelectric point only dissolved solids are present (i.e. total solid equal to dissolved solid). The reduction of the total solids was of 5% for MF process using ZrO₂ membranes. The UF ZrO₂ membrane allowed to reduce the total solids content of 7.5%.

Table 2.6. Total and dissolved solids in the OMWWs as received and after pre-treatment step.

Sample	Total solids (%)	Dissolved solids (%)
OMWWs before treatment	9.04	2.28
OMWWs after filtration through 35 micron filter	4.71	2.28
OMWWs after acid pre-treatment and filtration through 35 micron filter	2.28	2.28

The TOC, TC and TIC (Table 2.7) not significantly change in samples after filtration of OMWWs raw with 0.45 filter and after acid treatment, meaning that the carbon content is just due to dissolved solids. The total, organic and inorganic carbon as well as the TOC

reduction percentage are reported in Table 2.7. Results indicated that the pre-treatment step allowed reducing the TOC of more than 70%. The rejection of the MF and UF membranes toward TOC show a similar trend to the rejection values observed for these membranes toward polyphenols (Table 2.3). The reduction of TOC was for the MF processes in the range between 10 and 15%, while for the UF processes it was in the range between 19 and 23%.

Table 2.7. Content of total, organic and inorganic carbon and % of TOC reduction in the OMWWs as received and after each treatment step.

Sample		TOC (g/L)	TC (g/L)	IC (g/L)	TOC Reduction (%)
Raw OMWWs as received		26.58	26.90	0.30	-
Raw OMWWs after 35 µm filter		13.00	13.14	0.13	51
Raw OMWWs after 0.45 µm filter		8.09	8.13	0.04	70
¹ OMWWs after acid pre-treatment and 35 µm filter		7.84	7.86	0.03	71
MF	ZrO ₂ 0.1 µm	7.07	7.08	0.01	10
	PVDF 0.3 µm	6.74	6.75	0.01	14
	PVDF-PP 0.2 µm	6.64	6.65	0.01	15
UF	ZrO ₂ 0.02 µm	6.37	6.38	0.01	19
	PAN 30 kDa	6.22	6.23	0.01	21
	PES 80 kDa	6.10	6.12	0.02	22
	PS 20 kDa	6.00	6.02	0.02	23

¹ Feed for microfiltration (MF) and ultrafiltration (UF) operations

2.4. Conclusions

In this part of work, suspension destabilization has been proposed as a new approach for OMWWs pre-treatment prior to membrane processes. This approach allowed to obtain higher flux respect to other pre-treatment process reported in literature.

The results in terms of the total and dissolved solids indicated that the pre-treatment step allowed to eliminate all the suspended solids and also the reduction of the content of pectins that are one of the main fouling source. Besides, the acid pH, lower than 2, preserves the biophenol by oxidation reactions and inhibits the mold growth.

Phenols measurements confirmed that the pre-treatment does not destroy the polyphenols, which can be recovered using the suitable membrane processes such as the nanofiltration process. The recovery of polyphenols by nanofiltration will be discuss in the chapter 3. The best fluxes up to 60 and 23 L/hm² were obtained in MF and UF respectively using a ZrO₂ membranes. The MF had shown better results in comparison with the other MF processes reported in literature data. This new approach is promising as pre-treatment step of wastewaters from different sources in fouling reduction during membrane processes. On the basis of the results obtained with MF ZrO₂ membranes on lab scale, the ceramic material was selected for the implementation of membrane technology in OMWWs treatment on lab-scale prototype. Besides, further study of OMWWs microfiltration were carried out according batch concentration and feed-and-bleed mode. Results will be discussed in detail in Chapter 3.

*Part of this chapter has been published: [Bazzarelli E.](#), [Poerio T.](#), [Mazzei R.](#), [D'Agostino N.](#), [Giorno L.](#), "Study of OMWWs suspended solids destabilization to improve membrane processes performance", *Separation and Purification Technology*, 149 (2015) 183-189.*

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Integrated membrane system for water recovery and concentrated polyphenols formulation

3.1. Introduction

Resource valorisation from waste represents one of the most important challenges for sustainable industrial processes. Limited resources and increasing interest in the use of bioactive compounds play an important role in the development of sustainable waste management practices. Several physical, chemical and biotechnological approaches, have been attempted to valorise OMWWs [1] resulting in an environment protection with zero discharge.

Membrane processes such as ultrafiltration (UF) and nanofiltration (NF) have been successfully used for over three decades in food and beverage industries for the treatment of several fluids and agricultural wastewaters [2]. Interesting applications have been developed for the separation, concentration and recovery of high-added value compounds, including pectins and polyphenols, from agro-food byproducts [3-4]. Possibility to operate at room temperature, small area-requirement, no phase change and chemical additive use, low investment and maintenance costs, high efficiency and low specific energy consumption are key advantages of membrane technology over conventional separation processes in many wastewater treatment processes [5]. The combination of different pressure-driven membrane processes for the recovery of polyphenols from OMWWs has been largely investigated [6-11]. Integrated systems in which membrane operations have been combined with other separation techniques such as supercritical fluid extraction [12] and chromatographic separations have been also proposed [13] for the recovery of antioxidants from OMWWs. By analyzing patent publications in this field, Takaç and Karakaya [14] concluded that the future direction of the processes for the recovery of antioxidant compounds from OMWWs is presumably toward the use of membranes in a sequential design. Membrane emulsification as encapsulation method has important potential in the valorization of recovered polyphenols for their application not only as food additives or as nutritional supplements, but also as active cosmetics or as drugs bioactive nutrients. The method allows the production of monodispersed droplets with controlled size in mild shear conditions [15-16]. The plethora of health benefits of biophenols coupled

with the benefit demonstrated by the encapsulation justifies the possible additional cost related to the use of this emerging technology. Several limitations are reported in the use of these valuable natural compounds: (a) according to the route of administration, their efficiency depends on their bioavailability and integrity; (b) during food products processing, distribution or storage or after oral administration, their instability in certain pH conditions or in the presence of enzymes or other nutrients, limits their activity and potential health benefits; (c) for topical use, their sensitivity to environmental factors, including physical, chemical, and biological conditions (i.e. Oxidation after light exposition) leading to the progressive appearance of brown colorant/or unwanted odors with a considerable loss in activity; (d) many polyphenols have an unpleasant taste which must be masked before their incorporation in food products or oral medicines [17]. Therefore, the use of encapsulated biophenolic compounds instead of free compounds (such as biophenols rich not-encapsulated solution and/or dry extracts powders) is a suitable method to protect these valuable natural compounds by maintaining their structural integrity until the consumption or the administration, mask its taste, increase the bioavailability. Among the existing encapsulation methods, membrane emulsification has been demonstrated as effective and reliable technique for the encapsulation of bioactive compounds [18-20]. Regarding the integrated membrane process, one of the major problems of the membrane operations is fast flux decline that reduce performance of the membrane. This phenomenon is caused by fouling phenomena and concentration polarization effects. The fouling is due to the adsorption of feed constituents on membrane surface and especially in microfiltration within the pore, while concentration polarization occurs following the accumulation of solute in solution phase in boundary layers adjacent to the membrane surface [21]. In an integrated membrane process is essential to achieve a high productivity for MF unit in order to ensure of subsequent operations. For this purpose, several method have been reported to overcome MF fouling such as feed pretreatment, flow manipulation, pressure relaxation, turbulence promoters, local vortex promotion, pulsatile flow and gas sparging, backwashing [22]. Air Back-flushing is an alternative method to fouling control and enhance the productivity of MF. The air backflushing is performed by injection of the air on the permeate side by using the air pressure greater than the operating pressure filtration for efficient fouling control. Several research groups have investigated the use of backflushing to improve the membrane performance. Cimini et al. [23] applied CO₂ backflushing technique for beer clarification using ceramic membrane. They reported that a periodic CO₂ backflushing resulted to be quite effective not only to

quasi-steady-state restore the permeation flux, but also to maximize the average permeation flux.

In this study conventional membrane operations, such as microfiltration (MF) and nanofiltration (NF), and relatively new membrane operations, such as osmotic distillation (OD) and membrane emulsification (ME), were combined in order to produce biophenols formulations of interest for food or nutraceutical applications from OMWWs. In order to maintain the high and stable flux during OMWWs treatment the operating backflushing cycle are introduced during the OMWWs microfiltration. The performance of the investigated membranes was evaluated in terms of fluxes, polyphenols, conductivity and TOC rejection (%) and encapsulation efficiency (%) in the selected operating conditions. A mass balance of the process was carried out based on water and biophenols recovered processing the OMWWs by the innovative sequential design.

Based on the results obtained from MF and NF on lab-scale prototype, an implementation of membrane plant was developed on productive scale at mill factory using the pre-filtration system, MF, NF and by a further step represented by reverse osmosis (RO).

3.2. Materials and Methods

3.2.1. Olive mill wastewaters

The OMWWs used to evaluate the potentialities of an integrated membrane system were pre-treated according to the procedure reported in the chapter 2. This method permits to remove completely suspended solids and therefore reducing fouling phenomena during the membrane filtration. The main characteristic of raw OMWWs are reported in Table 3.1.

pH	4.76
Conductivity (mS/cm)	11.6
Total phenols (g/L)	3.1
Total solids (%)	5.7
TOC (g/L)	34.5

3.2.2. Integrated membrane system

OMWWs were treated according to the combination of membrane operations depicted in Figure 3.1. In particular, after the removal of suspended solids by an acidification step, the OMWWs were first submitted to a MF operation; the MF permeate was fed to a NF process and finally the NF retentate was further concentrated by OD. After that, the concentrated solution enriched in polyphenolic compounds was used for the formulation of emulsion using membrane emulsification (ME). The optimum conditions for achieving higher permeate flux for MF, NF and higher rejection for NF were investigated. The rejection (R) of the selected membranes towards specific compounds was determined according to equation already reported in the previous chapter and here recalled:

$$R_j = \left(1 - \frac{C_p}{C_f} \right)$$

where C_p and C_f are the permeate and feed concentration of component of interest, respectively.

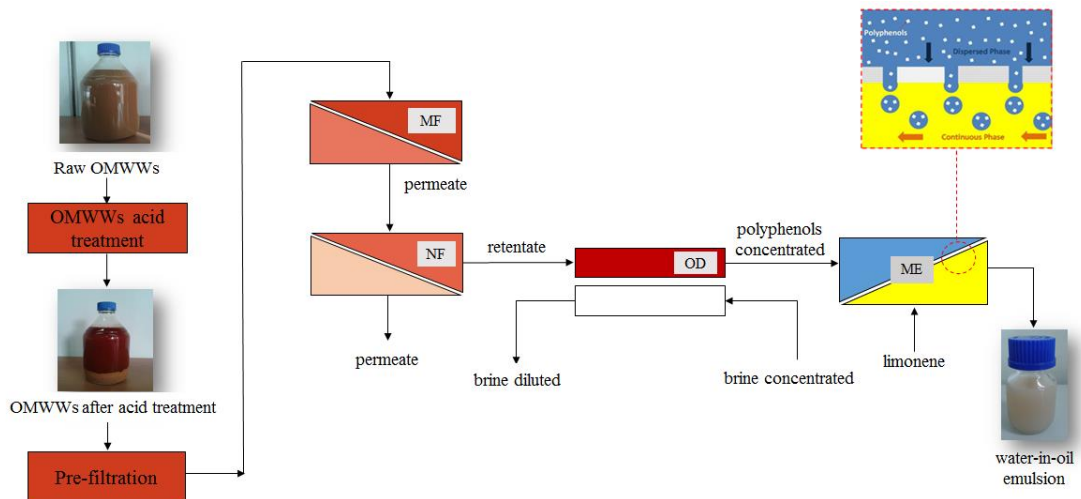


Figure 3.1. Schematic representation of investigated integrated membrane processes.

3.2.3. Upscaling integrated membrane process

Development of integrated membrane process on productive scale was designed using the MF and NF membranes with better performance reported in the previous sections and combined with other unit constituted by reverse osmosis (RO). Same pre-treatment of OMWW was used, as previously described. The step of RO was developed using polyamide thin-film composite membrane in spiral wound configuration (99.5% rejection for NaCl). The flow sheet in Figure 3.2 shows the components of upscaled membrane plant.

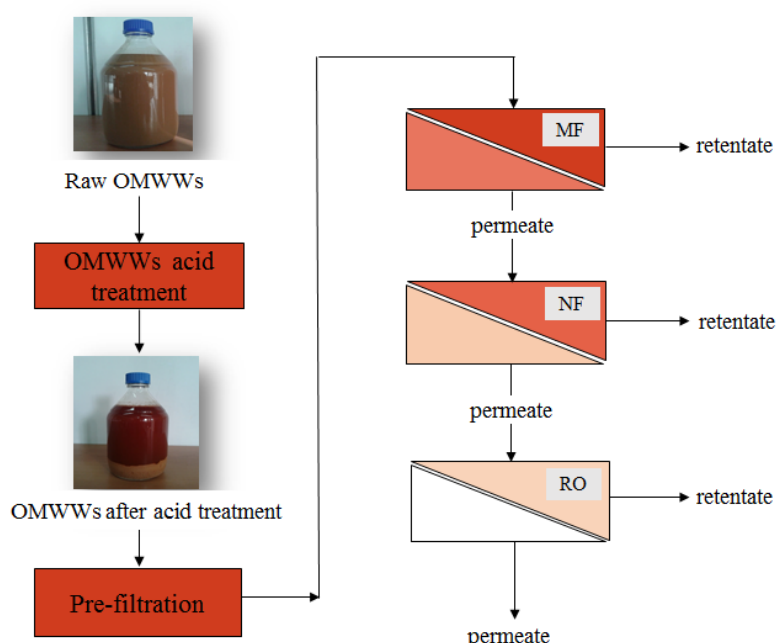


Figure 3.2. Upscaled investigated integrated membrane processes.

3.2.4. Microfiltration set-up

MF experiments were performed by using a lab-scale prototype equipped with a feed tank of 25 L, a centrifuge pump (LOWARA, CEA 120/5), a stainless steel housing to accommodate the tubular multichannel membrane and a permeate tank. Two manometers located before and after the membrane module allow measuring the inlet and outlet pressure. The transmembrane pressure (TMP) value was regulated by a pressure control valve on the retentate side. The feed flow rate (Q_f) was measured by a digital flowmeter; the permeate flow rate was automatically measured and registered by a mass flow controller. The plant is equipped with an automated air backflushing system in line with

the permeate side in order to remove foulants accumulated on the membrane surface during microfiltration run. The air backflushing was set at 6.5 bar for 60 s each 10 minute of OMWWs microfiltration. The feed temperature was controlled by a heat exchanger fed with tap water. MF experiments were carried out according two different operative modes: batch concentration and feed and bleed. In the batch concentration configuration the retentate was returned to the feed reservoir while the permeate was collected separately: this configuration was used in order to define the highest volume reduction factor (VRF) in combination with a suitable productivity of the MF process. The VRF is defined as the ratio between the initial feed volume and the volume of retentate according to the following equation:

$$\text{VRF} = \frac{V_f}{V_r}$$

where V_f and V_r represent the initial feed volume and volume of retentate, respectively.

The feed-and-bleed configuration was investigated to simulate the operative mode usually used for continuous operation when a higher recovery rate must be obtained. According to this configuration, the permeate was removed from the system together with a small part of the retentate. Most of the retentate was recycled and mixed with the feed solution to maintain a high tangential velocity in the membrane module.

The MF process was operated at a TMP of 1.7 bar, an axial feed flow rate of 6 m³/h (corresponding to an axial feed velocity of 7.5 m/s) and a temperature of 25±2 °C.

The hydraulic permeability of the MF membrane was measured before each experiment. After the OMWWs processing the membrane was cleaned with a 1% (w/w) alkaline detergent (Ultrasil 50, Henkel) and a 1% (w/w) NaOH solution at a temperature of 60 °C for 30 min.

Different types of ceramic membranes were used for MF experiments and their performance was compared. Their main characteristics are reported in Table 3.2.

Table 3.2. Characteristics of selected MF membranes.

Membrane process	Microfiltration		
Manufacturer	Tami Industries	Tami Industries	Inopor
Membrane material	TiO ₂ (Isoflux)	ZrO ₂	Al ₂ O ₃
Membrane configuration	Tubular Multichannel	Tubular Multichannel	Tubular Multichannel
pH operating range	0-14	0-14	0-14
Membrane surface area (m ²)	0.35	0.35	0.10
Pore size (micron)	0.14	0.10	0.10

3.2.5. Nanofiltration set-up

The MF permeate was subsequently processed by NF. The experiments were performed in a lab-scale prototype system. The plant consists of a feed tank with a capacity of 25 litres, a gear pump (LOWARA), two different stainless steel-flow housings able to accommodate a spiral wound and a tubular multichannel membrane, respectively. Two manometers located before and after the housing were used to measure the inlet and outlet pressure and consequently the transmembrane pressure (TMP). The feed flow-rate and the TMP values were regulated by a pressure control valve, on the retentate side, and by regulating the velocity of the pump. A cooling system, fed with tap water, was used to keep constant temperature of the feed solution at 25±1 °C. All NF experiments were performed according to a batch concentration configuration in selected operating conditions. TMP and feed flow rates were fixed at 10 bar and 400 L/h for the polymeric membranes and tubular multichannel, and 7 bar and 200 L/h for the monochannel tubular ceramic ones. NF membranes were cleaned with a 1% (w/w) alkaline detergent (Ultrasil 50, Henkel) and a 0.1% (w/w) NaOH solution at a temperature of 40 °C for 30 min. Their hydraulic permeability was measured before and after cleaning procedure. The fouling index (*FI*) of NF membranes was calculated by comparing the pure water permeability before and after the OMWWs treatment [24] based on following equation:

$$FI = \left(1 - \frac{WP_1}{WP_0}\right) \cdot 100$$

where WP_0 and WP_1 are the pure water permeability before and after the OMWWs filtration.

The cleaning efficiency (CE) was evaluated by measuring the water permeability after the cleaning procedure (WP_2) in comparison with the initial water permeability (WP_0):

$$CE = \left(\frac{WP_2}{WP_0} \right) \cdot 100$$

The membranes used and their main characteristics are reported in Table 3.3

Table 3.3. Characteristics of selected NF membranes.

Membrane process	Nanofiltration			
Manufacturer	GE Osmonics	Filmtec-Dow	Membraflow	Inopor
Membrane material	cross-linked aromatic polyamide	polyamide thin-film composite	TiO ₂	TiO ₂
Membrane configuration	spiral wound	spiral wound	tubular monochannel	tubular multichannel
Max. operating pressure (bar)	27.60	41	70	-
Max. operating temperature (°C)	50	45	350	350
pH operating range	2-10	2-11	0-14	0-14
MgSO ₄ rejection (%)	96	>97	-	-
Membrane surface area (m ²)	0.32	2.6	0.00187	0.1
Pore size (nm)	-	-	2	0.9
MWCO (Da)	150-300	200	-	450

3.2.6. Osmotic distillation set-up

The NF retentate was submitted to an OD treatment using a lab-scale prototype equipped with polypropylene hollow fiber membrane modules having a pore size of 0.03 μm , a porosity of about 40% and a membrane surface area of 1.4 m^2 .

The retentate was pumped through the shell side of the membrane module; calcium chloride dihydrate at 60 w/w%, used as stripping solution, was pumped through the fibre lumens (tube side). Both solutions were recirculated back to their reservoirs after passing through the contactor. Circulation of both brine and juice was counter-current.

Inlet and outlet pressures for both tube side and shell side streams were registered by pressure gauges in order to control the pressure differentials between the two sides of the membrane. OD system was generally operated with a slightly higher pressure on the shell side of the module than the lumen side in order to avoid the leakage of the brine strip into the product.

The NF retentate was recirculated in the shell side of the OD membrane module with an average flow rate of 27.4 L/h. The stripping solution was recirculated in the tube side with an average flow rate of 37.5 L/h. The temperature of both feed and brine was 22 ± 1 $^{\circ}\text{C}$, whereas the average transmembrane pressure (TMP) was 0.38 bar.

The flow rate of extracted water was measured with a digital balance placed under the feed tank and it was used to calculate the evaporation flux (J_{ev}).

After each trial, the plant was cleaned first by rinsing the tube side and shell side with de-ionised water. Then, a KOH solution at 2% w/w was circulated for 1 hour at 40 $^{\circ}\text{C}$. After a short rinsing with de-ionised water a citric acid solution at 2% w/w was circulated for 1 hour at 40 $^{\circ}\text{C}$. Finally, the circuit was rinsed with de-ionised water.

3.2.7. Membrane emulsification set-up

Water-in-oil (W/O) emulsions were prepared by pulsed back-and-forward cross-flow batch membrane emulsification [25]. The OD retentate was used as dispersed phase in combination with PVA 20% w/w in order to have a final ratio OD retentate: PVA equal to 1:3. The continuous phase was limonene containing Span 80 (2% w/w) as emulsifier. Shirasu porous glass hydrophilic tubular membranes (SPG, Miyazaki, Japan) with a pore size of 3.1 μm was used for the preparation of the emulsion. The OD retentate was injected through the membrane with a flux of 6.6 $\text{L h}^{-1}\text{m}^{-2}$, while limonene was agitated within the

membrane lumen at fixed amplitude and frequency of 4.7 cm and 2.53 Hz, respectively. The emulsification process was stopped when 10% v/v of dispersed phase was emulsified. Emulsion droplets were analyzed by optical microscopy (Zeiss, model Axiovert 25) and the pictures captured by a camera (JVC, model TK-C1481BEG) connected with the microscope were analyzed by the Scion Image program. For each sample more than 800 droplets were counted and measured. Droplet diameter is given in D[3,2] (surface weighted mean diameter or Sauter diameter) and size distribution is given as span. Phases separation of emulsion was induced by centrifugation and the aqueous dispersed phase was collected to determine the encapsulation efficiency (EE%):

$$EE\% = \frac{C_0}{C_i} \cdot 100$$

where C_0 and C_i are the measured and theoretical concentration of polyphenols in the dispersed phase, respectively.

3.2.8. Analytical measurements

The samples collected from the different membrane operations were analysed to quantify total soluble solids (TSS), total organic carbon (TOC), conductivity, polyphenols and low molecular weight phenolic compounds.

TSS were measured by using a hand refractometer (Atago Co., Ltd., Tokyo, Japan) with a scale range of 0-32 °Brix (1 °Brix corresponds to 1g TSS/100 g of sample).

Total carbon (TC) and inorganic carbon (IC) were analyzed by a TOC analyzer (TOC-VCSN, Shimadzu). TOC values were obtained by difference between TC and IC.

Total phenols were estimated calorimetrically while low molecular weight polyphenols were analysed by using an HPLC according the methods reported in the chapter 2.

3.3. Results and discussion

In the following paragraphs, a comparison of the performance of different ceramic membranes used in OMWW treatment. In addition, the potentiality of air backflushing cycles in order to improve the permeate flux of ceramic microfiltration unit and limit the membrane fouling are reported.

Regarding nanofiltration membranes, the influence of materials, configurations, molecular weight cut-off or pore size on permeate flux, conductivity removal, TOC removal, polyphenols rejection and fouling index are illustrated.

Furthermore, development of integrated membrane process based on microfiltration, nanofiltration, osmotic distillation and membrane emulsification aimed at recovery, concentration and polyphenols formulation in W/O emulsion are illustrated. Finally, MF and NF operations on lab-scale prototype are upscaled to the productive scale using a further step consists of reverse osmosis.

3.3.1. Microfiltration

Figure 3.3 shows the permeate flux of MF of OMWWs processes carried out in both feed-and-bleed and batch concentration modes up to a VRF of 5 for selected membranes. The highest steady state permeate flux was obtained using TiO₂ membrane (Isoflux) for both modes. In particular, steady-state permeate fluxes of 46.7 and 14.1 L/h m² were obtained in feed-and-bleed and batch concentration, respectively. Instead, membrane ZrO₂ showed the lowest permeate flux during OMWWs treatment, in this case steady state permeate flux for feed and bleed mode was about 21 L/hm² while for batch concentration was 11.1 L/hm². A similar result was obtained for the Al₂O₃ membrane working in batch concentration mode. Best results obtained with TiO₂ isoflux membranes can be due to the membrane structure. Skrzypek et al. [26] reported that the membrane Isoflux compared with classical ceramic MF membranes, allows to obtain constant flux along the length of membrane element, because the thickness of the separating layer decreases from the inlet to the outlet in the way that the pressure to layer thickness ratio remains constant. Besides the TiO₂ membrane (Isoflux) showed higher hydraulic permeability compared to other ceramic membranes (Figure 3.4).

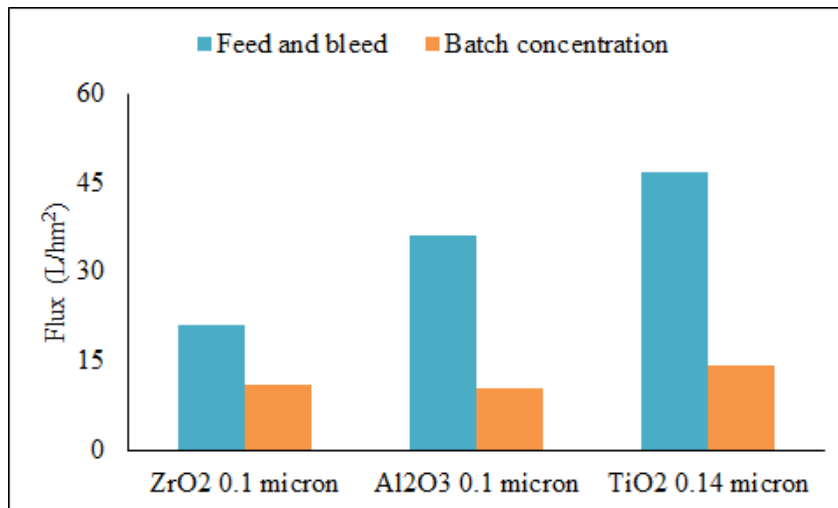


Figure 3.3. Flux vs time of MF processes carried out in constant feed concentration mode and batch concentration mode

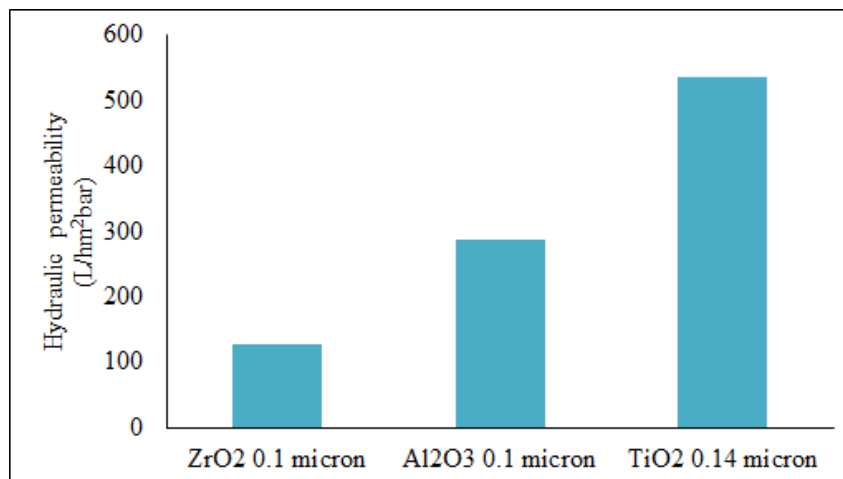


Figure 3.4. Hydraulic permeability of MF membranes

Interestingly, the initial hydraulic permeability of the MF TiO₂ membrane was almost fully restored with the adopted cleaning protocol. Previous works on this topic reported that MF is a critical step of the integrated process for the selective separation of OMWWs for the rapid decrease of the permeate flux and irreversible fouling of polymeric membranes. In particular, Russo et al. [9] reported only a 40% recovery of the hydraulic permeability of a polymeric 500 kDa membrane by using alkaline and acid solutions 0.1-0.2 M. The MF process carried out with ceramic membranes of different materials, as expected, had a negligible rejection to polyphenolic compounds less of 8%; therefore, most of phenolic compounds were recovered in the permeate stream.

3.3.2. Microfiltration with air backflushing cycles

The time evolution of permeate flux during the MF process carried out in both feed-and-bleed and batch concentration modes is shown in Figures 3.5 and 3.6. These figures illustrate also the effect of air backflushing on the flux of OMWWs processing. In particular using the TiO₂ membrane, the flux reached the steady state a 60 L/hm² and it remained constant up to 8 hours in feed and bleed mode, when air backflushing cycle (BF) was applied. When air was injected in the opposite direction of MF during OMWWs processing in both feed-and-bleed configuration, the permeate flux a steady state was about 30% higher than that observed without injection of air (Figure 3.5). On the other hand, working in batch concentration mode an acceptable permeate flux value of 20 L/hm² is obtained after 8 hours reaching a final VRF of 12. Experimental result showed that steady state permeate flux was higher using the air BF cycles during OMWWs processing, compared to non-backflushed ones. In fact, an improvement of the productivity of 112% at VRF 12 is obtained.

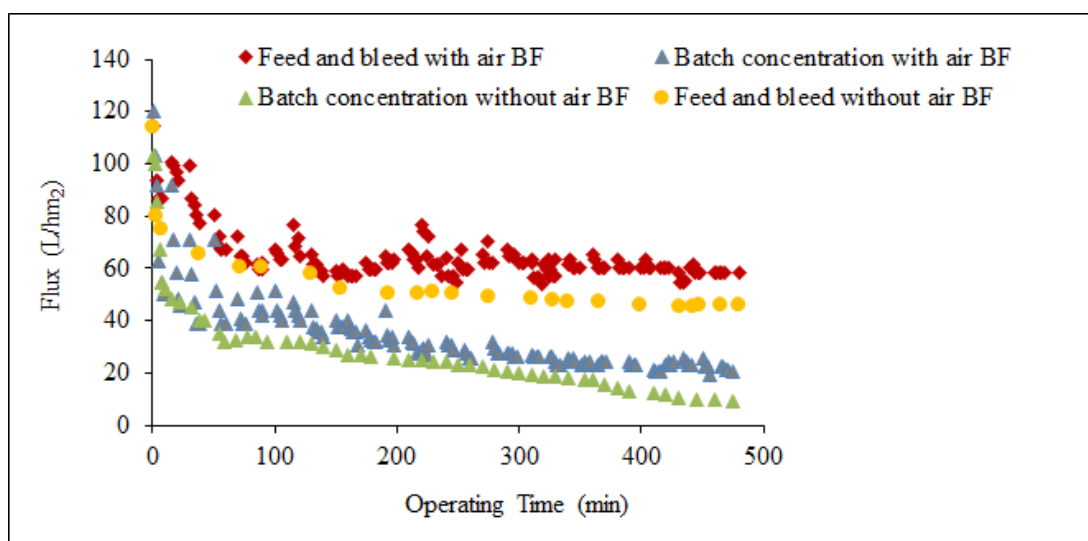


Figure 3.5. Flux vs time of MF (TiO₂) processes carried out in constant feed concentration mode and batch concentration mode up to VRF 12.

Regarding the ZrO₂, the air backflushing cycles has allowed to keep the flux above of 17.6 L/hm² in batch concentration mode when the VRF was 5. An improvement of permeate flux of 60% compared to OMWWs processing without the application of air backflushing was reached (Figure 3.6). Instead in feed and bleed mode, the steady-state flux of about 26 L/hm² was obtained using the air backflushing. In this case, the initial flux of 39 L / hm²

was reduced of 35% after 8 hours of OMWWs processing with increasing of productivity of 30% compared the OMWWs microfiltration run without air backflushing.

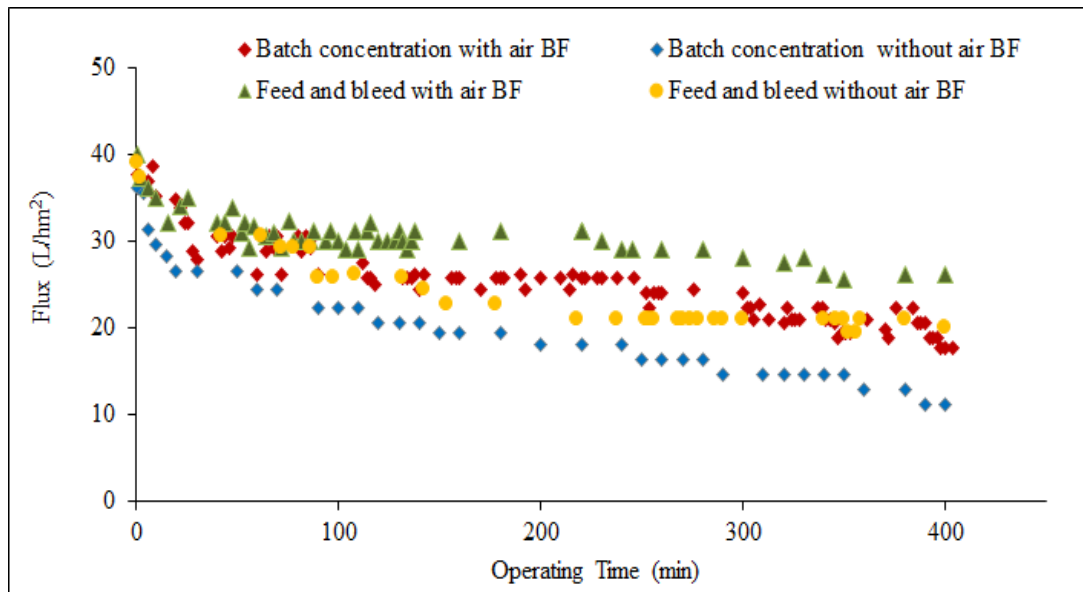


Figure 3.6. Flux vs time of MF (ZrO₂) processes carried out in constant feed concentration mode and batch concentration mode up to VRF 5.

For the Al₂O₃ membrane, the air backflushing cycle was not efficient by setting the air pressure at 6.5 bar. It is due probably more thick ceramic support compared to others selected membrane, or for high affinity of Al₂O₃ to water. Therefore, with Al₂O₃ membrane is necessary increase the air pressure to replace water in the pores in order to obtain an efficient flow of air. The data obtained using TiO₂ membrane, demonstrated the easy scale-up of the MF process for lab-scale prototype OMWWs processing, In particular, the flux of 60 L/hm² has been obtained using a membrane module with a membrane surface area (0.35 m²) 194 times higher than the one (18 cm²) used in the batch scale. The results obtained in this work are comparable with literature data using different stream in which it is clearly demonstrated that air backflushing cycles improve the flux at steady state. In particular, Sondhi et al. [27] used ceramic membranes for crossflow filtration experiments with Cr(OH)₃ suspension as synthetic electroplating wastewater. The filtration experiments with and without backflushing showed that backflushing was effective in reducing the fouling phenomenon resulting in up to five fold increase in steady-state flux and 100% flux recovery. Almandoz et al. [28] report the results of corn syrup clarification in bench-scale microfiltration experiments. These data showed the effectiveness of the

backflushing technique resulting an increase steady-state flux and 73–83% initial flux recovery.

3.3.3. Nanofiltration

The MF permeate was processed by NF in order to concentrate the phenolic compounds in the retentate stream. Figure 3.7 shows the time course of the permeate flux obtained for the NF membranes in the selected operating conditions up to a VRF of 3. Results show that the ceramic membrane exhibits a higher flux compared to polymeric membranes. For NF membrane with the pore size of 2 nm, the initial permeate flux decreased about 67% and it reaches a steady state value of 34 L/h m². On the other hand, the NF 0.9 nm showed a lower permeate flux decay and higher steady state permeate flux compared to NF 2 nm. Reduction of the pore size allowed to limit the fouling phenomena such as pore blocking, resulting in a higher flux with NF 0.9 nm compared with NF 2 nm. Regarding, the polymeric membranes in polyamide, the permeate fluxes at VRF 3 were 10.1 and 7.2 L/hm² for membrane with MWCO of 150-300 and 200 Da, respectively. The NF 200 Da showed a lower permeate flux in comparison with other NF membrane but on the contrary, the retention of all compounds was significantly higher. Results obtained using different membranes in terms of material, pore size/MWCO and configurations were compared in terms of polyphenol, TOC, TS, conductivity rejections of each NF membranes (Figure 3.7)

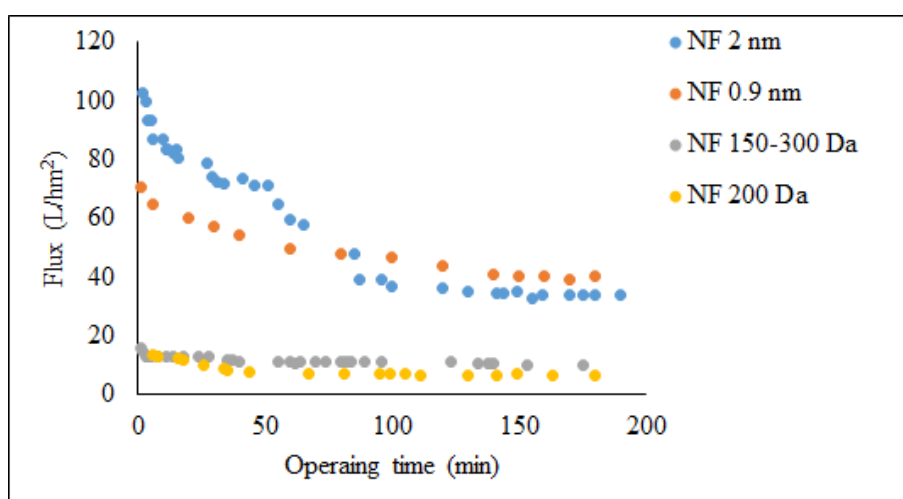


Figure 3.7. Flux at VRF of 3 for NF membranes.

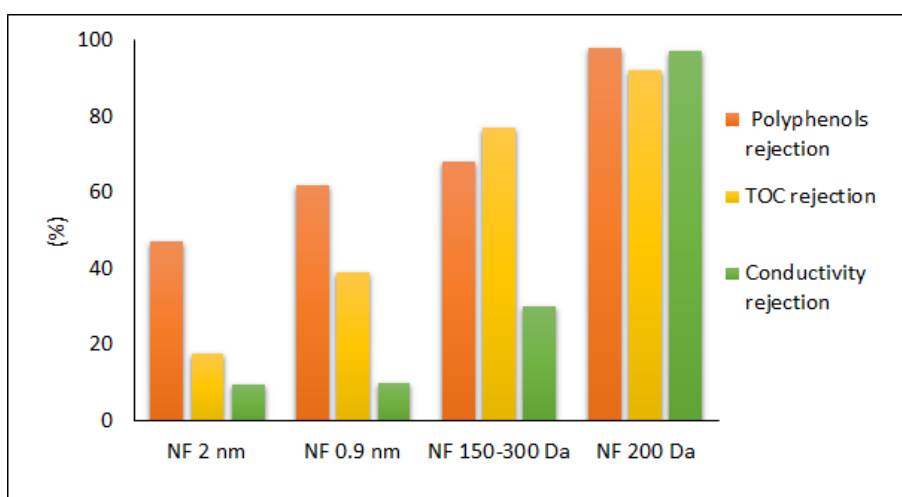


Figure 3.8. Polyphenols, total organic carbon (TOC) and conductivity rejection for NF membranes (pore size of 2 and 0.9 nm, MWCO of 150-300 and 200 Da) VRF: 3, 25°C.

Since the aim of the work is to completely recover the phenolic fraction of the raw OMWWs for their reuse in high-added value final formulations, the 200 Da membrane was selected as the most suitable membrane to be used before the final concentration by OD. Very high rejections are achieved using the membrane with MWCO of 200 Da. For this membrane, TOC, conductivity and polyphenols rejection were 92, 96 and 98%, respectively. By referring to the low molecular weight of polyphenols, the NF 200 Da membrane exhibited rejections between 90 and 98% for identified polyphenols. Regarding the others NF membranes, the rejection towards different analysed low molecular weight polyphenols was lower than 26% (Figure 3.9).

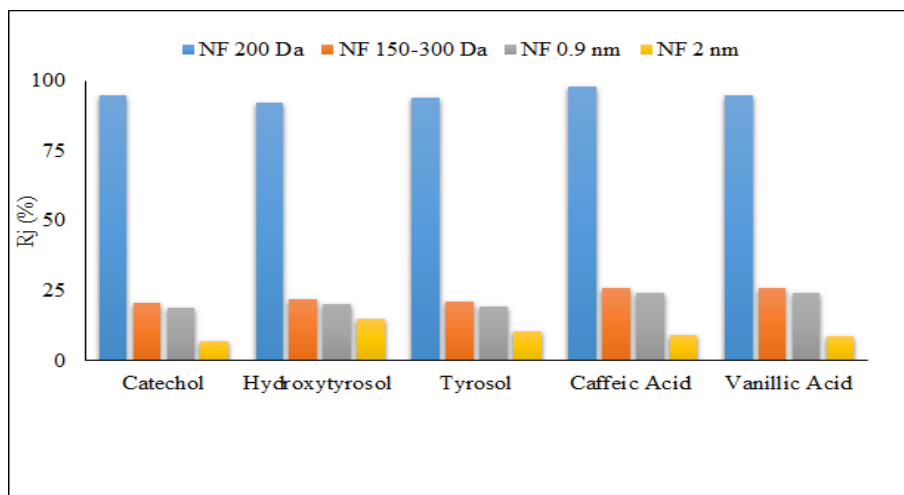


Figure 3.9. Low molecular weight biophenols detected in samples of OMWWs processed with NF membranes.

The most suitable NF membrane was also chosen on the basis of water permeability measurements by calculating their fouling index. Table 3.4 reports the hydraulic permeability measurements of virgin membrane, after OMWWs processing and after cleaning procedures.

The cleaning efficiency (CE) indicated that, as a consequence of a reversible fouling, the water permeability of virgin membranes was completely recovered after the cleaning procedure. In addition, the NF 200 Da membrane exhibited the lowest fouling index.

Table 3.4. Hydraulic permeability measurements of NF membranes, cleaning efficiency (CE) and fouling index (FI) (WP_0 = hydraulic permeability before treatment of OMWWs; WP_1 = hydraulic permeability after treatment of OMWWs).

Type of membrane	WP_0 (L/m ² hbar)	WP_1 (L/m ² hbar)	CE (%)	FI (%)
NF 2 nm	30.0	17.0	100	43
NF 0.9 nm	18.8	9.8	100	48
NF 150-300 Da	5.1	2.6	100	49
NF200 Da	5.9	4.6	100	36

According to data reported in literature, high polyphenols rejections are reached in spite of low permeation fluxes. In addition, in most cases, severe and/or expensive multistep treatments are needed before the NF process in order to obtain high polyphenols rejections. In particular, Zirhepour et al. [29] obtained a polyphenols rejection of about 90% (at a VRF 3) and a permeate flux of 5.2 L/m²h with the NF90 membrane (MWCO 150-200 Da) when the raw solution was pre-treated by 3 different MF membranes followed by an UF (MWCO 35 kDa) and an NF step (MWCO 200-250 Da). Cassano et al. [7] obtained a polyphenols rejection of 93% with a permeate flux of 3 L/m²h using as a cotton fabric filter and two UF steps as pre-treatment before the NF process. The present work allowed simplifying the pre-treatment step of the raw OMWWs before the final NF step. Indeed a permeate flux of 7.2 L/m²h, combined to a polyphenols rejection of 98%, was achieved by using the NF with MWCO of 200 Da after the destabilization of the raw OMWW suspension through an acid pre-treatment followed by a MF step. It must point out that the acid pre-treatment allowed to overcome the main drawbacks encountered in the recovery of biophenols from OMWWs by membrane operations through a complete removal of suspended solids and the preservation of polyphenols from their oxidation.

3.3.4. Osmotic distillation

A further concentration of the phenolic fraction was carried out by treating the NF retentate with an OD unit. Figure 3.10 shows the time evolution of the OD process within a closed loop in which the NF retentate is concentrated from 4 to 17 °Brix.

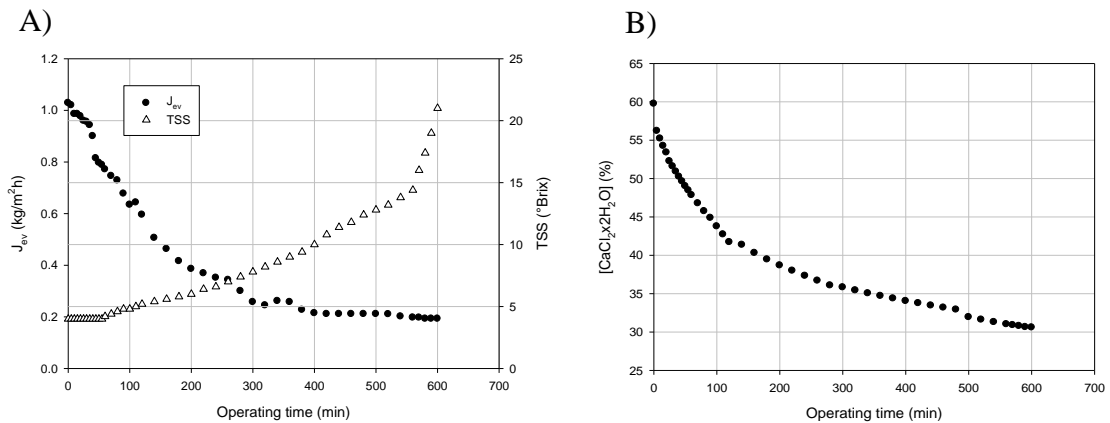


Figure 3.10. Osmotic distillation of NF retentate. Time course of (a) evaporation flux and TSS concentration; (b) brine concentration.

In this process, 6 kg of NF retentate were reduced at about 857 g through the water removal in an operating time of 600 min. Throughout the experimental run brine and feed temperature were maintained almost constant at 22 ± 1 °C. At the first the brine concentration was 60 w/w% producing an evaporation flux of about 1 $\text{kg/m}^2\text{h}$. In the range 0-300 min the decrease of the evaporation flux has a very similar behaviour to that observed for the dilution of the stripping solution. In particular, a reduction of brine concentration of 40% produced a decrease of the evaporation flux of 75% and an increase in TSS from 4 to 20.5 °Brix. In the range 300-600 min the evaporation flux reached a steady-state value of about 0.2 $\text{kg/m}^2\text{h}$ while the brine concentration was reduced from 36 to 30% w/w. Results confirm that the flux decay can be attributed to the dilution of brine solution and, consequently, to the reduction of the driving force of the OD process which is represented by the difference in water activity between brine and feed solution [30]. The concentration polarization phenomenon and the presence of free oils which can coat hydrophobic membranes are additional factors contributing to the permeate flux decay [31-32]. By using the same equipment and in quite similar operating conditions Garcia-

Castello et al. [31] obtained initial and steady-state evaporation fluxes of 1.00 and 0.35 kg/m²h, respectively, in the concentration of a NF permeate from 2 to 8 °Brix. El Abbassi et al. [33] obtained higher steady-state evaporation fluxes (of about 2.2 kg/m²h) in the concentration of crude OMWW with a 0.2 µm polytetrafluoroethylene (PTFE) membrane at a temperature of 30 °C and a permeate CaCl₂ concentration of 5 M. In another work the same authors measured steady-state evaporation fluxes of about 1.44 L/hm² in long term direct contact membrane distillation (DCMD) experiments of micro-filtered OMWWs with a flat-sheet membrane (TF200, Gelman Science) made of PTFE polymer and supported by a polypropylene net [32]. Despite evaporation fluxes were higher than those measured in the present system it is noticeable that the biophenol concentration factor was much higher (about 7.0) if compared with data reported by El Abbassi et al. [32-33] (about 1.9). This high concentration factor produced an enriched fraction of low molecular weight polyphenols as reported in Table 3.5.

Table 3.5. Low molecular weight biophenols detected in samples of NF retentate processed by OD.

Phenolic compounds	Feed (mg/L)	Retentate (mg/L)
Catechol	54.1	362.67
Hydroxytyrosol	60.7	406.89
Tyrosol	266.4	1785.08
Caffeic acid	7.3	49.04
Vanillic acid	1.00	6.7

3.3.5. Membrane emulsification

The enriched phenolic fraction from the OD process was used as dispersed phase in the preparation of W/O emulsions selected as a formulation model for the encapsulation of bioactive compounds. The W/O emulsion produced by pulsed back-and-forward cross-flow batch membrane emulsification is shown in Figure 3.11 together with particle size distribution. The mean particle size was 6.8 μm that was approximately two times the pore size of the membrane. A uniform W/O emulsion was produced as indicated by the span of size distribution, which was 0.3. The encapsulation efficiency calculated according the equation (3) was 90%.

Results demonstrated the suitability of membrane emulsification as polyphenols encapsulation method for potential applications in functional foods, nutraceutical and pharmaceutical industries. The method is extremely attractive in this area because it permits to produce uniform particles with controlled size operating in mild conditions and with low energy consumption. Advantages from the encapsulation include: i) protection for adverse environmental conditions, such as undesirable effects of light, moisture, and oxygen, thereby contributing to an increase in the shelf life of the product, and promoting a controlled liberation of the encapsulated compounds, ii) lowering of unpleasant tastes and flavours, iii) improvement of the bioavailability and half-life of the polyphenols.

Further investigations were carried out in order to evaluate the effect of dispersed phase fraction and operating conditions (dispersed phase flux and shear stress) to control particle size and particle size distribution. Moreover, encapsulation efficiency one of the major phenolic compounds contained in OMWWs represented of catechol and release studies were performed. The results of these studies will be discussed in detail in Chapter 4.

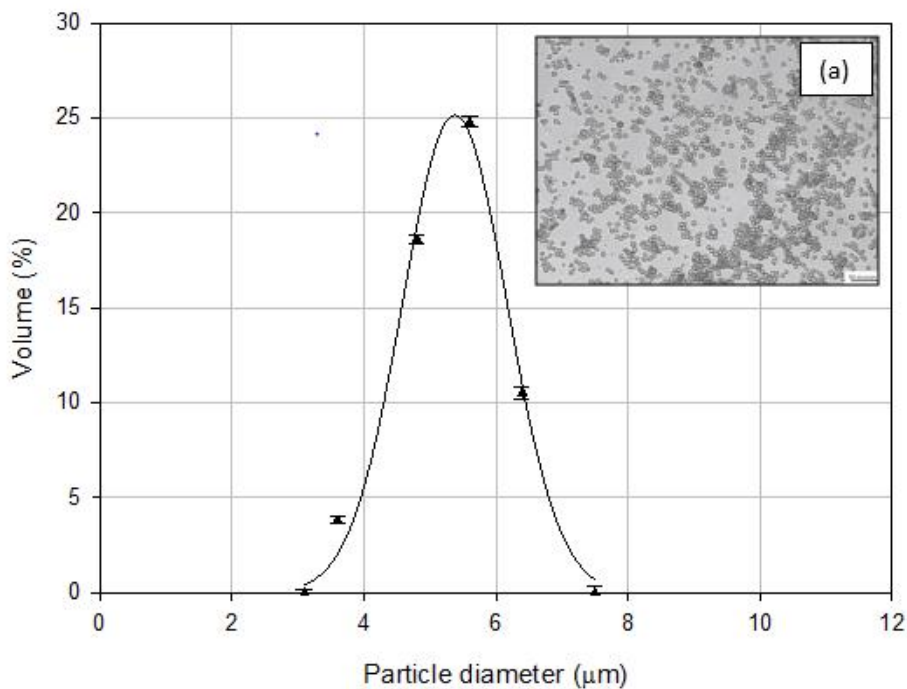


Figure 3.11. Particle size distribution of W/O emulsions produced by pulsed back-and-forward cross-flow batch membrane emulsification (a) optical microscope image of O/W emulsion.

3.3.6. Mass balance of water and phenolic compounds recovery

The recovery of biomolecules and water from waste represents one of the most important challenges for sustainable resource exploitation. This responds to the scarce or limited availability of renewable resources and to the increased interest towards natural biomolecules for their reuse in food, cosmetics and nutraceutical products. This strategy has an additional advantage in terms of environmental protection through waste minimization.

For this purpose, a mass balance of the process was carried out considering the amount of water and biophenols recovered in the processing of OMWWs with the proposed sequential design. In particular, the mass balance of the integrated membrane process, related to an initial OMWWs volume of 1.000 L, is reported in Figure 3.12. The volume of permeate and retentate streams together with the concentration and the amount of phenolic compounds recovered for each step are highlighted. According to the mass balance most of phenolic compounds contained in the raw OMWWs pass through the MF membrane producing a permeate stream with a concentration of phenolic compounds very similar to

that of the initial feed solution. On the basis of the final VRF about 80 L of MF retentate are obtained as residual sludge that can be reused as raw material for biogas production through an anaerobic/aerobic digestion process. The remaining permeate, about 917 L that has total polyphenols concentration of 1.6g/L, is used as feed solution for the following NF step. In this process 117 L of retentate containing 12.5g/L of polyphenols are obtained according to the final VRF. A purified water stream (800 L) is recovered on the permeate side. This solution, depleted in phenolic compounds, can be reintroduced in the oil mill working cycle (i.e. olive washing, membrane and plant cleaning) or reused for other applications such as agriculture (i.e. irrigation) and food (i.e. beverage formulations) [34-35]. A retentate volume of 16.7 L (corresponding to the 1.67% of the initial volume of treated OMWWs), consisting in an enriched fraction of phenolic compounds (87.5 g/L), is obtained from a further concentration of the NF retentate by OD. The diluted brine can be reconcentrated by the thermal evaporation so minimizing environmental problems due to the discharge of the extracting solutions. The OD concentrated fraction is formulated by the ME process for the production of a W/O emulsion with an encapsulation efficiency of 90%.

According to the mass balance, carried out processing 1.000 L of OMWWs, the amount of recovered polyphenols is equal to 1.463g corresponding to 85% of the initial amount of polyphenols in the raw wastewater. Many papers reported a techno-economical study of OMWW treatment showing that the cost of treatment with membrane filtration can be covered by exploitation of the products recovered, leading to possible profit [36-37]. In particular, hydroxytyrosol and tyrosol are among the most economically relevant components of OMWW phenolic fractions. Their price is 2.700,00 and 220,00 € per gram, respectively (Extrasynthese and Sigma Aldrich quotation). By treating 1000 L of waste, were covered 60 g of hydroxytyrosol (162.000 €) and 266 g of tyrosol (52.000 €). Considering an olive mill of medium size that produces 30.000 L of wastewaters per year, the operating costs of the proposed system can be easily covered also considering a further purification steps.

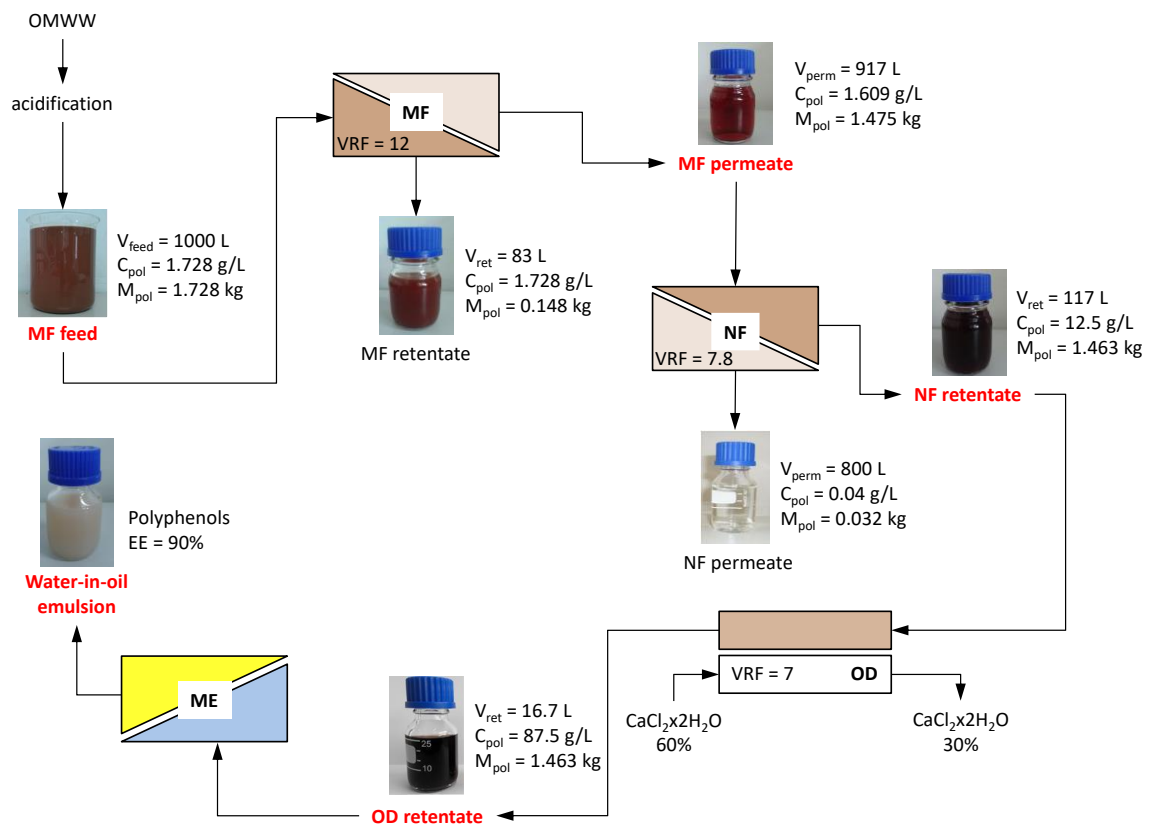


Figure 3.12. Mass balance based on water and polyphenols recovery processing 1000 L of OMWWs with the integrated membrane system.

3.3.7. Upscaling OMWWs treatment plant

On the basis of the results obtained with MF and NF operations on lab scale prototype, upscaling of integrated process on productive scale was developed. A further unit consisting of RO operation was added to achieve a higher purification the NF permeate. The system permitted the polyphenols recovery/concentration on large scale, as well as water purification. It consists of the following unit:

- 1) Pre-filtration unit using bag filter of 25 and 5 micron in series mode
- 2) Microfiltration (MF) using a ceramic multichannel membrane in TiO_2 (12.95 m^2 membrane surface, pore size of 0.14 μm);
- 3) Nanofiltration (NF) using a spiral wound membrane in PA (38 m^2 membrane surface, MWCO of 200 Da);
- 4) Reverse osmosis (RO) using a spiral wound membrane in PA (53.2 m^2 membrane surface, NaCl rejection of 99.5%).

In Figure 3.13 is shown a picture of integrated membrane system on productive scale at olive mill.

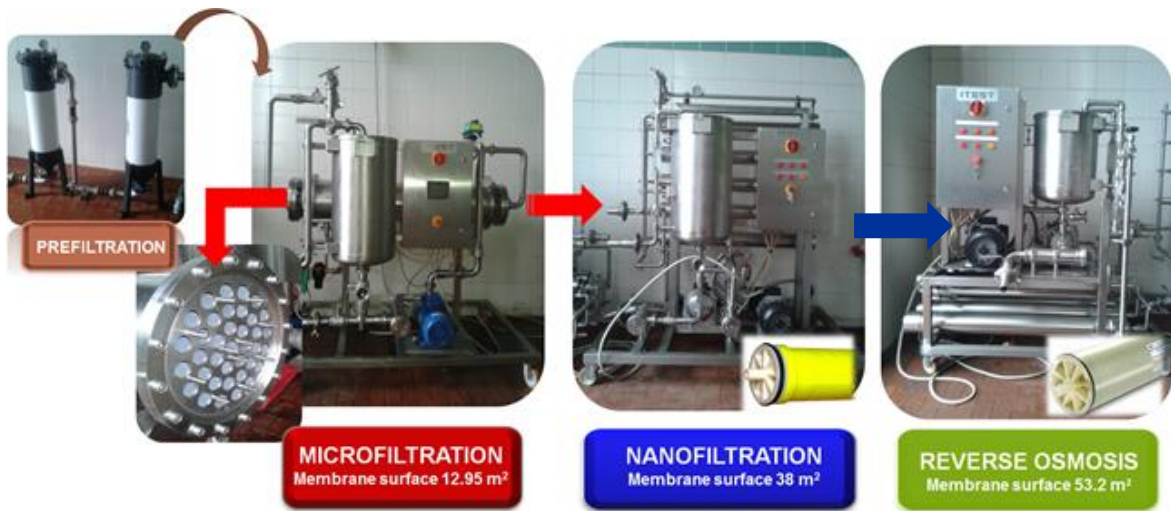


Figure 3.13. Integrated membrane process on productive scale.

The plant allowed the treatment of OMWWs up to 2.5 m³/day. MF operation was carried out according the feed-and-bleed mode using the air backflushing cycle, while the NF and RO operations were performed in batch concentration mode. The VRF of 5 and 12.5 were reached for NF and RO process, respectively. In particular, the nanofiltration unit permitted to obtain a concentrated polyphenols solution from retentate side with polyphenols rejection of 98% and achieve TOC removal efficiency higher than 93% in the permeate stream. Therefore, the NF process produced water from permeate side with acceptable quality for safe disposal to the environment. A further purification of NF permeate stream was obtained by the RO unit. The RO membrane showed a high retention toward TOC and conductivity up to 98%. Furthermore, a complete retention of polyphenols in the retentate of RO unit were detected. (Figure 3.14).

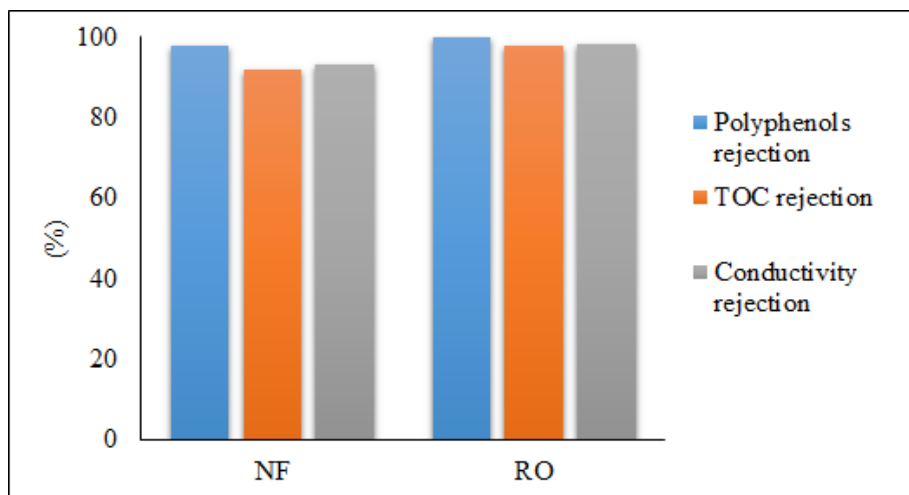


Figure 3.14. Rejection of polyphenols, total organic carbon (TOC) and conductivity for NF (MWCO 200 Da) and RO (NaCl rejection of 99.5 %) membranes.

In Table 3.6 the HPLC analyses of the main polyphenols detected in the initial and collected samples for all integrated membrane process are reported. In the MF permeate were recovered up to 91% of polyphenols respected to the initial feed. Regarding the NF and RO process, in the retentate were recovered up to 96.7% of polyphenols for NF unit and 100% for the RO. The quality of the permeate produced by the RO unit was acceptable for reuse in the cycle of oil production process such as olive washing, plant cleaning and olive paste dilution in the continuous process.

Table 3.6. Low molecular weight biophenols detected in samples of OMWWs processed with integrated membrane process (MF-NF-RO).

Membrane process	Sample	Catechol	Hydroxytyrosol	Tyrosol	Caffeic Acid	Vanillic Acid	Coumaric Acid
MF TiO ₂ 0.14 micron	Feed (mg/L)	3.73	52.96	18.6	1.38	2.33	0.96
	Permeate (mg/L)	3.44	48.78	17.18	1.28	2.13	0.91
	Rejection (%)	7.8	7.9	7.6	7.2	8.6	5.2
NF PA 200 Da	Permeate (mg/L)	0.15	1.6	0.77	0.05	0.08	0.03
	Rejection (%)	95.6	96.7	95.5	96.1	96.2	96.7
RO PA NaCl rejection 99.5%	Permeate (mg/L)	0	0	0	0	0	0
	Rejection (%)	100	100	100	100	100	100

3.4. Conclusions

A new integrated membrane system for water recovery and polyphenols encapsulation has been proposed and studied. In particular, polyphenols after their recovery and concentration were encapsulated by means of membrane emulsification process for a potential development in formulations suitable for different applications fields. The integrated membrane process is composed by four main units:

- a pre-treatment unit composed by an acidification and a MF step for the removal of all suspended solids,
- a nanofiltration unit that permitted to obtain, water from permeate side, and a concentrated of polyphenols solution from retentate side,
- an osmotic distillation unit that uses the nanofiltration retentate to further concentrate the polyphenols
- a membrane emulsification process to encapsulate the recovered bioactive compounds in a water-in-oil (W/O) emulsion.

Microfiltration step has been successfully applied in the integrated membrane system, obtaining best performance in terms of flux on lab-scale prototype. For this step, the first time air backwashing was applied on OMWWs processing by MF unit in order to reduce the membrane fouling effect. Experiments on different microfiltration membrane shown that air backflushing was effective method to minimize membrane fouling and improve the productivity of OMWWs treatment. The air backflushing allowed the removal of fouling particulate on membrane surface, resulting a flux enhancement of 2 times in the MF step using TiO₂ membrane with pore size of 0.14 micron.

From the comparison with literature data resulted that all the nanofiltration processes usually gives high polyphenols rejections with low fluxes or viceversa. Then, the choice between them, must be addressed basing on the final aim. In the present work, the highest flux (7.2 L/hm²) has been reached with an almost complete biomolecules retention using membrane with MWCO of 200 Da.

By osmotic distillation process a concentration factor of 7 has been reached that is about 3.5 times more compared with literature data. The membrane emulsification process permitted polyphenols encapsulation efficiency of 90%, resulting a highly innovative and effective technique for the application in the valorisation of wastewater coming from olive mills.

According to the results achieved with the MF and NF on lab scale prototype, an integrated membrane system on productive scale based on MF, NF operations was developed in combination with an additional step of RO. The process allowed the purification of OMWWs with the complete recovery of phenolic compounds in the retentate stream of NF and RO unit. In addition, reverse osmosis membranes was capable of produce a high quality effluent from OMWWs. A mass balance, carried out processing 1000 L of OMWWs by the proposed integrated membrane system demonstrated that it was possible to recover about 800 L of water and 1.463 g of polyphenols.

*Part of this chapter has been published: Bazzarelli F., Piacentini E., Poerio T., Mazzei R., Cassano A., Giorno L., “Advances in membrane operations for water purification and biophenols recovery/valorization from OMWWs”, *Journal of Membrane Science*. 1 (2016) 402-409*

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Olive polyphenols microencapsulation using water-in-oil emulsions and solid lipid particles

4.1. Introduction

Olive polyphenols are known to play a role in preventing diseases such as cardiovascular diseases and inflammatory diseases. Several studies confirmed that phenolic compounds from OMWWs reduce the effect of oxidative stress associated with pathological disorders including atherosclerosis, cancer, inflammatory diseases, neurodegenerative diseases, and they have some anti-microbial and anti-viral properties [1]. Nevertheless, polyphenolic compounds have limited stability in environmental conditions, such as temperature, light, moisture, pH, oxygen concentration and unpleasant taste. These drawbacks might be overcome by using microencapsulation system. This technology has promoted the development of new products and improvement of existing products by solving unique challenges such as converting liquids to solids, separating reactive components, protecting ingredients from the environment, controlling release, or masking ingredients as well as improve the bioavailability of the compound.

There have been major advances in the development of delivery systems to encapsulate lipophilic bioactive components while there is still a pressing need to develop novel manufacturing conditions and formulations to prepare effective delivery systems for hydrophilic bioactive compounds such as biophenols [2]. Major challenges in the design of delivery systems suitable for the encapsulation of bioactive compounds includes the control of particle size and surface properties as well as fundamental physicochemical phenomena associated with encapsulation and release (i.e. partitioning and mass transport release of active ingredients) for the production of particles based end-products with target functionality. A wide variety system such as emulsions, liposomes, lipid and polymer particles have been used as components of food, cosmetics, and drugs enhancing the bioavailability of hydrophilic or lipophilic active compounds. Monodisperse particles gives better control over the dose and release behavior of the encapsulated active compounds, yields higher encapsulation efficiency and better biocompatibility. Conventional devices for preparing particles such as high pressure, ultrasonic homogenizers, colloid mills, rotor–stator systems and microfluidizers require high-energy input for the production of droplets,

leading to droplets with a wide size distribution and degradation of temperature/shear sensitive compounds that should be encapsulated. An alternative method for particles preparation is membrane emulsification (ME). It is a dispersion process to produce uniform droplets of one liquid phases (e.g. water) in a second immiscible liquid phase (e.g. oil) using low energy per unit volume [3-4]. This process is increasingly used for the preparation of emulsions and micro/nano particles, such as lipid nanoparticles, nanocapsules, gel microbeads, microcapsules and liposomes. The shear stress is applied on the membrane surface and the droplet size is controlled by the pore size of the membrane [5]. In this work, for the first time, microparticles production by ME, containing biophenols recovered from OMWWs by integrated membrane processes has been achieved. OMWWs used in the present work were produced from the biologic olive oil production (toxic pesticides are not used) and supplied by Olearia San Giorgio (San Giorgio Morgeto, Italy). The process used for OMWWs biophenols recovered and concentration has been previously described. Briefly, after suspended solids removal by an acidification/microfiltration (MF) step, concentrated polyphenols were obtained by nanofiltration (NF) followed by osmotic distillation (OD). The enriched biophenolic fraction was used for the preparation of a water-in-oil (W/O) emulsion by ME. Moreover, considering that the bioactive molecule distribution between two phases plays an important role in determining its stability, retention and release, coefficient partition of both catechol, as biomolecule model, and biophenols recovered and concentrated by advanced membrane process has been measured. The feasibility to valorize biophenols coming from OMWWs in bio-functional particles has been evaluated. This aspect is a crucial issue with a view to create a drug controlled delivery system.

In addition, less hydrophilic biophenols such as hydroxytyrosol and oleuropein aglycone have been encapsulated in solid lipid particles (SLPs) based on cocoa butter produced using two methods such as ME and homogenizer. This study was aimed to evaluate the influence of these two manufacturing method on size distribution and biophenols encapsulation efficiency of SLPs.

Hydroxytyrosol (HT) and oleuropein aglycone (Agly) were used for encapsulation in lipid particles for their physical properties. Chatzidaki et al. [6] reported for HT a partition coefficient of $\log P_{o/w} = 1.1$, that indicates its amphiphilic character, while oleuropein aglycone produced from oleuropein hydrolysis, producing is more hydrophobic [7]. Rodis et al. [8] evaluated the partition coefficient ($K_p = O/W$) of the phenolic compounds

between aqueous and olive oil phase, demonstrating that aglycone partition coefficient ($K_p = 1.49$) is higher compared to oleuropein partition coefficient ($K_p = 0.0006$).

Solid lipid particles (SLPs) are carrier systems based on a high melting point lipid as a solid core. They are derived from an oil-in-water (O/W) emulsion by exchanging the liquid lipid (oil) by a solid lipid.

Lipid matrices made from biodegradable and biocompatible lipids are very well tolerated carrier systems suitable for many applications such as dermal application of cosmetics and pharmaceuticals [9]. Encapsulation of hydrophilic materials such as biophenols into a hydrophobic matrix is a major challenge as the drug tends to partition toward the aqueous phase during the production process. Usually, encapsulation of hydrophilic drugs is achieved by using double emulsion W/O/W to reach satisfactory loading efficiency. In this work, we evaluated the potentiality of an alternative formulation to load olive phenolic compounds in lipid matrix.

The SLPs present a high potential for dermal application in the pharmaceutical and cosmetic field for enhancing the penetration of compounds for specific skin layers [10-11]. Furthermore, SLPs exhibit many advantages such as controlled release, negligible skin irritation, drug targeting, incorporation of lipophilic and hydrophilic drugs, and enhancement of bioavailability of the entrapped bioactive and finally protect the chemically labile compounds such as phenolic compounds from degradation [12].

Only few works are related to the use of lipid particles for the encapsulation of hydrophilic compounds. Reithmeier et al. [13] investigated the potential of lipids such as triglycerides as a parenteral controlled release device for peptides (thymocartin and insulin) using a solvent evaporation and a melt dispersion method for S/O/W formulation.

Chambi et al. [14] produced solid lipid microparticles by spray chilling using mixtures of lipid to load hydrophilic compounds as the core materials including glucose or casein or hydrolysed casein. In another work, Shah et al. [15] prepared ciprofloxacin hydrochloride based solid lipid nanoparticles (SLNs) by solvent diffusion technique in order to improve ophthalmologic bioavailability of drugs and the therapeutic action. Other SLPs formulations are developed as a carrier for water-soluble compounds such as hydroquinone [16], streptomycin sulfate [17], diclofenac sodium [18] and isoniazid [19].

In this work, the pulsed back-and-forward ME has been selected as low shear encapsulation method because it is particularly attractive for the production highly concentrated microemulsions without causing coalescence [20].

The best operating conditions to control particles size and size distribution of the W/O and SLPs were studied, as well as the encapsulation efficiency (EE) of biophenols. Moreover, the comparison of two emulsification methods (pulsed back-and-forward ME and rotor-stator homogenizer) regarding particles size, size distribution and EE are presented.

4.2. Materials and Methods

4.2.1. Chemicals for water-in- oil emulsion formulation

A water-in-oil emulsion was prepared using PVA (average MW 13,000-28,000 Da, Sigma-Aldrich, Italy) 15% (wt.) as dispersed phase and Span 80 (Fluka, Italy) 2% (wt.) in limonene (97%, Sigma-Aldrich, Italy) as continuous water phase. Catechol (Sigma-Aldrich, Italy) has been selected as biophenol molecule model because it was one of the most representative biophenols contained in OMWW [7] and it was dissolved in PVA solution. Alternatively, a biophenols concentrated solution obtained from olive mill wastewaters (OMWWs) by integrated membrane operations as reported in the previous chapter 3 was also used. Considering an initial OMWWs volume of 1.000 L, it was possible to obtain an enriched fraction of biophenolic compounds of 87.5 g/L after NF and OD concentration steps. All the solvents used for HPLC mobile phase preparation (acetonitrile, acetic acid and methanol) were purchased from Sigma-Aldrich (Italy).

4.2.2. Chemicals for solid lipid particles formulation

Solid lipid particles were developed using cocoa butter (Online market Mystic Moments). The main constituents of cocoa butter include triglycerides of palmitic, stearic and oleic acids [21]. An aqueous phase containing polyoxyethylene (20) stearyl ether (Brij 78, Sigma-Aldrich) was used as emulsifier. The solid lipid mixture is used to encapsulate hydroxytyrosol (Sigma-Aldrich Italy) and oleuropein aglycone. The β -glucosidase (Sigma-Aldrich) from almond were used to produce oleuropein aglycone (Oleuropein from Extrasynthase).

Sodium dihydrogen phosphate anhydrous and disodium hydrogen phosphate anhydrous (Sigma Aldrich) were used to prepare phosphate buffer solution for enzymatic reaction. Acetonitrile and o-phosphoric acid for HPLC mobile phase preparation were purchased from Carlo Erba and VWR International, respectively. Ethyl acetate (VWR International) was used for oleuropein aglycone extraction and preparation of the lipid phase containing the bioactive compounds.

4.2.3. Oleuropein aglycone production

Oleuropein aglycone was obtained from oleuropein by enzymatic reaction by using β -glucosidase in a discontinuous reactor (batch reactor). The reaction was carried out at 37°C for 24 hours using 49 mL of oleuropein and 1 mL of β -glucosidase at a concentration of 1.35 and 1g/L respectively. After the reaction, the enzyme was removed from the solution by ultrafiltration with a 30 kDa regenerated cellulose membrane (Vivaspin[®], Sartorius). Subsequently, the aglycone was extracted in ethyl acetate using an aqueous phase/ethyl acetate ratio equal to 1:4 by using the rotor-stator homogenizer. The ethyl acetate containing aglycone was then mixed with the lipid phase and used as dispersed phase for the preparation of solid lipid particles as illustrated in the section 4.2.6.

4.2.4. Formulation by pulsed back-and-forward cross-flow batch membrane emulsification

SPG (Shirasu porous glass) tubular membranes (8.7 mm inner diameter, 0.65 mm wall thickness) with an effective membrane area of 31.3 cm² from SPG Technology Co., Ltd. (Japan) were used. A hydrophobic membrane with nominal pore size of 3.1 μ m were used for the production of the water-in-oil emulsion while a hydrophilic membranes with nominal pore size of 1.1 μ m were used for the production of the solid lipid particles. Before each experiment, the membrane was wetted in the continuous phase in an ultrasonic bath. For the W/O emulsion, the dispersed phase was injected from the shell side of the membrane by using a peristaltic pump (Gilson, Minipuls 3), while for the production of the solid lipid particles the dispersed phase was pressurized through the membrane under nitrogen pressure. The dispersed phase flux (J_d) was calculated as follows:

$$J_d = \frac{Q_d}{A_m}$$

where Q_d [L h⁻¹] is the dispersed phase flow rate and A_m [m²] is the membrane area.

The continuous phase for both formulations was agitated along the lumen side of the membrane by a programmable peristaltic pump (Digi-Staltic double-Y Masterflex[®] pump Micropump, model GJ-N23.JF1SAB1) at fixed amplitude and frequency. The schematic figure of the apparatus used for pulsed back-and-forward cross-flow batch membrane emulsification to produce w/o emulsion is illustrated in Figure 4.1. A similar system was used to develop the solid lipid particles, in this case the plant was equipped with nitrogen

cylinder to press the dispersed phase through the pores of the membranes. Membrane module and dispersed phase for SLPs production were immersed into a heated bath at 40°C.

The maximum shear stress (τ_{\max}) [Pa] is a function the amplitude (a) and the frequency (f) of the pulsed flow according to the following equation:

$$\tau_{\max} = 2 a (\pi f)^{\frac{3}{2}} (\mu_c \rho_c)^{\frac{1}{2}}$$

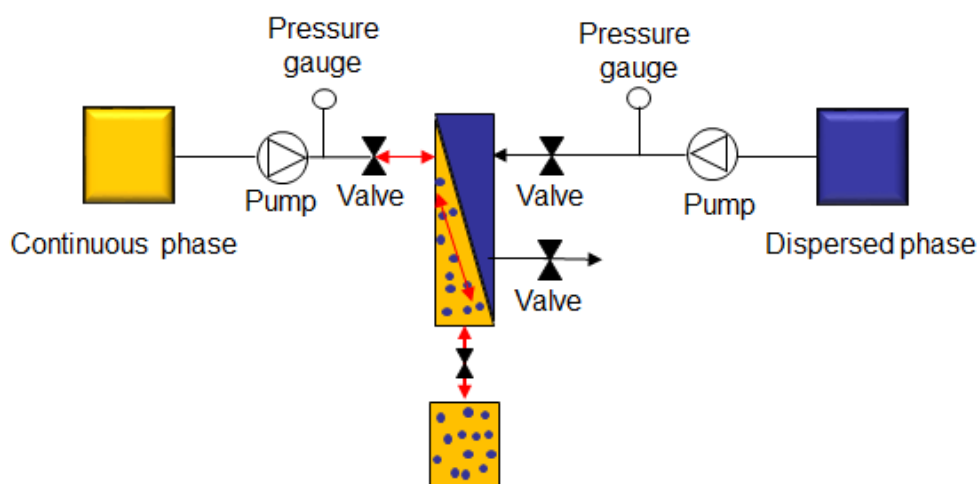


Figure 4.1. Membrane emulsification plant for formulations preparation.

4.2.5. W/O emulsion preparation

The membrane emulsification process was carried out as function of dispersed phase flux and shear stress, in order to evaluate the effect of these parameters on particle size and size distribution. The operating conditions providing the best uniformity of the droplets have been used for the production of W/O emulsion containing biophenols. Catechol or OMWWs concentrated were dissolved in the aqueous dispersed phase. Catechol and OMWWs concentration used were 10 g L⁻¹ and 3 g L⁻¹, respectively. The emulsification process was stopped when the dispersed phase percentage was 10% v/v. At the end of the emulsification, 10 mL of emulsion were centrifuged at 10.000 rpm for 10 minutes to promote phases separation and the separated water phase was analyzed to measure the concentration of catechol or biophenols (according to the type of encapsulated materials used) by HPLC and UV spectrophotometry, respectively. The experimental setup used for release experiments consisted of a cylindrical glass tube put in a thermostatic bath used to

keep the temperature at 25°C. 4 ml of emulsion containing catechol or biophenols were filled in a dialysis bag (Spectra/Por Dialysis Membrane, $z_{a} < \text{MWCO } 15.000$) and immersed in 18 mL of receptor solution (ultrapure water). Aliquots of receptor solution were withdrawn for determination of catechol or biophenols concentration by HPLC and UV spectrophotometer. The aliquot withdrawn was replaced with the same volume of pure water in order to maintain constant the volume of the receptor solution. The amount of catechol or biophenols released (C_r) to the receptor solution is expressed as the ratio between the fraction of catechol (or biophenols) released and their initial encapsulated concentration during the time:

$$C_r = \frac{C_t}{C_{IN}}$$

where C_t and C_{IN} are the concentration of catechol (or biophenols) released at t time and initially encapsulated in the aqueous dispersed phase, respectively.

The bioactives partition between different phases depending on their relative thermodynamic affinity for each phase. Considering that the location of a bioactive within a delivery system plays an important role in determining its stability, retention, and release, the partition (or distribution) coefficient (K_D) of catechol and biophenols was measured.

K_D gives an indication of the substance solubility in the two phases and it is defined by the equilibrium concentration ratio of the component A in the organic ($[A]_o$) and aqueous phases ($[A]_w$):

$$K_D = \frac{[A]_o}{[A]_w}$$

Another important parameter is the degree of extraction E defined as:

$$E = \frac{(n_A)_o}{(n_A)_{w}^{IN}}$$

where $(n_A)_{w}^{IN} = (n_A)_w + (n_A)_o$ are the initial moles of the component A in the aqueous phase and $(n_A)_o$ are the moles at equilibrium.

If V_w and V_o are the aqueous and organic phase volumes, then:

$$E = \frac{(n_A)_o}{(n_A)_w + (n_A)_o} = \frac{[A]_o V_o}{[A]_w V_w + [A]_o V_o} = \frac{\frac{[A]_o}{[A]_w}}{\frac{[A]_o}{[A]_w} + \frac{V_w}{V_o}}$$

The methodology used to calculate the partition coefficients involved the formation limonene/water mixtures containing catechol or biophenols. In particular, catechol was

dissolved in a known volume of distilled water at concentrations of 5 mg/L and then mixed with limonene, instead the aqueous solution of biophenols is coming from olive mills wastewaters. The flasks were immersed in a constant temperature bath (25°C) and stirred for at least 4 hours, long enough to approach equilibrium. After phase separation, catechol concentrations were determined quantitatively in both phases using high-performance liquid chromatography (HPLC). When biophenols from OMWWs have been encapsulated, biophenols concentrations were determined by using Folin-Ciocalteu method. The distribution coefficient was calculated using different volume ratios of aqueous and organic phases (with a constant initial concentration of catechol in the aqueous phase) [22]. The catechol concentration in limonene was plotted as function of catechol concentration in the aqueous phase measured at equilibrium after each single extraction. The same procedure was used in the case of biophenols.

4.2.6. Solid lipid particles preparation

As a comparison, solid lipid particles (SLPs) loading biophenols were prepared by two methods such as rotor-stator homogenizer and emulsification membrane. The solid lipid particles were evaluated for their size particles, size distribution, biophenols encapsulation efficiency. Preliminary studies have been carried out by using rotor-stator homogenizer (OV5 Velp Scientifica) in order to optimize the composition of the dispersed phase used for solid lipid particles (SLPs) preparation and reach high biophenols EE. Subsequently the SLPs were produced using the technique of emulsification membrane. For the encapsulation of hydrophilic phenolic compounds (hydroxytyrosol and oleuropein aglycone) in a hydrophobic matrix based of cocoa butter, three different strategies have been evaluated (Figure 4.2):

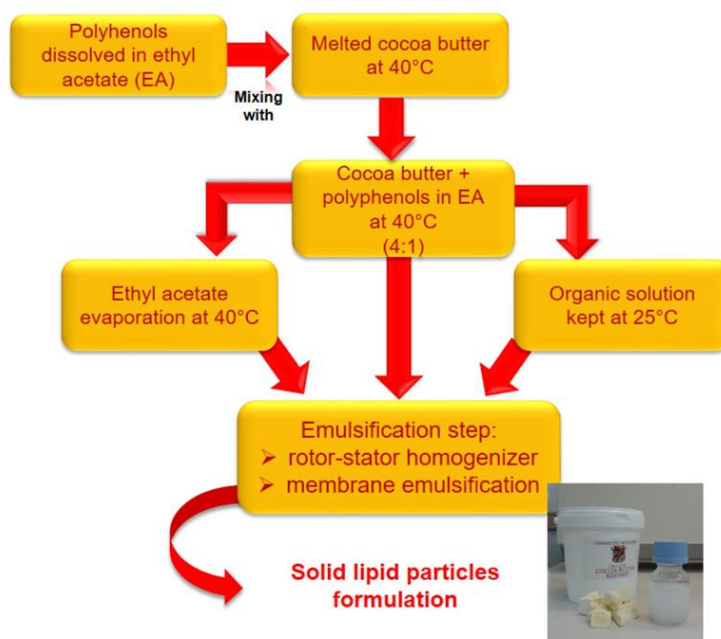


Figure 4.2. Strategies used to produce solid lipid particles load with polyphenols.

- Composition A: melted lipid (40°C) has been mixed with ethyl acetate (containing biophenolic compounds, hydroxytyrosol or oleuropein aglycone) in a ratio equal to 4:1 and kept at 40°C during the emulsification process.
- Composition B: melted lipid (40°C) has been mixed with ethyl acetate (containing biophenolic compounds, hydroxytyrosol or oleuropein aglycone) in a ratio equal to 4:1. The mixture has been cooling down to 25°C and then used for the emulsion preparation. At this temperature the cocoa butter mixed with the ethyl acetate maintains the liquid state for at least 10 hours, therefore the emulsification processes were carried out without temperature control.
- Composition C melted lipid (40°C) has been mixed with ethyl acetate (containing biophenolic compounds, hydroxytyrosol or oleuropein aglycone) in a ratio equal to 4:1. The ethyl acetate has been evaporated at 40° to obtain a dispersion of biophenolic compounds in the lipid melt. The dispersion has been used as dispersed phase in emulsion preparation.

Since the solubility of ethyl acetate in water is 8.7 (wt.%), aqueous phase was saturated with the organic solvent in order to prevent that it acts as a vehicle for the diffusion of

biophenols toward the aqueous phase during the SLPs preparation. This procedure was performed when the organic phase consisted of melted lipid mixed with ethyl acetate.

At the end of the emulsification process, the solid lipid particles were filtered using regenerated cellulose membrane (MWCO 30 kDa, Vivaspin[®], Sartorius) to recovery the aqueous phase. The free polyphenolic compounds in the aqueous phase was analyzed by HPLC to evaluate the EE. In order to remove the organic solvent, the SLPs were washed with water distilled several times and then recovered by centrifugation using regenerated cellulose filter. Alternatively, the SLPs were stirred overnight at room temperature to allow the evaporation of the solvent. The lipid content in the formulation was measured using thermobalance (Ohaus Model MB45) where samples were heated isothermally at 100°C until constant weight was reached.

Viscosity measurements of dispersed phase are performed with a LVDV-III digital viscometer (Brookfield) attached with thermostatic bath.

The hydroxytyrosol and oleuropein aglycone concentration used was 55 and 85 mg L⁻¹, respectively. Ethyl acetate was chosen as solvent for biophenolic compounds because it has been demonstrated exhibits higher extraction power respect to other solvents towards biohenols [23].

4.2.6.1. Solid lipid particles preparation by rotor-stator homogenizer

Solid lipid particles were prepared by rotor-stator homogenizer (OV5, Velp Scientifica) using different dispersed composition as described above. The organic phase was add dropwise to 32 mL aqueous phase containing the surfactant (4 wt./wt. %, Brij78) and mixing a high speed of 10.000 rpm for 1 min. The particles of the dispersed phase are broken up to a large extent by forces of inertia and shearing in turbulent flow, which in turn depends on the geometry of rotor-stator as well as on the rotational speed.

4.2.6.2. Solid lipid particles preparation by membrane emulsification

Membrane emulsification was investigated as innovative process to achieve an accurate control of solid lipid particles production at lower shear stress conditions compared to conventional method. The SLPs were produced using a volume of 25 mL of aqueous continuous phase, which was agitated into lumen of the membrane by using a peristaltic pump with flow rate reversion function, while the lipid phase (compositions A, B and C) was pressed through the membrane pores allowing the formation of small particles. The

emulsification process was go on until to reach concentration of the dispersed phase of 30 % (wt./wt.). In order to identify the optimum operating conditions for the production of monodispersed lipid particles, the emulsification process was carried out as function of dispersed phase flux, the shear stress of continuous phase and as a function of the percentage of the dispersed phase.

4.2.7. Determination of particle size and particle size distribution

W/O emulsions were observed by optical microscopy (Zeiss, model Axiovert 25), equipped with a camera (JVC, model TK-C1481BEG). Pictures were analyzed by the Scion Image program as described in the previous chapter. The resulting values were elaborated in Excel to calculate the mean diameter.

Light scattering system (Mastersizer 2000, Malvern Instruments) was used for the characterization of the solid lipid particles in terms of size and size distribution. The mean particle size was expressed as the surface weighted mean diameter (or Sauter diameter) expressed by $D[3,2]$ and as the volume weighted mean diameter (or De Brouckere diameter) expressed by $D[4,3]$. $D[3,2]$ and $D[4,3]$ were determined, respectively, as follows:

$$D[3,2] = \frac{\sum D_i^3 n_i}{\sum D_i^2 n_i}$$

$$D[4,3] = \frac{\sum D_i^4 n_i}{\sum D_i^3 n_i}$$

where D_i = particle diameter of class i and n_i = number of particle in class i . The width of droplet size distribution was expressed as a Span number, calculated by the following expression:

$$Span = \frac{D[90] - D[10]}{D[50]}$$

where $D[x0]$ is the diameter corresponding to $x0$ vol.% on a relative cumulative droplet size curve.

4.2.8. Analytical measurements

The content of biophenols recovered and concentrated from OMWW were determined by using Folin-Ciocolteau method while HPLC analysis were carried out to evaluate biophenols compounds (Catechol, hydroxytyrosol and oleuropein aglycone) concentration. A HPLC system equipped with an UV detector (Agilent 1200 Series) and a reversed phase Luna C18 column (Phenomenex, Torrance, CA) were used. The analysis was carried out at temperature of 25°C, pressure of 100 bar and wavelength of 280 nm. The composition of the mobile phases used for different phenolic compounds is reported as follows:

Catechol: a mixture of water/acetic acid (99/1, v/v) (70 %, solvent A) and methanol/acetonitrile/acetic acid (90/9/1, v/v/v) (30%, solvent B) was used as mobile phase. The analysis was made at a flow rate of 1.0 mL/min.

Hydroxytyrosol: the mobile phase was a mixture of acetonitrile/water (10:90 v/v) acidified with o-phosphoric acid (up to pH 3). The flow rate was set at 1.0 ml/min

Oleuropein aglycone: the mobile phase was a mixture of acetonitrile/water (21:79 v/v) acidified with o-phosphoric acid (up to pH 3). The flow rate of analysis was 1.2 ml/min.

Total polyphenols were estimated colorimetrically by using the Folin-Ciocalteu method. The absorbance was measured by using a UV-visible spectrophotometer (Lambda EZ201, Perkin Elmer) at 765 nm.

The encapsulation efficiency of phenolic compounds (EE%) for W/O and SLPs have been evaluated according to equations 1 and 2, respectively.

$$EE\% = \frac{C_0}{C_i} \times 100 \quad (1)$$

where C_0 and C_i are the measured and initial concentration of phenolic compounds in the aqueous dispersed phase, respectively.

$$EE (\%) = \frac{(W_t - W_f)}{W_t} \times 100 \quad (2)$$

where W_t is the initial mass of biophenols in the dispersed phase and W_f is the free amount of biophenols (no encapsulated) in the aqueous phase of the formulation.

4.2.9. Energy consumption in the production of solid lipid particles

Estimation of energy consumption for the production of solid lipid particles by membrane emulsification and rotor-stator homogenizer was evaluated considering the production of formulation at 30% of dispersed phase. For each emulsification method the energy required was calculated in terms of energy density (Ev) according to the following equation:

$$E_v = \frac{P}{V}$$

where P is the effective power input ($J s^{-1}$) and V is the volume flow rate of emulsion ($m^3 s^{-1}$)

For the ME process carried out at 25°C, the power input was calculated as the pressure drop along the membrane (ΔP) multiplied by flow rate of peristaltic pump (Q):

$$P = \frac{\Delta P * Q_f}{\eta}$$

Where ΔP is the pressure drop along the membrane module, Q_f is the flow rate of the peristaltic pump and η is the pump efficiency. Besides, when the membrane emulsification process was performed at 40°C, the power demand to heat the water (about 6 kg) in the thermostatic bath was also considered. In case of rotor-stator homogenizer, the power input corresponding to 10.000 rpm (the stirring speed used to produce the formulation) was given by the supplier.

4.4. Results and discussion

In the following paragraphs are reported the results achieved for water-in-oil (W/O) and solid lipid particles (SLPs) formulations loaded with biophenols compounds. The purpose of experiments was to:

- investigate the effects of dispersed phase fraction and process parameters on particle size and size distribution of W/O emulsion by using membrane emulsification;
- apply the optimized process to the encapsulation of an enriched fraction of biophenols in W/O emulsion;
- study biophenols release for W/O emulsion;
- evaluate the encapsulation efficiency and particles distribution of two emulsification method (membrane emulsification and rotor-stator homogenizer) for SLPs loaded with low hydrophilic biophenols;
- optimize process parameters of membrane emulsification for the development of SLPs. Furthermore, the influence of dispersed phase fraction on particle size and size distribution of SLPs was evaluated.
- evaluate energy consumption for the production of solid lipid particles by membrane emulsification and rotor-stator homogenizer.

4.4.1. W/O emulsion preparation

4.4.1.1. Effect of dispersed phase flux on particle size and size distribution

The influence of dispersed phase flux on particle size and particle size distribution is shown in Figure 4.3. Particle size was constant ($7.3 \pm 0.2 \mu\text{m}$) in the range of flux between 2 to $14.3 \text{ L h}^{-1} \text{ m}^{-2}$ and uniform drops were produced with the span of distribution of 0.4. Particles size ($D[3,2]$ and $D[4,3]$ of $12.8 \mu\text{m}$ and $16.1 \mu\text{m}$, respectively) and particles size distribution (span 1) increase were obtained when the flux was increased to $20 \text{ L h}^{-1} \text{ m}^{-2}$. Results demonstrated that it is possible to increase the dispersed phase flux up to $14.3 \text{ L h}^{-1} \text{ m}^{-2}$ without significantly influence the control of particles production by using ME process. The shear stress used (5.8 Pa) determined droplets detachment before that the increase of droplet volume, gives coalescence at the membrane level while, the further increase of dispersed phase flux lead to the formation of larger droplets before they are detached. In this case, droplet–droplet interactions at the pore level was responsible of broadening the droplet size distribution.

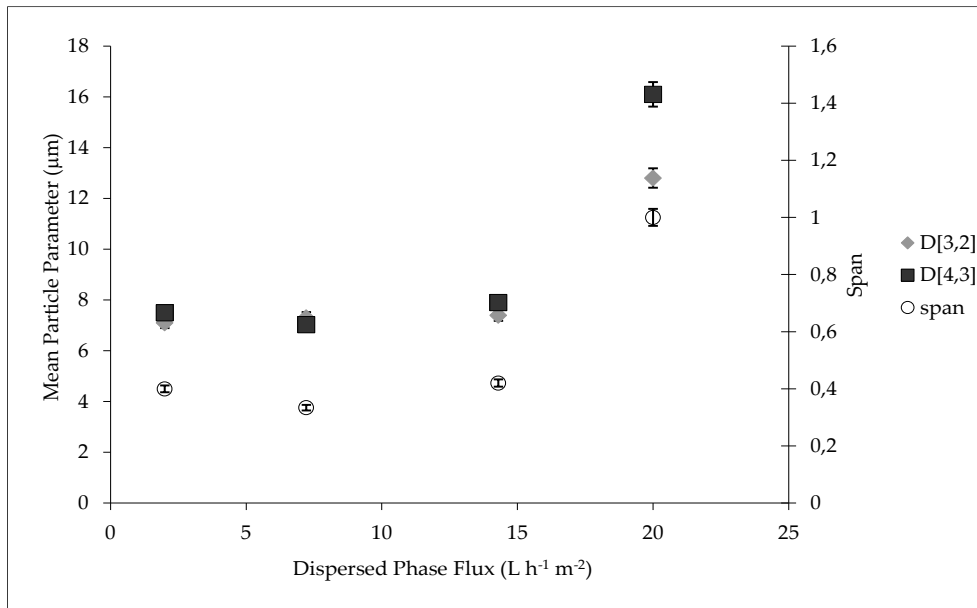


Figure 4.3. The effect of dispersed phase flux on particle size and particle size distribution of W/O emulsion prepared by using pulsed back-and-forward membrane emulsification (shear stress: 5.8 Pa, membrane pore size: 3.1 μm).

4.4.1.2. Effect of shear stress on particle size and size distribution

Figure 4.4 shows the effect of the shear stress on particle size and particle size distribution. It was observed that the largest droplets ($D[3,2] = 10.9 \mu\text{m}$) were produced at the lowest shear stress value (1 Pa) while in the range between 2.3 and 5.8 Pa the particles size was approximately 2.3 times the membrane pore diameter (7.4 μm). At low shear, larger droplets are produced because they are break away from the membrane not fast enough to prevent the droplet coalescence at the membrane pore level. On the contrary, when the shear stress was increased to 2.3 Pa, droplets detachment is faster and the further increase of the shear does not significantly modify the droplet diameter. Particles with wide size distribution (span = 1.46) were obtained only at low value of shear stress. On the contrary, the particle size distribution was almost independent (span = 0.33-0.40) of the shear in the range between 2.3 to 5.8 Pa because of the decreased probability of droplet coalescence at the membrane surface. Figure 4.5 shows the microscope image of W/O.

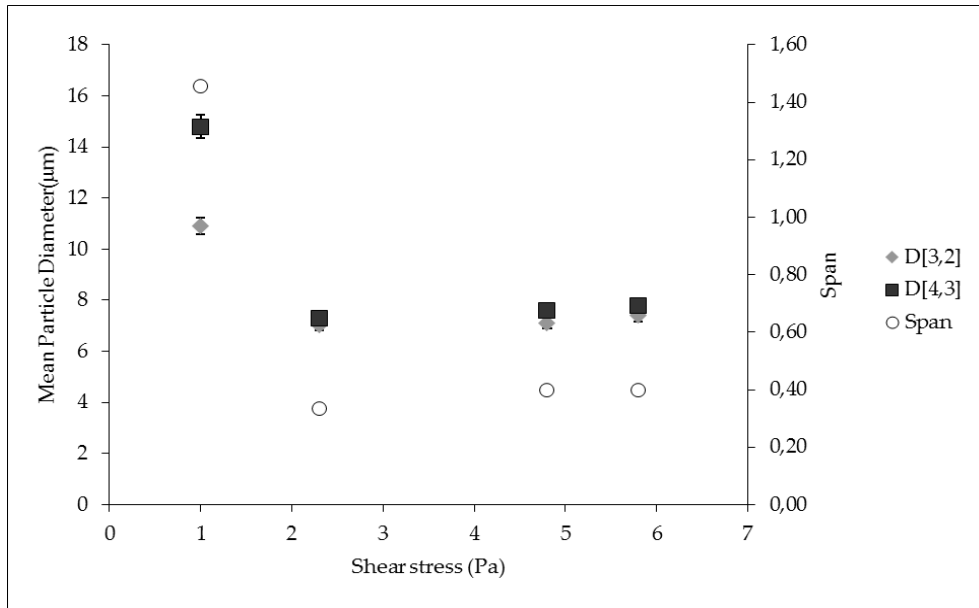


Figure 4.4. The effect of shear stress on particle size and particle size distribution of W/O emulsion prepared by using pulsed back-and-forward membrane emulsification (dispersed phase flux: $7.2 \text{ L h}^{-1} \text{ m}^{-2}$, membrane pore size: $3.1 \text{ }\mu\text{m}$).

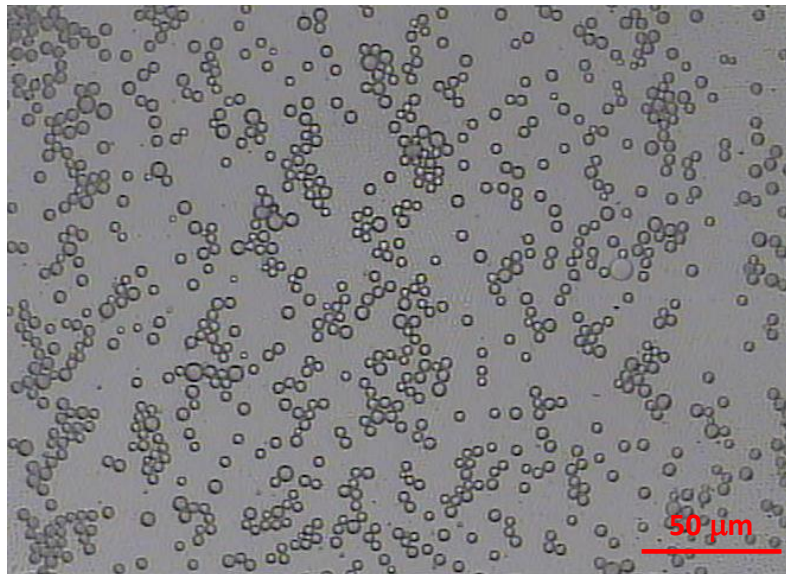


Figure 4.5. Optical microscope image of W/O emulsion prepared by pulsed back and forward membrane emulsification.

4.4.1.3. Effect of dispersed phase fraction on particle size and size distribution

The dispersed phase volume fraction in an emulsion plays an important role in determining its stability due to the increased probability of collision frequency or contact between the droplets promoting coalescence, flocculation or sedimentation. The dispersed phase fraction was changed in the range between 9 to 30 % v/v. Figure 4.6 shows mean particle diameter and span versus the percentage of the water content within the W/O emulsion.

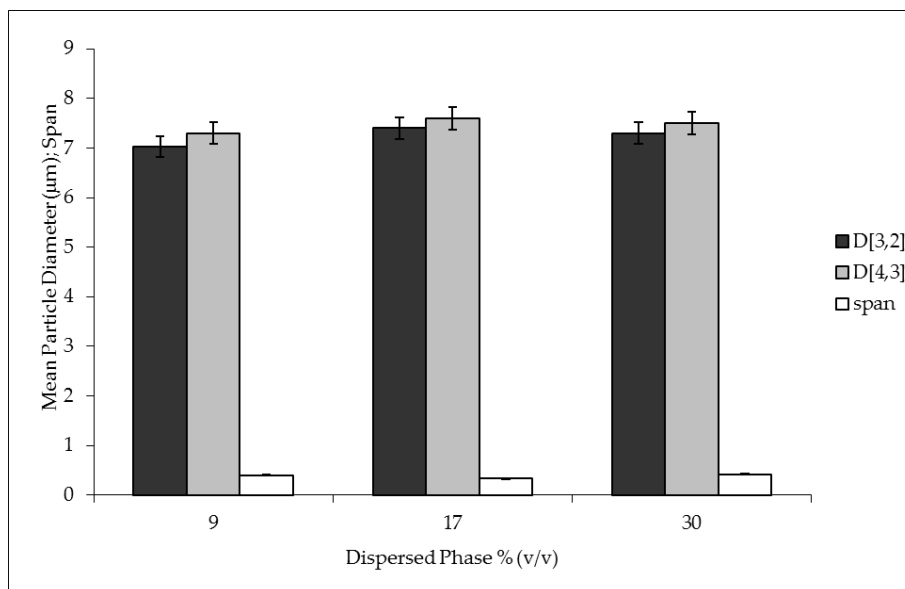


Figure 4.6. The effect of dispersed phase % (v/v) on particle size and particle size distribution of w/o emulsion prepared by using pulsed back-and-forward membrane emulsification (dispersed phase flux: $7.2 \text{ L h}^{-1} \text{ m}^{-2}$, shear stress: 5.8 Pa , membrane pore size: $3.1 \text{ }\mu\text{m}$).

The average size of $7.2 \text{ }\mu\text{m}$ for D[3,2] and $7.5 \text{ }\mu\text{m}$ for D[4,3] with a span of 0.38 was kept constant when the water content was increased. Because also phases separation was not observed, results indicated that the emulsifier (Span 80) accumulates at the interfacial film between water droplets dispersed phase and limonene continuous phase stabilizes the water droplets and avoiding the coalescence mechanism of water droplets phase. In emulsification process by using membranes, changes in terms of droplets size and size distribution are observed when high concentrations of dispersed phase are reached because of droplets breakup due to the shear during long time of operation [12]. The results demonstrated that it is possible to control the shear stress conditions on the generated drops by appropriately selecting the suitable emulsification membrane-based method to produce highly concentrated formulations with tuned size and size distribution. The dispersed phase content is important because determines the initial concentration of active compounds (i.e.

flavor molecules, vitamins, drugs) in the emulsion which has important implications for formulation functional properties (i.e. taste and aroma of food emulsions; nutraceutical or pharmaceutical activity in emulsion-based delivery system).

4.4.1.4. Encapsulation efficiency and release studies

Catechol and biophenols partition coefficient were determined prior to the release studies. Catechol equilibrium isotherm at 25 °C is reported in Figure 4.7. The figure illustrates a straight-line passing from zero and its slope corresponds to the distribution coefficient of catechol between the two phases, limonene and water. A K_D of 0.0034 was obtained with a $R^2 = 0.9936$, showing a good correlation between the data with the fitted regression line. A similar trend was obtained also by calculating polyphenols K_D by using the real OMWWs stream with a K_D value equal to 0.012. Data demonstrate the high affinity of biophenols studied in the present work (catechol and biophenols from OMWWs), toward the water phase.

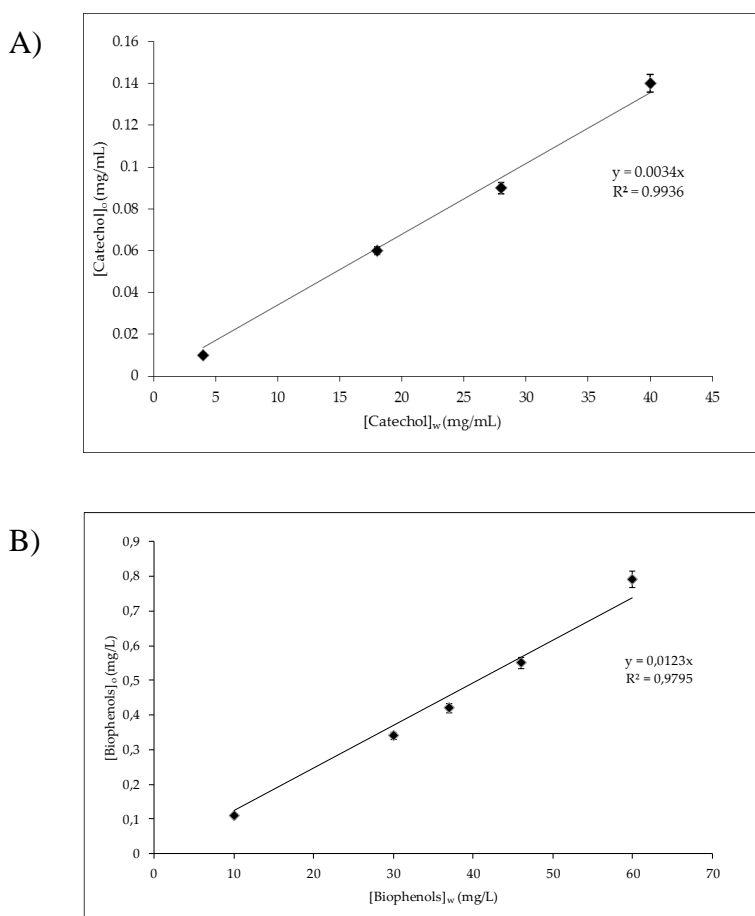


Figure 4.7. Extraction isotherm at 25 °C between aqueous and limonene phases of A) Catechol and B) biophenols coming from OMWWs.

The different solubility in the two phases and the hydrophilic/lipophilic properties of the biomolecules contained in the real stream are responsible of the higher value of K_D obtained for biophenols coming from OMWWs. The K_D of biophenols between the dispersed water phase and the continuous oil phase is a key issue for their successful encapsulation in droplets emulsion and their following release. According to this, drug encapsulation efficiency is influenced by the solubility of the drug in the continuous phase. The drug will easily diffuse into the continuous phase or will be retained in the dispersed phase as a function of its solubility in both phases. In emulsion systems, the drug diffusion into the continuous phase occurs until the bioactive partition equilibrium between the two phases is reached. In the present work, the encapsulation efficiency for catechol and biophenols was 98% and 92%, that correspond to a partition coefficient between oil and water phase of 0.02 and 0.09, respectively. The partition coefficient estimated in the emulsion system is affected by the compositions of both continuous phase and dispersed phase more complex than the limonene/water mixture. However, data confirmed the high affinity of bioactive molecules toward the water phase.

The catechol release from dispersed aqueous phase as a function of time is shown in Figure 4.8. At the beginning of the release experiment, diffusion of catechol was fast and slow down slightly after 24 hours when the amount of released drug of 70% is achieved. A similar trend was observed for the biophenols recovered from OMWWs. Although biophenolic compounds are dissolved in the aqueous dispersed phase previously, they partition among the aqueous and the oil component of the emulsion according to the structure and composition of the oil continuous phase (as indicated by the K_D) and they diffuse through the dialysis membrane before to be extracted in the receptor solution. As the amount of biophenols from oil phase is decreased as a consequence of the extraction in the receptor solution, the concentration at equilibrium, corresponding to the partition coefficient, is restored. In these conditions, the emulsion droplets will act as drug reservoirs providing biophenols, which will diffuse across the interfacial film until the equilibrium concentration is restored. The porous cellulose membrane, chosen as dialysis tubing, has a large molecular weight cutoff, which guarantees a minimum barrier effect on the diffusing molecules. The fast release observed in the case of biophenols recovered from OMWWs can be related to the different affinity of each compound of the complex matrix toward the oil phase and the receptor solution.

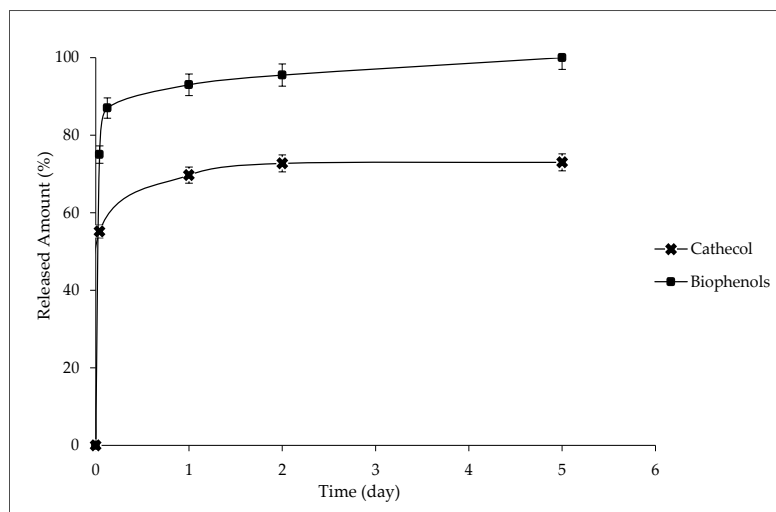


Figure 4.8. Catechol and Biophenols (concentrated of polyphenols) released amount as a function of time.

4.4.2. Solid lipid particles (SLPs) production

4.4.2.1. SLPs production by membrane emulsification and homogenizer

The effect of dispersed phase composition on biophenols-loaded SLPs on particle size and particle size distribution was studied by ME. Different composition based on cocoa butter have been evaluated. Cocoa butter is a vegetal fat with a melting point just below human body temperature. Considering the poor solubility of biophenolic compounds in cocoa butter, a mixture of cocoa butter and ethyl acetate (containing biophenolic compounds) in a ratio equal to 4:1 was used. The mixture was used as dispersed phase keeping constant the temperature at 40° C for the time of the experiment. Alternatively, the mixture was used as dispersed phase after cooling down to the room temperature (25°C) or the ethyl acetate has been first evaporated at 40°, to obtain a dispersion of biophenolic compounds in the melted cocoa butter, and then used as dispersed phase, keeping constant the temperature at 40° C for the time of the experiment.

A shear stress of 1.36 Pa and a dispersed phase flux of 2.4 L h⁻¹ m⁻² were used in all the experiments.

Uniform particles (span ≈ 0.5) in the same range of size (mean particle diameter ≈ 4 μm) were produced as reported in Table 4.1. Table 4.1 reports also a drastic reduction in energy consumption from 4.0*10⁷ to 1.1*10⁶ for SLPs production at 30% of dispersed phase when the emulsification was carried out at room temperature. The use of cocoa butter-ethyl acetate mixture enabling a process at about ambient temperatures allows also reducing the

duration of the heating step shortening the overall processing time without needing a special plant design for temperature control. The EE of hydroxytyrosol was 90% when the process was carried at room temperature and it was very close to the value obtained by using the dispersion of the biophenolic compounds in the melted cocoa butter after the ethyl acetate evaporation. It is notable that the viscosity of the dispersed phase increases in case of cocoa butter alone compared with the cocoa butter/ethyl acetate mixture at 25°C, from 18.8 to 41.5 cP. The increase of dispersed phase viscosity is expected to delay the drug diffusion within the droplets [24]. However, the high solidification rate obtained when the process was carried out at about ambient temperature determined and increase of the EE because the faster solidification of the lipid matrix prevents the drug diffusion across the phase boundary [25]. This is also confirmed by the further decrease of the EE when the cocoa butter /ethyl acetate mixture was used at 40°C and the dispersed phase viscosity was reduced down to 10.6 cP.

SLPs were also produced by using the homogenization method by using the same phase compositions used in membrane emulsification experiments. If we refer to the $D[4,3]$ value as the mean particle size, particles in the same range of size were produced by using the two emulsification methods (Table 4.1). The most significant difference was observed in particle size distribution (span). The homogenization method given a poor control of the particle size, and resulted in a wide distribution of particle size. A comparison of the size distribution of SLPs prepared by membrane emulsification and mechanical method is shown in Figure 4.9. A span of distribution in the range from 1.55 to 2.00 were obtained by using the homogenizer device while uniform particle size distribution (span ≈ 0.45) was obtained in all the experiments carried out by using ME process. Significant difference was also observed in the $D[3,2]$ value that is more sensitive to the presence of smaller droplets than $D[4,3]$ indicating that the homogenizer generate smaller particles but less homogeneous. In addition, the reduction in energy requirements by using ME is very significant when compared with other homogenization processes. Santos et al. [26] reported energy densities required typically range from 10^4 to 10^6 J m^{-3} to produce droplets size of 1–10 μm by premix ME, while is range from 10^6 to 10^8 J m^{-3} for rotor-stator devices and high pressure homogenizers. Table 4.1 reports also a drastic reduction in energy consumption from $6.4 \cdot 10^8$ to $1.1 \cdot 10^6 \text{ J m}^{-3}$ when the emulsification was carried out by membrane emulsification. Conventional homogenization devices apply high specific energy inputs to disrupt droplets [27], which leads to an increase in the temperature of an

emulsion, because a significant amount of the mechanical energy is converted into heat due to viscous dissipation [28].

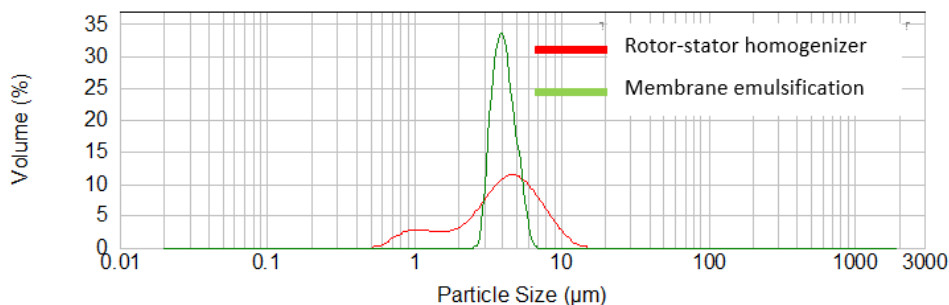


Figure 4.9. Size distribution of SLPs prepared by membrane emulsification and rotor-stator homogenizer.

Table 4.1 reported also the EE of hydroxytyrosol obtained by using the homogenizer. Data demonstrate that membrane emulsification process allowed improving the EE of hydroxytyrosol of about 2 times compared with the homogenizer when cocoa butter-ethyl acetate mixture was used at 40° C. The high energy inputs applied during the homogenization was responsible of droplets disruption and hydroxytyrosol diffusion from the lipid dispersed phase toward the water continuous phase. Hydroxytyrosol is reported to have a partition coefficient of $\log P_{o/w} = 1.1$ [6] that indicates that it have a certain solubility in both water and oil phases which influence the EE. On the other end, the EE increased as a function of the dispersed phase viscosity, as expected. Membrane emulsification is recognized to provide high encapsulation yields of multiple emulsions [29-30-31] as a result of the mild applied shear stress [32]. To the best of our knowledge, the EE obtained by using membrane emulsification and conventional mechanical stirring methods has never been compared for the preparation of simple emulsions. Our results indicate that membrane emulsification process can also provides highly efficient encapsulation of bioactive compounds partially soluble in the continuous phase as in the case of hydroxytyrosol. The combination of droplets formation by drop-by-drop mechanism and process and formulation parameters which accelerate the solidification of the particles (such as dispersed phase viscosity increase) contribute into improving the EE of amphiphilic and hydrophilic drugs in lipid matrices.

Table 4.1. Phenolic compounds encapsulation efficiency (EE), particle size and particle size distribution of SLPs, energy consumption for membrane emulsification (ME) and homogenizer (H) related SLPs production at 30% of dispersed phase (DP)

DP composition	T (°C)	DP viscosity (cP)	Emulsification Method	D[3,2] (µm)	D[4,3] (µm)	Span	Energy Consumption (Jm ⁻³)	EE%
Cocoa butter /ethyl acetate (4:1)	40	10.6	ME	3.95±0.09	4.05±0.08	0.42±0.02	4.0*10 ^{7a}	85
			H	2.85±0.32	4.50±1.10	1.57±0.08	6.4*10 ⁸	39
Cocoa butter /ethyl acetate (4:1)	25	18.8	ME	3.92±0.11	4.02±0.12	0.45±0.02	1.1*10 ⁶	90
			H	2.79±0.80	4.66±0.80	1.77±0.30	6.4*10 ⁸	65
Cocoa butter (after ethyl acetate evaporation)	40	41.5	ME	4.22±0.08	4.35±0.09	0.47±0.02	4.0*10 ^{7a}	92
			H	2.98 ±0.2	5.93±1.9	2.00±0.70	6.4*10 ⁸	80

^aIncluding the energy required to heat the water in the thermostatic bath

4.4.2.2. Optimization *fluid-dynamic conditions* of membrane emulsification process

The influence of dispersed phase flux and shear stress on particle size and particle size distribution was investigated. The experiments have been carried out over a range of dispersed flux of $0.44\text{-}3.2\text{ L h}^{-1}\text{ m}^{-2}$ and shear stress of $0.4\text{-}5\text{ Pa}$. At first, the transmembrane pressure was increased in the range from 0.25 to 0.55 bar while the shear stress has been kept constant at a value of 1.36 Pa . Uniform particles (span = 0.42) and an average diameter of approximately 3.6 times greater than the pore size of the membrane ($D[4,3]$ and $D[3,2] = 4\text{ }\mu\text{m}$) were produced when the dispersed phase flux was increased from 0.44 to $2.4\text{ L h}^{-1}\text{ m}^{-2}$. The further increase of the dispersed flux until $3.2\text{ L h}^{-1}\text{ m}^{-2}$ determines particle size and particles size distribution increase. In particular, the size distribution becomes bimodal (Figures 4.10 and 4.11) with a span of the distribution equal to 1.7 with a marked increase in average particle diameter ($D[4,3] = 20.4\text{ }\mu\text{m}$) indicating the development of coalescence at the membrane pore level. Two operational zones, the size-stable and outflow zone, are identified in membrane emulsification process as a function of dispersed phase flux increases [33]. It has been observed that unimodal droplet size distribution is possible within the size-stable zone, in which the mean droplet size is almost independent of the dispersed phase flux and the droplet size is mainly controlled by the equilibrium interfacial tension. In the outflow zone, the transition from dripping to jetting regime occur when the dispersed flux increases and the droplet detachment point moves further downstream from the pore usually resulting in the formation of a larger droplet. The production of polydisperse droplets at the higher dispersed phase flux can be also due to the activation of additional pores that take part in droplet formation [34].

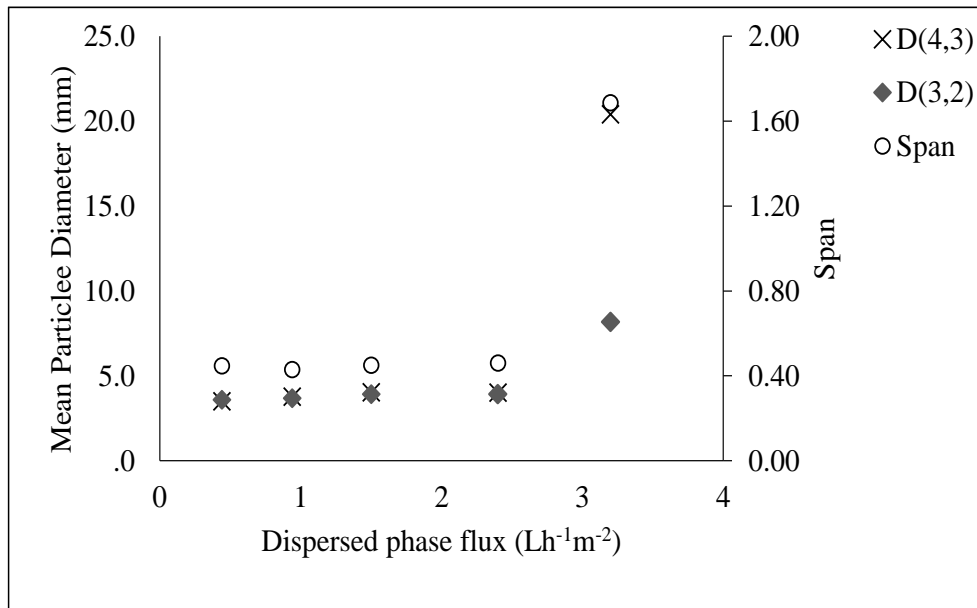


Figure 4.10. The effect of dispersed phase flux on particle size and particle size distribution of SLPs prepared by using pulsed back-and-forward membrane emulsification (shear stress: 1.36 Pa, Temperature dispersed phase 25°C, membrane pore size: 1.1 μm).

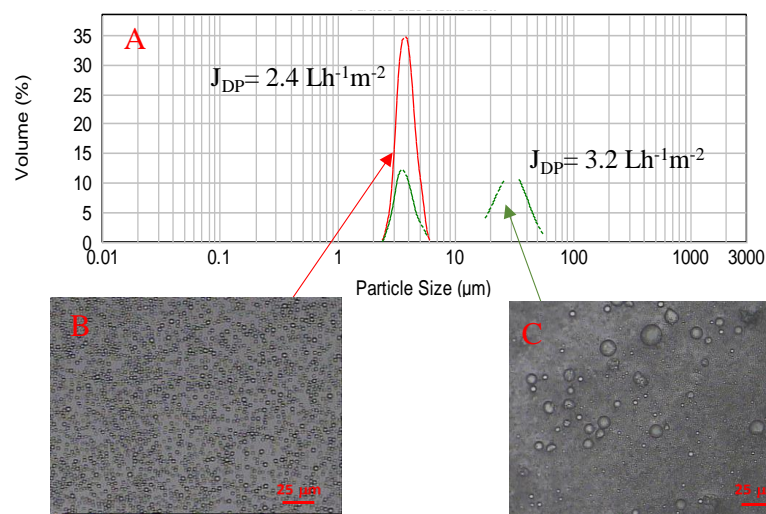


Figure 4.11. Solid lipid particles distribution (A) prepared at different value of dispersed phase flux (J_{DP}) and constant shear stress (1.36 Pa), B) optical microscope image of SLPs prepared at dispersed phase of $2.4 \text{ Lh}^{-1} \text{ m}^{-2}$, C) optical microscope image of SLPs prepared at dispersed phase of $3.2 \text{ Lh}^{-1} \text{ m}^{-2}$.

The effect of shear stress on particle size and size distribution was studied at a constant dispersed phase flux of $2.4 \text{ L h}^{-1}\text{m}^{-2}$ (Figure 4.12). In this study, the shear stress was increased in a range between 0.4 and 5 Pa. The droplet size decreases as the shear stress increase and reaches a size where it becomes independent of

shear stress. An increase of the shear stress reduces the time for droplet growth prior to detachment, reducing the size of droplet formed. This determines an average of distance between two neighbouring droplets. Therefore, the probability of interaction among of droplets at the membrane surface decreases and both smaller and monodisperse droplets are formed. The largest change in particles size occurs between 0.4 and 1.36 Pa. At low shear stresses (0.40 Pa) the particles grow and coalesce at the membrane surface before finally being removed by the action of the shear force of continuous phase, resulting a largest particles ($D[3,2] = 4.8 \mu\text{m}$, $D[4,3] = 8.9 \mu\text{m}$).

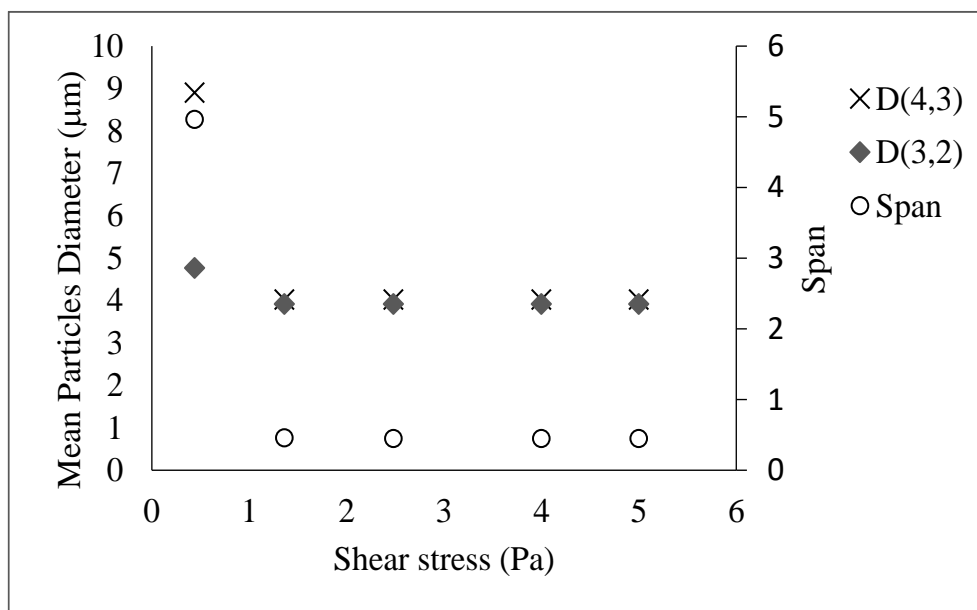


Figure 4.12. The effect of shear stress on particle size and particle size distribution of solid lipid particles by using pulsed back-and-forward membrane emulsification (dispersed phase flux: $2.40 \text{ L h}^{-1} \text{ m}^{-2}$, Temperature dispersed phase 25°C , membrane pore size: $1.1 \mu\text{m}$).

4.4.2.3. Effect of dispersed phase fraction on particle size and size distribution

In order to investigate the effect of cocoa butter amount on particle size and size distribution, the formulations were produced up to 40% of cocoa butter. Figure 4.13 shows particle size and size distribution as a function of the percentage of the cocoa butter at 40°C or when the mixture of cocoa butter/ ethyl acetate was used at 25 and 40°C . As it can be observed, increasing the concentration of lipid content there were no differences in particles size and size distribution of lipid particles up to 40% of cocoa butter. In

particular, uniform particles with size of 4 μm (D4.3) and span of 0.45 using the mixture of cocoa butter/ethyl acetate (ratio 4:1) at 25°C (Figure A) and 40°C were obtained (Figure B). While suspensions were produced with a size of 4.4 μm (D4.3) and a span of 0.47 using the cocoa butter at 40°C (Figure C).

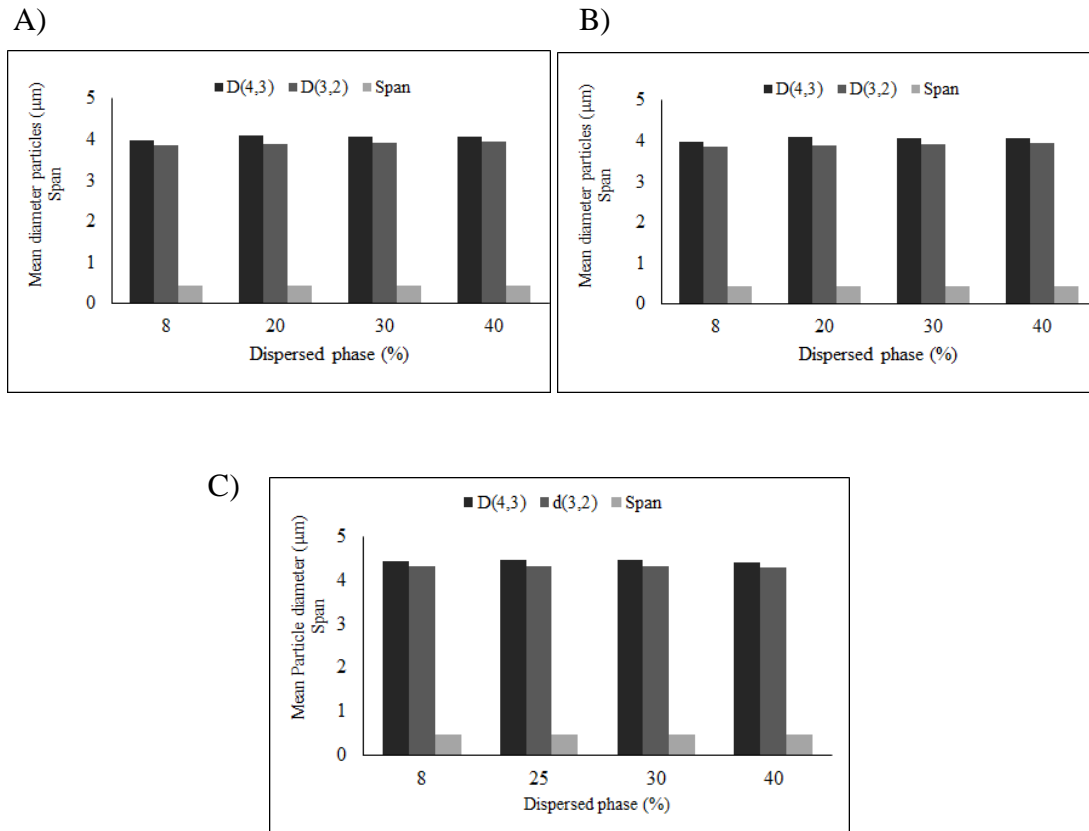


Figure 4.13. The effect of dispersed phase % (w/w) on particle size and particle size distribution of solid lipid particles prepared using cocoa butter/ethyl acetate (4:1) at 25°C (Figure A) at 40°C (Figure B) and using cocoa butter at 40°C (Figure C) prepared by using pulsed back-and-forward membrane emulsification (dispersed phase flux of cocoa butter: 2.40 L h⁻¹ m⁻², shear stress: 1.36 Pa, membrane pore size: 1.1 μm).

4.4.2.4. Encapsulation efficiency of oleuropein aglycone in solid lipid particles

Table 4.2 reported the encapsulation efficiency (EE) of oleuropein aglycone in solid lipid particles (SLPs) produced by using the homogenizer and membrane emulsification methods. The effects of various formulation as reported above (section 4.2.6) on the efficiency of biophenols loading in SLPs were investigated. The results are in accordance with a previous study on HT encapsulation. Since two phenolic compounds such as HT and oleuropein aglycone show a similar partition coefficient ($K_d=1.1$ for HT and $K_d=1.49$ for oleuropein aglycone). In comparison with the two emulsification devices, it was found that SLPs achieved by membrane emulsification presented highest value of biophenols encapsulation efficiency. In particular, the EE of oleuropein aglycone was 93% when the process was carried out using the dispersion of the biophenolic compounds in the melted cocoa butter at 40°C, which was used as dispersed phase in SLPs preparation after the ethyl acetate evaporation (composition C). Instead, the encapsulation efficiency of oleuropein aglycone was equal to 90% using as dispersed phase (composition B), the mixture consisting of the melted lipid (40°C) and ethyl acetate (containing biophenolic compounds) in a ratio equal to 4:1. The obtained mixture was then cooled to 25°C and emulsified to produce SLPs. On the other hand, when a mixture of melted lipid and ethyl acetate was emulsified at 40°C (composition A), the encapsulation efficiency decreased slightly to 86%. The effect of viscosity of dispersed phase on EE was more evident with SLPs produced using homogenizer method. The encapsulation efficiency increased from 43% to 72%, when the viscosity of dispersed phase was increased from 10.6 cP (composition A) to 18.8 cP (composition B). This is attributed to the delay phenolic compounds diffusion from the organic phase into the water phase during the production process. Furthermore, the increase of the viscosity of the dispersed phase allows faster solidification and reduces the leaching of biophenols to the continuous phase, with consequent improvement of EE.

Table 4.2. Encapsulation efficiency (EE) of oleuropein aglycone in SLPs prepared by using homogenizer (H) and membrane emulsification (ME)

DP ¹ composition	T (°C)	DP ¹ viscosity (cP)	Oleuropein aglycone EE (%)	
			H	ME
Cocoa butter /ethyl acetate (4:1)	25	18.8	72	90
Cocoa butter /ethyl acetate (4:1)	40	10.6	43	86
Cocoa butter (after ethyl acetate evaporation)	40	41.5	83	93

¹DP dispersed phase

4.5. Conclusions

In the present work, membrane emulsification has been successfully used for the encapsulation of single biophenolic compounds (catechol, hydroxytyrosol and oleuropein aglycone) and enriched phenolic fraction coming from OMWWs. W/O emulsion has been prepared by using limonene, containing Span 80 (2% wt.), as continuous phase and PVA 15 % wt. as dispersed phase. Results showed that the optimum conditions to prepare uniform emulsion with controlled droplets size are: shear stress in the range between 2.3 to 5.8 Pa and dispersed phase flux in the range between 2 to 14.3 L h⁻¹ m⁻². Emulsions with high dispersed phase fraction (30 % v/v) were produced without phases separation and any changes in terms of droplets size and size distribution was observed. High encapsulation efficiency (EE) was also obtained for catechol (98%) and biophenols fractions purified and concentrated from OMWWs (92%) demonstrating that the formulation have a potential use in food, pharmaceutical or cosmetic field. The presence of additional high-added value components (such as sugars, fats and minerals) in the polyphenols enriched fraction [35-36] as well as the absence of toxic pesticides (due to the use of OMWWs produced from a biologic olive oil production) contributes to improve the quality of encapsulated ingredients for the production of by-products with a potential health benefit. Regarding the solid lipid particles, in the present work was studied a novel procedure for encapsulation of olive polyphenols with high EE into solid lipid particles using traditional method (rotor-stator homogenizer) and membrane emulsification.

The results showed that the ME process was more efficient than the homogenizer in producing monodisperse particles by using mild operating conditions (shear stress of 1.36

Pa). This has allows to limit the diffusion of bioactive compounds toward the aqueous phase with consequent improvement of encapsulation efficiency. In particular, the influence of emulsification technique on the EE becomes evident using the dispersed phase at 40°C. The homogenizer given an EE of 39% compared to the 85% obtained by ME. Both the lower mechanical stress of membrane emulsification method and the fast solidification step of lipid particles has allowed improvement of HT entrapment in the hydrophobic matrix as demonstrated by using a cocoa butter/ethyl acetate mixture at room temperature (EE of 92%). The optimization of ME process was carried out and uniform particles with mean particle size of 4 µm (3.6 times the pore size of the membrane) and a span of 0.45 are produced up to 40% cocoa butter using a mild shear stress of 1.36 Pa and a dispersed phase flux lower than 3.2 L h⁻¹m⁻².

The studies performed to produce oleuropein aglycone-loaded SLPs, showed EE up to 93% using ME. The emulsification process has allowed increasing the encapsulation efficiency up to two times compared with the homogenizer when the process was carried out at 40°C. Results obtained encourage further studies on release of biophenols loaded into SLPs.

Regarding the energy consumption for production of solid lipid particles, energy density of membrane emulsification at 25°C was about 40 times less than those performed at 40°C. Furthermore compared to rotor-stator homogenizer method, energy requirement of ME was about two order of magnitude lower than that of the conventional method.

Membrane emulsification can satisfy the demand of pharmaceutical and fine chemical industry for improved sustainable manufacturing processes that allow to tune the properties of particulate carrier systems by reducing the energy consumption and improving the encapsulation efficiency.

Part of this chapter has been published: Piacentini E., Poerio T., Bazzarelli F., Giorno L., "Microencapsulation by Membrane Emulsification of Biophenols Recovered from Olive Mill Wastewaters", Membranes, (2016) 6, 25.

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OVERALL CONCLUSIONS

This work developed an integrated membrane process for olive mill wastewater (OMWW) valorization through the enhancing of water recycling, polyphenols recovery and formulation of emulsions loaded with biophenols. The system proposed based on a pre-treatment unit and a combination of different membrane operations including microfiltration (MF), nanofiltration (NF), osmotic distillation (OD) and membrane emulsification (ME). In particular, the OMWW was first pre-treated to remove suspended solids and then submitted to a microfiltration step, the permeate stream coming from microfiltration unit was used to feed the nanofiltration process, after that the nanofiltration retentate was further concentrated by osmotic distillation. The final step of downstream processing is the enriched polyphenols fraction formulation in water-in-oil (W/O) emulsions by membrane emulsification.

The identified drawbacks for the processing of OMWW using membrane technology are related to the rapid flux decrease due to fouling phenomena. For this reason, a new approach to the OMWW pre-treatment based on suspension destabilization has been developed and applied upstream the membrane operations.

The suspension destabilization method provides a general approach to treat wastewater with large suspended solids content. The pre-treatment proved to be relevant to achieve the complete elimination of all the suspended solids, making the OMWW more suitable for the following microfiltration and ultrafiltration processes.

The performance of different membrane materials during microfiltration and ultrafiltration processes was evaluated in terms of permeate flux, total organic carbon (TOC) reduction and phenols rejection. The highest permeate fluxes were obtained in the microfiltration and ultrafiltration processes using ceramic membranes. The performance of different nanofiltration membranes was also evaluated. On the basis of this study, a lab scale prototype has been designed and tested. The plant was equipped with MF ceramic module with area up to 1 m², NF polymeric spiral wound module with area up to 2.6 m² and with polymeric hollow fiber module for osmotic distillation (area of 1.4 m²). Instead, the ME experiments were performed using porous glass tubular membrane.

The integrated membrane system resulted efficient in all the operation units. Indeed, relatively high fluxes, with respect to literature data, were obtained in both MF and NF operations, respectively of 60 and 7.2 L/h m². Furthermore, introduction of air backflushing cycles during OMWW microfiltration was demonstrated an useful strategy

for minimizing and controlling the fouling phenomena. In particular, the permeate flux at the steady-state in feed and bleed mode was about 30% higher than that observed without injection of air. Instead, working in batch concentration mode, an improvement of permeate flux up to two-fold was obtained using air backflushing.

Regarding the NF unit, high polyphenols and TOC rejections was achieved. Thus, NF operation has demonstrated to be efficient for water and polyphenols recovery. By properly tailoring the operation conditions of NF (200 Da) unit, TOC and polyphenols rejections were of 92% and 98%, respectively. The NF retentate was further concentrated with a factor of 7 by OD at low operating temperature, producing an enriched fraction of biophenols. The membrane emulsification (ME) process resulted a highly innovative and effective technique for the valorisation of biophenols concentrated by the integrated membrane process. Pulsed back-and-forward ME allowed to produce of water-in-oil (W/O) emulsions with narrow distribution and high encapsulation efficiency (EE) using biophenols mixture (EE of 92%). Furthermore, catechol compound has been used as biophenol model for development of water-in-oil (W/O) emulsions formulations (EE of 98%).

In addition, an alternative and new approach was presented to encapsulate also less hydrophilic phenolic compounds in solid lipid particles (SLPs) by membrane emulsification (ME) or rotor-stator homogenizer devices.

The results showed that the membrane emulsification process was more efficient than the homogenizer in producing monodisperse particles and also provides highly efficient encapsulation of bioactive compounds. In particular, the highest polyphenols encapsulation efficiency of 93% was reached by membrane emulsification using mild shear stress and low energy input.

On the basis of the results achieved with the lab scale integrated membrane system prototype, a productive scale plant was developed at olive mill factory. More specifically, the integrated membrane system was based on OMWW acid treatment step, preliminary filtration using bag filter in series mode, microfiltration unit using tubular multichannel ceramic membrane (TiO₂) with area of 12.95 m² and spiral wound membrane (polyamide thin-film composite) in both NF (MWCO of 200 Da) and RO (NaCl rejection of 99.5%) processes with area of 38 and 53.2 m², respectively. The upscaled integrated process allowed to treat up to 2.5 m³/day of OMWW with completely recover polyphenols and water after the final unit of reverse osmosis. In particular, the quality characteristics of water stream respect the limits imposed by legislation law, therefore the water fraction can

be discharged in aquatic system or reintroduced in olive oil production cycle.

APPENDIX

Award

- Travel award for the participation to the XXXII Summer School 2015 “Integrated and Electromembrane processes”, Straz pod Ralskem/Liberec (Czech Republic). 21-26 June, 2015

Education and training from 2014 to 2017

- Journal club, University of Calabria, 2014-2016.
- Course “English advanced scientific”, Prof.ssa T. Ting, University of Calabria, 2015;
- Course “Molecular structure and materials properties” Prof.ssa A. Crispini, University of Calabria, 2015;
- XXXII EMS Summer School on “Integrated and Electromembrane processes”, Straz pod Ralskem/Liberec (Czech Republic), 2015;
- Course “Laboratory for synthesis and materials Manipulation”, Prof. N. Godbert, University of Calabria, 2015;
- Course “Molecular modelling”, Prof. B. Rizzuti. University of Calabria, 2015;
- XXXI EMS Summer School on “Innovative Membrane Systems”, Cetraro (CS); 2014;
- Course “Research management, knowledge of research systems and funding systems” Dott.ssa L. Giorno, Prof. R. Bartolino, Prof. V. Chiarella, University of Calabria - Institute on Membrane Technology, 2014;
- Course “Advanced methods of numerical calculation” Prof. F. Stabile, University of Calabria, 2014;
- Course “Exploitation of research results and intellectual property” Prof. R.Barberi, University of Calabria, 2014;
- Course “Chemistry of polymeric materials”, Prof. N. Godbert, University of Calabria, 2014;
- Course “Advanced spectroscopy”, Prof. E. Cazzanelli, University of Calabria, 2014;
- Course “Membrane technology and nanostructured materials” Dott.ssa L.Giorno, Cetraro (CS), 2014.

Other activity: teaching support activity, partnerships

- Participation in the organization of the final workshop of the project “Olio Più” PON 01545;
- Participation to research project “Olio Più” PON 01545;
- Supervisor of master thesis, Student Bi Jing. 2014;
- Support to experimental activities for master student of science degree.

Workshop and seminary

- Seminary “ITM-CNR seminary day”, 1 December 2016, Rende 2016;
- Workshop “Roadshow-Material Characterization”, 17 June 2015, Bologna (BO);
- Seminary “Non-equilibrium structural states in zeolite materials by temperature-and time-resolved diffraction and innovative applications for zeolite materials” 19 November 2014, Rende (CS).
- Workshop “Final activities of the project PON Olio Più” 14 May 2014, Reggio Calabria (RC);
- Seminary “Use of White Rot Fungi for the treatment of industrial waste” 6 May 2014, Rende (CS).
- Workshop “Focus on Microscopy”, 30 January 2014, Rende (CS);

Publications

Articles in International Journals

- Bazzarelli F., Piacentini E., Poerio T., Mazzei R., Cassano A., Giorno L., Advances in membrane operations for water purification and biophenols recovery/valorization from OMWWs, *Journal of Membrane Science*, 497 (2016) 402–409.
- Piacentini E., Poerio T., Bazzarelli F., Giorno L., Microencapsulation by Membrane Emulsification of Biophenols Recovered from Olive Mill Wastewaters, *Membranes*, (2016) 6, 25.
- Bazzarelli F., Poerio T., Mazzei R., D’Agostino N., Giorno L., Study of OMWWs suspended solids destabilization to improve membrane processes performance, *Separation and Purification Technology*, 149 (2015) 183-189.
- Bazzarelli F., Piacentini E., Giorno L., Biophenols-loaded solid lipid particles (SLPs) development by membrane emulsification. *Journal of Membrane Science*. 2017. In press.

Other Articles in International Journals

- Tocci E., De Lorenzo L., Bernardo P., Clarizia G., Bazzarelli F., Mckeown N. B., Carta M., Malpass-Evans R., Friess K., Pilnáček K., Lanč M., Yampolskii Y.P., Strarannikova L., Shantarovich V., Mauri M., Jansen J.C., Molecular Modeling and Gas Permeation Properties of a Polymer of Intrinsic Microporosity Composed of Ethanoanthracene and Tröger's Base Units, *Macromolecules*, 47 (2014) 7900-7916.

Contribution to Encyclopedia of Membranes

- Bazzarelli F., Giorno L., *Nanocomposite membranes*. Encyclopedia of Membranes. (2016) 1356-1358, Springer;
- Bazzarelli F., Mazzei R., Giorno L., *Cellular membranes*. Encyclopedia of Membranes. (2016) 345-347, Springer;
- Giorno L., Piacentini E., Bazzarelli F., *The Principle of Microfiltration*. Encyclopedia of Membranes. (2016) 1669-1672, Springer;
- Piacentini E., Bazzarelli F., Giorno L., *Dense membranes*. Encyclopedia of Membranes. (2016) 530-532, Springer;
- Giorno L., Piacentini E., Bazzarelli F., *Porous membranes*. Encyclopedia of Membranes. (2016) 1625-1627, Springer;
- Bazzarelli F. and Giorno L., *Aquaporins (AQPs) or Water Channels*. Encyclopedia of Membranes. (2016) 110-111, Springer;
- Giorno L., Piacentini E., Bazzarelli F., *Macroporous, Mesoporous, and Microporous Membranes*. Encyclopedia of Membranes. (2016) 1116-1117, Springer;
- Piacentini E., Giorno L., Bazzarelli F., *Dialysis*. Encyclopedia of Membranes. (2016) 537-539, Springer.

Proceedings

Oral Presentation

- Bazzarelli F., Poerio T., Mazzei R., Giorno L., Development of an integrated membrane process for olive mill wastewater purification and polyphenols recovery. Conference Society of Chemistry, 3-4 December 2015, Catanzaro;
- Bazzarelli F., Poerio T., Mazzei R., Giorno L., Olive mill wastewater treatment and recovery of biophenols by integrated membrane process, ITM-CNR Seminar Days, 18-19 December 2014, Rende (CS).

Poster presentation

- Bazzarelli F., Piacentini E., Poerio T., Mazzei R., Cassano A., Giorno L. Advanced integrated membrane system for recovery and encapsulation of biophenols from olive mill wastewaters. Giornata DSCTM 2016 - from Biomolecules to Society, from Society to Biomolecules, 23-24 June 2016, Cetraro (CS);
- Bazzarelli F., Poerio T., Mazzei R., Giorno L., Development of an integrated membrane system for olive mill wastewater treatment and recovery of high added value bioactive compounds, ITM-CNR seminar day, November 25-26, 2015, Rende (CS);
- Bazzarelli F., Poerio T., Mazzei R., Giorno L., Purification of olive mill wastewater and recovery of biophenols using integrated membrane process. XXXII EMS Summer School 2015 “Integrated and Electromembrane processes”, Straz pod Ralskem/Liberec, Czech Republic, June 21-26, 2015;
- Bazzarelli F., Poerio T., Mazzei R., Giorno L., Integrated membrane process to treat olive mill wastewater and recover biophenols, XXXI EMS Summer School on “Innovative Membrane Systems”, Cetraro (CS), 28 September 03 October 2014.