UNIVERSITAT POLITÈCNICA DE VALÈNCIA

ESCUELA TÉCNICA SUPERIOR INGENIERÍA INDUSTRIAL VALENCIA

CHEMICAL ENGINEERING MASTER THESIS

STUDY OF THE RECOVERY OF PHENOLIC COMPOUNDS FROM SEMISOLID WASTES FROM OLIVE OIL PRODUCTION BY MEANS OF SOLID-LIQUID EXTRACTION AND MEMBRANE TECHNOLOGY

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ABSTRACT

The production of olive oil generates a semi-solid waste rich in phenols which happens to be phytotoxic. These phenolic compounds have attracted the attention of pharmaceutical, cosmetic and the food industry due to their antioxidant, anti-inflammatory and anticarcinogenic properties.

The objective of this master`s thesis is to study the operating conditions of the ultrafiltration process for the recovery of phenols present in the olive pomace. This was done by making a solid-liquid extraction of the phenolic content from the olive pomace and making ultrafiltration experiments with three different flat membranes having different pore sizes; UP005, UP150 and UH050 membranes from Microdyn-Nadir. The best operating conditions were also determined testing different cross flow velocities (CFV) (1.5, 2.5 and 3.5 m/s) and trans-membrane pressures (TMP) from 0.75 to 5.5 bar.

The solid-liquid extraction was carried out with the aid of the Ultrasonic Assisted Extraction technique using distilled water as the solvent at 40^{o}C for 45 minutes. The extract was passed through a centrifuge and filtered to remove bigger solids that would affect the ultrafiltration process. Next was the ultrafiltration test with the various membranes altering the cross flow velocity and trans-membrane pressure. The UH050 produced the highest permeate flux (150.96 L/m^2·h) obtained from all the ultrafiltration tests as the UP150 produced a much lower permeate flux (26.71 L/m^2·h) than expected due to severe fouling. The UP005 produced a maximum permeate flux of 31.51 L/m^2·h, lower than the UH050 due to its smaller pore size.

The feed and the permeates were characterized to determine the rejection percentage of the colour, total solids, COD, phenolic content, and the phenolic family compounds. Colour was the most rejected parameter by all membranes with at least 72% rejection. The rejection of total solids was lower but was seen to increase with the increase in TMP and it varied from 53 – 79% with the UP005 membrane, the UH050 from 21 – 91% and the UP150 around 61%. The rejection of COD increased as the TMP and CFV raised, being the UH050 the membrane that experienced the highest variation of COD rejection, which increased from 28% at 1.5 m/s and 0.75 bar to 72% at 2.5 m/s and 2.5 bar. The UP005 gave the highest rejection at the highest CFV and TMP and it varied from 57 – 72% and the UP150 produced a 64% rejection. In terms of phenolic content, the UP005 produced the highest phenolic content rejection of all membranes (73 - 90%), while the rejection varied from 19 – 72% for the UH050, and the UP150 produced 65.29% rejection. Increasing the TMP at all CFVs resulted in an increase of phenolic content rejection due to the fouling factor.

With all the results obtained, the UH050 membrane was selected at 2.5 m/s, 2.5 bar thanks to the high permeate flux (150.96 L/m^2·h), high COD rejection (72%), high total solids rejection (91%) and low phenolic family rejection (simple phenols – 28%, phenolic acids and aldehydes – 6%, secoiridoids – 26%, flavonoids – 28%, free fatty acids - 71% and unknowns – 35%).

Keywords: Olive Oil, Olive Pomace, Phenolic Compounds, Ultrafiltration.

RESUMEN

La producción de aceite de oliva genera un residuo semisólido rico en fenoles que resulta ser fitotóxico. Estos compuestos fenólicos han llamado la atención de la industria farmacéutica, cosmética y alimentaria por sus propiedades antioxidantes, antiinflamatorias y anticancerígenas.

El objetivo de esta tesis de máster es estudiar las condiciones de operación del proceso de ultrafiltración para la recuperación de los fenoles presentes en el alperujo. Para ello se ha realizado una extracción sólido-líquido del contenido fenólico del alperujo y se han realizado experimentos de ultrafiltración con tres membranas planas de diferente tamaño de poro: membranas UP005, UP150 y UH050 de Microdyn-Nadir. También se determinó la mejor condición de funcionamiento probando diferentes velocidades tangenciales (CFV) (1.5, 2.5 y 3.5 m/s) y presiones transmembranales (TMP) de 0.75 a 5.5 bar.

La extracción sólido-líquido se realizó con la ayuda de la técnica de extracción asistida por ultrasonidos utilizando agua destilada como disolvente a 40^{o}C durante 45 minutos. El extracto se pasó por una centrifuga y se filtró para eliminar los sólidos más grandes que pudieran afectar al proceso de ultrafiltración. A continuación, se realizó la prueba de ultrafiltración con las distintas membranas alterando la velocidad tangencial y la presión transmembranal. La membrana UH050 produjo la densidad de flujo de permeado más alta (150.96 L/m^2·h) obtenida en todas las pruebas de ultrafiltración, mientras que la UP150 produjo un flujo de permeado mucho menor (26.71 L/m^2·h) de lo esperado debido a un grave ensuciamiento. La membrana UP005 produjo una densidad de flujo de permeado máxima de 31.51 L/m^2·h, inferior a la de la UH050 debido a su menor tamaño de poro.

La alimentación y los permeados se caracterizaron para determinar el porcentaje de rechazo del color, los sólidos totales, la DQO, el contenido fenólico y los compuestos de la familia fenólica. El color fue el parámetro más rechazado por todas las membranas, con al menos un 72% de rechazo. El rechazo de los sólidos totales fue menor, pero se observó que aumentaba con el incremento de la TMP y variaba del 53 al 79% con la membrana UP005, del 21 al 91% con la UH050 y para la UP150 alcanzó un valor en torno al 61%. El rechazo a la DQO se incrementó al aumentar la TMP y el CFV, siendo la UH050 la membrana que experimentó la mayor variación de rechazo de la DQO, que pasó del 28% a 1.5 m/s y 0.75 bar al 72% a 2.5 m/s y 2.5 bar. La UP005 presentó el mayor rechazo a los valores más altos de CFV y TMP y varió del 57 al 72% y la UP150 produjo un rechazo del 64%. En términos de contenido fenólico, la UP005 produjo el mayor rechazo de contenido fenólico de todas las membranas (73 - 90%), mientras que el rechazo varió del 19 - 72% para la UH050, y la UP150 produjo un rechazo del 65.29%. El aumento de la TMP en todas las CFV produjo un aumento del rechazo del contenido fenólico debido al mayor ensuciamiento.

Con todos los resultados obtenidos, se seleccionó la membrana UH050 a 2.5 m/s, 2.5 bar gracias a la alta densidad de flujo de permeado (150.96 L/m^2·h), al alto rechazo a la DQO (72%), al alto rechazo de los sólidos totales (91%) y al bajo rechazo de la familia fenólica (fenoles simples - 28%, ácidos fenólicos y aldehídos - 6%, secoiridoides - 26%, flavonoides - 28%, ácidos grasos libres - 71% y desconocidos - 35%).

Palabras clave: Aceite de oliva, alperujo, compuestos fenólicos, ultrafiltración.

RESUM

La producció d'oli d'oliva genera un residu semisòlid ric en fenols que resulta ser fitotóxic. Aquests compostos fenòlics han cridat l'atenció de l' indústria farmacèutica, cosmètica i alimentària per les seues propietats antioxidants, antiinflamatòries i anticancerígenes.

L'objectiu d'aquest treball de fi de màster és estudiar les condicions de funcionament del procés d'ultrafiltració per a la recuperació de fenols presents en l'alperujo d'oliva. Això es va fer fent una extracció sòlid-líquid del contingut fenòlic de l'orujo d'oliva i fent experiments d'ultrafiltració amb tres membranes planes diferents que tenen diferents mesures de porus; Membranes UP005, UP150 i UH050 de Microdyn-Nadir. També es va determinar la millor condició de funcionament provant diferents velocitats de flux creuat (CFV) (1,5, 2,5 i 3,5 m/s) i pressions trans-membranals (TMP) de 0,75 a 5,5 bar.

L'extracció sòlid-líquid es va dur a terme amb l'ajuda de la tècnica d'extracció assistida ultrasònica utilitzant aigua destil·lada com a dissolvent a 40^{o}C durant 45 minuts. L'extracte es va passar a través d'una centrifuga i es va filtrar per eliminar sòlids més grans que afectarien el procés d'ultrafiltració. A continuació es va fer la prova d'ultrafiltració amb les diverses membranes variant la velocitat de flux creuat i la pressió de la trans-membranal. La membrana UH050 va produir el flux de permeat més alt (150,96 L/m2·h) obtingut de totes les proves d'ultrafiltració, ja que el UP150 va produir un flux de permeat molt més baix (26,71 L/m2·h) del esperat a causa d'embrutiments greus. La membrana UP005 va produir un flux màxim de permeat de 31,51 L/m2·h, inferior a l'UH050 a causa de la seua menor mesura dels porus.

L'alimentació i els permeats es van caracteritzar per determinar el percentatge de rebuig del color, els sòlids totals, la DQO, el contingut fenòlic i els compostos de la família fenòlica. El color va ser el paràmetre més rebutjat per totes les membranes amb almenys un 72% de rebuig. El rebuig dels sòlids totals va ser menor, però es va veure que augmentava amb l'augment de TMP i va variar del 53 al 79% amb la membrana UP005, del 21 al 91% amb la membrana l'UH050 i al voltant del 61% per a la membrana UP150. El rebuig del DQO va augmentar a mesura que la TMP i la CFV van augmentar, sent l'UH050 la membrana que va experimentar la major variació de rebuig de DQO, que va augmentar del 28% a 1,5 m/s i 0,75 bar al 72% a 2,5 m/s i 2,5 bar. La membrana UP005 va donar el major rebuig a les CFV i TMP més altes i va variar del 57 al 72% i la membrana UP150 va produir un rebuig del 64%. Pel que fa al contingut fenòlic, la membrana UP005 va produir el major rebuig del contingut fenòlic de totes les membranes (73 - 90%), mentre que el rebuig va variar del 19 al 72% per a la membrana UH050, i la membrana UP150 va produir un rebuig del 65,29%. L'augment de la TMP en totes les CFVs va resultar en un augment del rebuig del contingut fenòlic a causa del major embrutiment.

Amb tots els resultats obtinguts, la membrana UH050 es va seleccionar en les condiciones de 2,5 m/s i 2,5 bar gràcies a l'alt flux de permeat (150,96 L/m2·h), alt rebuig del DQO (72%) al alt rebuig del sòlids totals (91%) i baix rebuig a les families fenòliques (fenols simples – 28%, àcids fenòlics i aldehids – 6%, secoridoides – 26%, flavonoides – 28%, àcids grassos lliures - 71% i desconeguts – 35%).

Paraules clau: Oli d'oliva, alperujo d'oliva, compostos fenòlics, ultrafiltració.

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CHAPTER 1. MOTIVATION

The master's thesis studies one of the stages of the research project titled ¨Study of the recovery of phenolic compounds from the by-products derived from the olive mill¨ (CTM2017-88645-R-AR) financed by the Ministry of Economy, Industry and Competitiveness under the programme ¨Aid for R&D&I projects¨ corresponding to the State Program of Research, Development and Innovation Oriented to Society's Challenges. This thesis will focus on the ultrafiltration process marked in red in Figure 1.

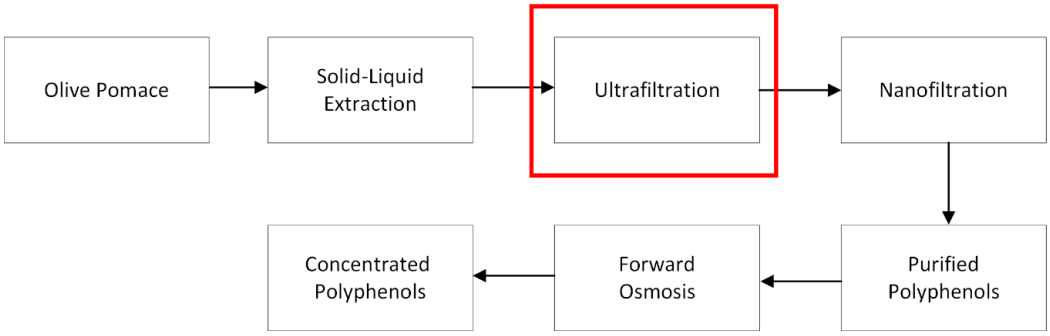


Figure 1. Scheme of global research project (CTM2017-88645-R-AR)

The olive oil industry has revamped the extraction of olive oil process in the last centuries. During the extraction, large quantities of waste are generated, which happen to be phytotoxic if not properly disposed because of its high content in polyphenols. To maintain a sustainable ecosystem and circular economy, there is a need for the valorisation and recycling of industrial waste which can be beneficial.

A major waste produced from the olive oil industry is the olive pomace, which is a semisolid waste rich in phenolic compounds, which are phytotoxic. One of the components of olive pomace that has been widely studied are the polyphenols due to their beneficial antioxidant, anticarcinogenic and anti-inflammatory properties, which are used in the cosmetic, pharmaceutical and food industries.

It is for this reason that this project centres on the study and optimization of the ultrafiltration process with the aim of extracting and purifying the polyphenols from the olive pomace in preparation of the nanofiltration stage.

CHAPTER 2. OBJECTIVES

The main objective of this Master´s Thesis is the study of the operating conditions of the ultrafiltration process for the recovery of phenols present in the olive pomace. At the end of the project, it is expected to determine the most suitable operating conditions in terms of trans-membrane pressure, and crossflow velocity and choose the most suitable membrane for the process.

The following steps will be taken to achieve the objective of this project:

- Perform a solid-liquid extraction to obtain an extract rich in phenolic compounds from the olive pomace.
- Perform ultrafiltration experiments with three different membranes altering the operating conditions (crossflow velocity and trans-membrane pressure) to determine their effect on the permeate flux and solute rejection.
- Recover the permeability using a chemical cleaning solution and select the membrane cleaning process.
- Characterize the feed and permeate from the ultrafiltration experiments to analyse the rejection of colour, total solids, Chemical Oxygen Demand (COD) and phenolic content.
- Choose the most suitable operating conditions and membrane for the recovery of phenolic compounds.

CHAPTER 3. INTRODUCTION

3.1. OLIVE OIL

Olive oil is the main source of fat in the Mediterranean diet. It is extracted from the olive seed which has an oil content of about 30%. Its composition varies with time of harvest and extraction process, which includes crushing of olive seeds, olive paste mixture, and uncoupling of solid-liquid by pressure or centrifugation (Maestri *et al.,* 2019).

The largest producer of olive oil in the world is the European Union (EU), contributing to 69% of global production. The olive oil producing countries in the EU are Spain, Italy, Greece, Portugal, France, Slovenia, Croatia, Cyprus, and Malta. These countries have 5 million hectares of olive groves. Spain is the largest contributor in the EU with more than half of the total EU production accounting for 63% (European Union, 2020).

In the last few years, the consumption of olive oil has seen a sharp increase due to its healthy properties. Therefore, the wastes and by-products derived from the olive production and the olive oil industry have also increased causing serious environmental and economic issues. However, the high content in bioactive compounds of these wastes and by-products makes its recovery both a great challenge and an excellent opportunity for the olive oil sector (Gullón Patricia *et al.,* 2020).

3.2. PRODUCTION OF OLIVE OIL

Olive oil production process (Figure 2) starts in the olive grove with maintenance and care of olive trees. During this phase, large amounts of solid waste are generated. This solid waste can contain thin branches (about 50% by weight), leaves (25%) and other (25%) formed by thick branches or wood (compositions depend on cultivation conditions, age of olive tree, etc.) (Romero-García *et a*l., 2014). The harvesting is optimized to obtain the maximum oil quantity with a predefined quality as this affects the quality and the cost of oil production (Nasini & Proietti, 2014).

Once the olive is harvested, the next phase is the storage and transportation process. This phase is crucial for the quality of the oil since an unconducive condition can trigger a sequence of degradation process that can lead to sensorial defects (musty or rancid), a free acidity increase, etc. (Proietti, 2014).

Next is the acceptance of the olives batch in the factory reception yard (Beltran *et al.,* 2016) and they go through a cleaning process to remove leaves and different impurities (wood portions, stones, damaged olives, etc.) and to eliminate the dust and soil. The separation should be done by mechanical and pneumatic methods and with water (Peri, 2014a).

After cleaning, the olives are stored in hoppers (Beltran *et al.,* 2016). Then, in the mill, the olives are grinded to a very fine particle size and the pits are reduced to relatively rough fragments to provide a good oil drainage network. At the end of this process, olives will be reduced to a homogeneous paste made up of olive solids, water, and oil (Leone, 2014).

The homogeneous paste goes to the malaxation process, which is one of the most important unit operations in the olive oil extraction, because it determines the olive oil's quality and nutritional properties (Kalogianni *et al.,* 2019). This process makes it easier for oil separation in the next unit operation. According to Gullón et al., at present, this phase is the only discontinuous unit operation in the modern olive oil production process due to the limited rate of transformations (need some time to be effectively carried out) (Gillón *et al.,* 2020).

The olive paste from the malaxing process is sent to the separation unit. This paste is a mix of three different phases: insoluble solids (woody bits and organic semisolid components), an aqueous phase (water and soluble components) and the oil phase (with triglycerides and minor components) (Baccioni & Peri, 2014). There are essentially three types of extraction processes: the traditional (pressing mills), and the two-phase and three-phase centrifugation systems, respectively (Matos *et al.,* 2010). These are explained below.

- The traditional system has been used for centuries with small modifications along in time, and this process is operated discontinuously. The olive paste is placed between the pressing mats and pressure is applied to produce a solid fraction (olive pomace), and a fraction consisting of an oil and water mixture, which is poured into a tank and allowed to settle for the oil and the water to separate (Roig *et al.,* 2006).

- The three-phase system is a process that takes place in a three-phase decanter where each phase (insoluble solids, oil phase and aqueous phase) is separated based on their densities (Baccioni & Peri, 2014). A significant amount of water is added to reduce the viscosity of the paste and favour the separation. According to Gullón et al., the insoluble solid (pomace) is recovered (could be used in subsequent processes), the aqueous phase (main wastewater of the process) has a large concentration of organic matter dissolved, and the oil phase, which contains 2–5% of water droplets and solids, needs a finishing centrifugation (Gullón *et al.*, 2020) using a vertical centrifuge. In this finishing process, a small quantity of water is introduced to separate and clean the oil, which results to a clarified oil, removing the solid impurities and producing residual water too (Baccioni & Peri, 2014). Due to the high amount of olive mill wastewater generated, there is a shift from the three-phase system to the two-phase system, because it is more environmentally friendly (Nunes *et al.,* 2016).

- The two-phase system uses more powerful decanters and water is not added. In this process a semi-solid slurry (vegetation water and insoluble solids) phase and an oil phase are obtained (Baccioni & Peri, 2014). Therefore, a new process waste, with specific physico-chemical properties, is generated in large quantities creating a serious management problem. Spain is one of the countries that has seen a quick change from the three-phase method to the two-phase system. This sudden shift has not happened in other olive oil producing countries, probably due to the management difficulties arisen with the new solid waste (Roig & Cayuela, 2006). The oil phase is then treated in

a centrifugal process with some water for cleaning and then recovered (Baccioni & Peri, 2014).

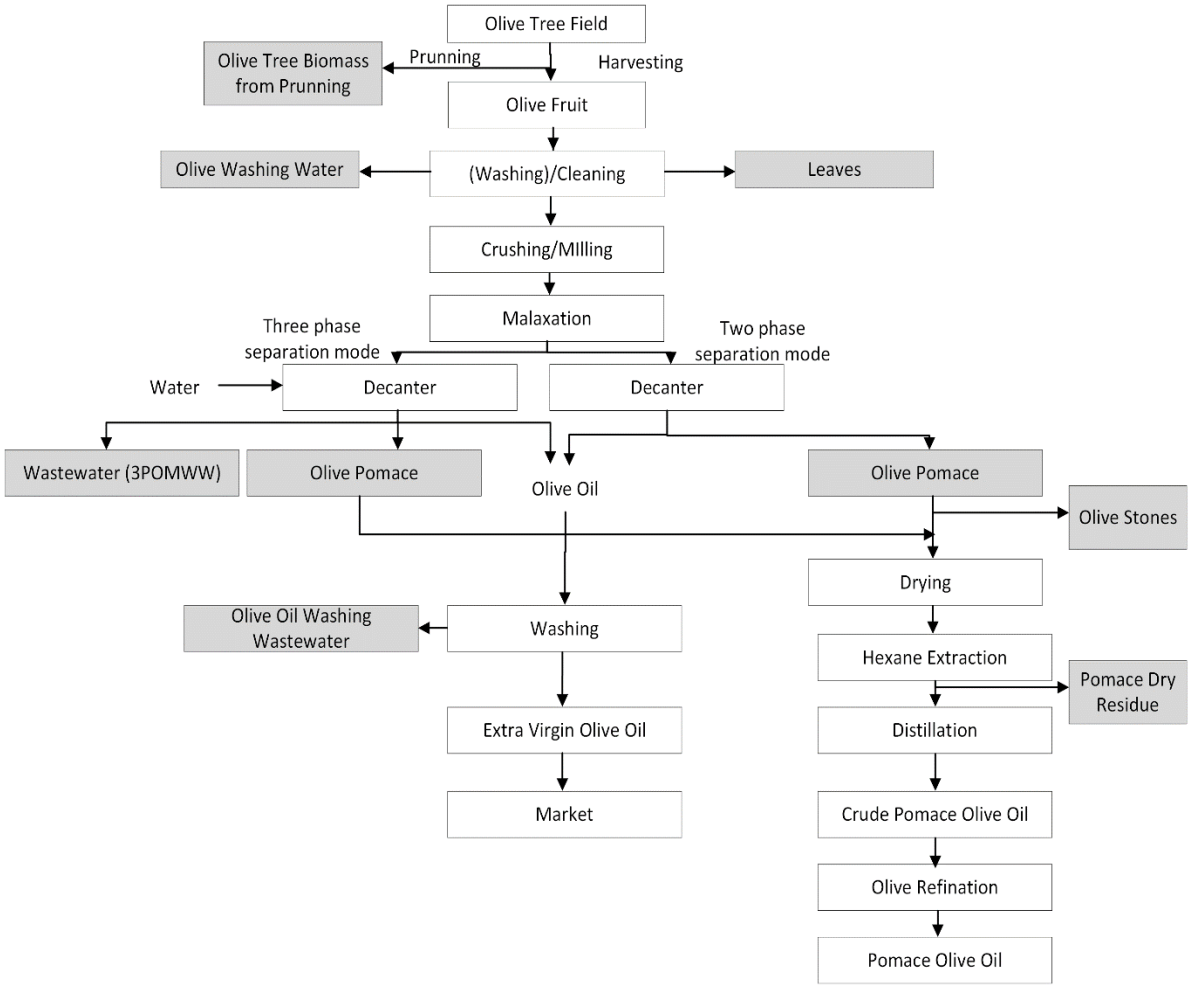


Figure 2. Scheme for industrial production of olive oil

3.3. OLIVE OIL BY-PRODUCTS

However, despite the economic benefits and the functional properties beyond the nutritional attributes of the olive oil, this sector is associated with environmental problems derived from the huge quantity of residues and by-products generated along the productive process (Manzanares et al., 2020). The major forms of industrial waste from the olive oil production industry are olive pomace and olive mill wastewater.

3.3.1. Olive Pomace (OP)

Olive pomace happens to be the main residue by weight of the two-phase extraction process and differs in composition depending on the olive cultivation or production process. In the two-phase separation mode, the OP (or alperujo) is a thick sludge with 55–70% of moisture content (Borja *et al.,* 2006), while the OP produced in the three-phase process has a 40–45% moisture content (Dermeche *et al.,* 2013). The residue is majorly composed of crushed olive stones, pulp

and skins and different quantities of water content based on the extraction methods used for the oil recovery (Romero-García *et al.,* 2014).

From research, it has been shown that olive pomace is an excellent source of valuable compounds such as phenols, carbohydrates, and proteins (Bermúdez-Oria *et al.,* 2019). It presents a typical composition of lignocellulosic biomass; in this sense, it contains lignin (30.0–41.6%), cell wall polysaccharides (35.3–49.0%) as cellulose, pectic polymers and hemicelluloses (xylans, glucoroxylans, xyloglucans and mannan), oil (7.5–14%) and minerals (4.4–6%) (Miranda *et al.,* 2019).

To eliminate the harmful effects it has on the environment if not properly disposed, OP is usually used in combustion. However, this method is not well accepted because it is a waste reduction strategy and not a recovery of valuable components method (Rubio-Senent *et al.,* 2015).

Considering the composition of the olive pomace, several authors have reported its use as raw material to obtaining multiple valuable compounds with health promoting properties. Based on Alu'datt, et al., they found a positive relation between the phenolic content and the antioxidant activity of olive cake extracts (Alu'datt, *et al.,* 2010). In another study, Bermúdez-Oria et al., obtained extracts that were rich in pectic polysaccharides related to polyphenols from OP with high potential in the medical field as antiproliferative or antioxidant agents (Bermúdez-Oria *et al*., 2019).

3.3.2. Olive Mill Wastewater (OMW)

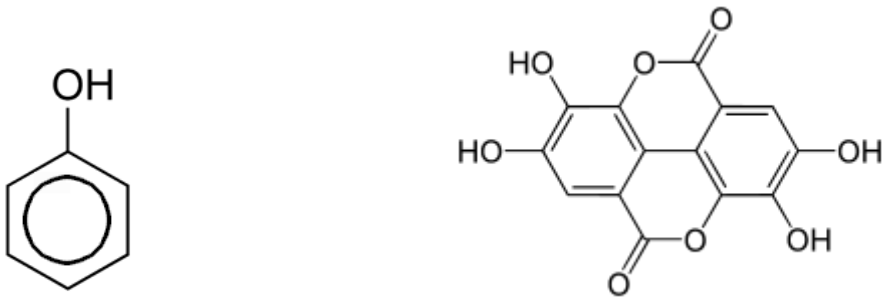
Olive mill wastewaters (OMW) happens to be the major residue from the three-phase extraction systems and traditional mills. They are made up of vegetable water of the fruit and the water used in different stages of the extraction. Their chemical composition varies depending on olive types, cultivation techniques, harvesting period and mostly the technology used for olive oil extraction (Paredes *et al.,* 1999).

The main features of OMW are the composition of organic compounds such as organic acids, lipids, alcohols, and polyphenols that makes OMW a phytotoxic material, turning it into an environmental hazard when it is not properly managed. OMW also contains valuable components such as a high organic matter concentration and several nutrients, especially potassium, that could be recycled and utilized as a potential fertiliser.

3.4. PHENOLIC COMPOUNDS

Phenolic compounds belong to one of the most important and widely distributed groups of natural products from plants. They possess a range of physiological properties such as antiallergenic, anti-inflammatory, antioxidant, antithrombotic and cardioprotective effects. They usually possess health benefits when high levels of fruit and vegetables are consumed (Sánchez-Arévalo *et al.,* 2021).

Structurally, phenolic compounds (Figure 3) contain an aromatic ring, bearing one or more hydroxyl groups, and range from elementary single-phenolic molecules to highly polymerized compounds.



(a) (b)

Figure 3. (a) Single-phenolic molecule (b) Polymerized compounds

According to Suarez *et al.,* 98% of phenolic compounds are retained in the OP wastes after extraction, making it an affordable and abundant source of biologically active phenolic compounds that contain promising compounds (Suarez *et al.,* 2009).

The major component of the phenolic fraction found in OMW is hydroxytyrosol, part of which comes from oleuropein, the most abundant phenol present in olive trees. Hydroxytyrosol is a strong antioxidant, with proven abilities to scavenge oxygen and nitrogen free radicals, to inhibit low-density lipoprotein oxidation, and to protect against DNA damage (Hu *et al.,* 2014; Zhang *et al.,* 2009). Despite these important benefits, hydroxytyrosol is yet to be commercially available in large amounts because all the synthetic or extractive systems have resulted to be difficult, costly, or not eco-friendly (Espín *et al.,* 2001; Fava *et al.,* 2017).

One of the main advantages on recovering phenolic compounds from OP is their use in functional foods but also, due to their physiological properties, pharmaceutical and cosmetic companies are paying a lot of attention on these compounds (Sánchez-Arévalo *et al.,* 2021). It has also been shown that as the phenolic compounds fraction reduces when added to food, hydroxytyrosol remains active for a longer period, thereby, preserving its properties (Ahmad *et al.,* 2020). In a study by Araújo et al., it was discovered that phenolic compounds extracted from the by-products of olive oil when added to different food matrixes such as sunflower oil, it improves their antioxidant properties as this is related to the capacity of hydroxytyrosol and oleuropein content (Araújo *et al.*, 2015). The recovery of polyphenols is important for the valorisation of these solid residues and would contribute to a more sustainable bioeconomy, reducing environmental issues related to these wastes and making them suitable for commercialization.

3.5. EXTRACTION OF PHENOLS

The valorisation of olive pomace has shown great potential for the recovery of its phenolic compounds. Eco-friendly and sustainable extraction methods could be carried out to ensure the high quality and antioxidant capacity of the phenolic compounds extracted. Hence, innovative extraction methods are being studied to obtain a high extraction performance within a shorter time while requiring lower energy consumption (Chanioti *et al.,* 2021).

Usually, the conventional extraction method is the use of organic solvents. The extraction process makes use of a solid-liquid extraction process commonly performed with a Soxhlet apparatus, where fresh solvent is repeatedly in contact with the solid component (Gullon *et al.,* 2017). The major disadvantages of this technique are due to the need for large quantity of solvents needed, the long processing time, the lack of stirring, a further solvent evaporation stage, and the possible degradation of the valorised compounds due to the high extraction temperature used (Azmir *et al.,* 2013). The efficiency of the extraction and the antioxidant potential are affected by the operation conditions, such as the temperature, the time, the liquid-to-solid ratio (L:S), and the type of solvent. The temperature should be optimized to improve the mass transfer of phenols by increasing their diffusion rate into the solvent. The choice of an optimized liquid-to-solid ratio also improves the diffusion rate and increased extraction efficiency since the presence of a high solvent volume improves the extraction process (Sahin *et al.,* 2013).

The recovery of phenolic compounds from olive pomace has been performed using various types of solvents including methanol, ethanol, acetone, water, and ethyl acetate (Gullon *et al.,* 2020). Böhmer-Maas et al., used methanol as a solvent to optimize the extraction of phenols from olive pomace at different concentrations of (40%, 60%, and 80% (v/v)), varying temperatures (45, 57.5, and 70 ^{o}C), and extraction times (60, 120, and 180 min). They arrived that total phenolic content was improved by using 40% (v/v) methanol at 70 ^{o}C and for 180 min (Böhmer-Maas *et al.,* 2020). Nakilcio˘glu and Semih studied the conditions of temperature (40, 50, and 60 ^{o}C), time (30, 60, and 90 min), and solvent type (methanol, ethanol, and acetone) for the extraction optimization of the phenolic compounds from olive pomace. They concluded that by using an extraction temperature of 40 ^{o}C, extraction time of 89.49 min, and methanol as solvent type, the obtained extracts contained a high total phenolic content, antioxidant activity, and concentration in individual phenolic compounds (Nakilcio˘glu and Semih, 2019). Finally, Aludatt et al. confirmed that the extraction of phenolic compounds from olive pomace at a temperature of 70 ^{o}C obtained the maximum phenolic content and antioxidant activity (Aludatt *et al.,* 2010). However, some researchers found that the best conditions for the maximum extraction of phenols are from 35 to 50 ^{o}C as the phenols begin to degrade from 45 ^{o}C (Sygouni *et al.,* 2019; Nipornram *et al.,* 2018).

Ultrasound-assisted extraction (UAE) technology is usually suggested for the recovery of valuable organic compounds from olive pomace. The sonic waves generate a high shear force which encourages the extraction by improving the mass transfer (Chemat *et al.,* 2017a) through cavitation and streaming (Leighton, 2007). UAE is considered as one of the best non-thermal technologies from an environmental perspective with numerous merits, such as lower extraction time, energy, and solvent usage (Chemat *et al.,* 2017b). The solid/liquid ratio has a great impact on the extraction yield and UAE parameters as amplitude and pressure can be easily changed to favour the target specific objectives (Chemat *et al.,* 2020). An ultrasound irradiation of 20–100 kHz delivers high results in shorter time, reduced solvent consumption and less energy input (Chemat *et al.,* 2008). Moreover, the energy from the ultrasound extraction also provides more effective mixing, faster energy transfer, and lower extraction temperature requirement (Azmir *et al.,* 2013).

UAE is highly impacted by various processing conditions such as time, temperature, solid/liquid ratio, power, and frequency hence, their optimization is key for a high extraction of valuable

organic compounds (Kumar *et al.,* 2021). Concerning olive pomace, Nunes et al. showed that UAE was an effective technique producing a high yield in a short time for the extraction of phenolic compounds from olive pomace such as hydroxytyrosol, maslinic acid, and oleanolic acid compared to the conventional technique (Nune *et al.,* 2018).

3.6. MEMBRANE TECHNOLOGY

A membrane is a selective boundary between two phases which performs a separation under the influence of a driving force (Bilad *et al.,* 2014) (Figure 4).

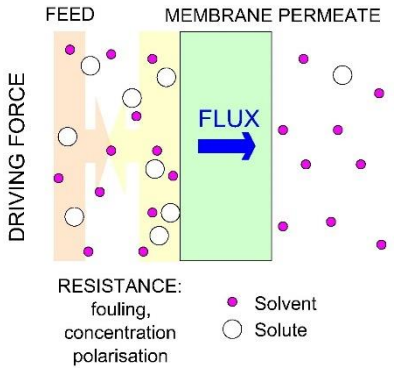


Figure 4. Scheme of membrane filtration process (Bilad et al., 2014)

The membrane technology has become an important separation technique over the past years. The uses of membranes are wide and become an essential separation technique. The separation of organic compounds from membrane technique shows a higher efficiency as compared to other conventional techniques and it depends entirely on the membrane itself. The process of separation is simple: the membrane behaves as a semi-permeable boundary between the two phases, and it controls the exchange between those two phases. The filter will let the liquid phase flow through the membrane, while it retains suspended solids and other substances (Padaki *et al.,* 2015).

The use of membrane-based processes has increased due to several advantages:

- Membrane separation can be carried out continuously.
- Requires low energy consumption.
- Can easily form hybrid processes by combining with other processes.
- The operational conditions are generally intrinsic and do not require additives.
- Membranes are assembled in modules, allowing easy up-scale up and capacity expansion.
- Operates at low temperatures which is essential in the food industry to avoid degradation of compounds.

Despite the several advantages, membranes also have some drawbacks, which prevents a more widespread application. These include concentration polarization, fouling, low membrane lifespan and low selectivity and permeance (Padaki *et al.,* 2015).

3.6.1. Types of Membranes

Membrane separation processes, with their intrinsic properties, meets the criteria for an environmentally friendly process, and has become a suitable alternative in the recovery of organic compounds from OP. The pressure-driven membrane processes mainly consist of microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO). They are conceptually similar processes, but the key difference is surface pore size of the membranes, which defines their applications as illustrated in Table 1 (Padaki *et al.,* 2015).

Table 1. Membrane separation characteristics

Pore type (Size range, nm)	Membrane type (Pore size, nm)	Species	Dimensions (nm)	Pressure Range
Macropores (>50)	Microfiltration (50-500)	Yeast & fungi	1000-10,000	1-3 bar
		Bacteria	300-10,0000	
		Oil emulsion	100-10,0000	
Mesopores (2-50)	Ultrafiltration (2-50)	Colloidal solids	100-1000	3-10 bar
		Viruses	30-300	
		Protein/polysaccharides	3-10	
		Humics/nucleic acids	<3	
Micropores (0.2-2)	Nanofiltration (≤2)	Common antibiotics	0.6-1.2	10-40 bar
		Organic antibiotics	0.3-0.8	>Osmotic pressure
	Reverse Osmosis (0.3-0.6)	Inorganic ions	0.2-0.4	
		Water	0.2	

3.6.2. Materials for membranes

Membranes are made up of organic or inorganic materials. Depending on the material and the membrane pore size, the passage of certain compounds is restricted in different ways due to the limiting operating conditions or properties of the membrane.

3.6.2.1. Organic membranes

The organic materials mostly used in industries for membranes are polymers. Very few polymer materials such as cellulose acetate, polyethersulfone, polyamide, polyvinylidene fluoride or polyacrylonitrile are suitable for membranes in the commercial applications despite a wide variety of polymers that can be used for membrane manufacturing (Hausmann *et al.,* 2013).

- Cellulose Acetate (CA): The main advantages of this polymer are the high water permeability, salt rejection and mechanical strength. The polymer is limited by its instability at high pH, high temperatures and incompatibility with chlorine.

- Polyethersulfone (PES): This polymer provides wide pH and temperature tolerance, chlorine resistance and wide pore sizes, which makes it a popular option when compared to other polymers for membrane manufacture.

- Polyamide (PA): These membranes can withstand high temperatures and less compaction compared to membranes made of cellulose acetate. Due to their good mechanical strength, they are mostly employed for reverse osmosis or nanofiltration membranes.

- Polyvinylidene Fluoride (PVDF): These membranes can withstand maximum temperatures of 50^{o}C and have good resistance to oxidising compounds and hydrocarbons compared to other materials, but tend to be difficult to handle for the membrane production.

- Polyacrylonitrile (PAN): This material has good solvent resistance properties and makes it a versatile polymer for membrane production. This makes it a suitable material for nanofiltration, reverse osmosis and ultrafiltration membranes, but it is more expensive than other membranes such as PES ones.

3.6.2.2. Inorganic membranes

Materials such as ceramics, metals and carbon can also be used to produce membranes.

- Ceramic membranes: These membranes consist of metal oxides materials and mostly made up of Al_2O_3, TiO_2, ZrO_2 etc. or a combination of these. They are mostly employed for microfiltration and ultrafiltration processes. They have a high tolerance for temperature and pH which makes them more suitable for conditions where organic polymers could not operate. The high capital cost limits the acceptance of ceramic membranes commercially.

- Metallic membranes: Like ceramic membranes, they can be used for microfiltration and ultrafiltration. They can operate at high temperature and pressure and depending on the metal, it can withstand a wide pH range. Metallic membranes are not used in the food industry because they are welded into one unit that cannot be disassembled for inspection.

3.6.3. Membrane geometry

The three main geometries of membranes are flat sheets, hollow fibres, and tubular. Flat sheets require a support which could be a porous backing or imbedded mesh to support the active layer. Both hollow fibres and tubular membranes have similar geometries, but they have different diameters. Hollow fibres have smaller diameters compared to tubular and the support layer serves as the active layer. The two membrane geometries can operate as ¨inside-out¨

where the inner walls are the active sides or ¨outside-in¨ where the outer walls are the active side (Hausmann *et al.,* 2013).

3.6.4. Membrane configuration

There are four main configurations of membranes for separation: spiral wound, tubular, plate and frame, and hollow fibre.

3.6.4.1. Spiral wound

Spiral wound membranes are made up of flat sheet membranes in layers and rolled around a central permeate collecting tube. The sheet membranes are separated by a highly porous support plate and have a permeate mesh that controls the flow path of the permeate. The advantages of this configuration are the high membrane packing density and low retention volume but is limited to be used with a relatively clean feed as the big particles could clog the sheets. This configuration is also not suitable if there is an intention to control the flow as large pressure differences across the membrane are not allowed.

3.6.4.2. Tubular

Tubular configurations consist of a shell design where tubular membranes are housed with a varying number of tubes depending on the design. Generally, the active layer of the tubular membranes is on the inside, and, as the feed and retentate flow through the tubular membranes, the permeate is collected within the shell. This configuration can be used with feeds having bigger particles and higher viscosities due to the large tube diameter. A problem with this design is that it has the lowest area to volume ratio of the four configurations, so, it requires the highest space.

3.6.4.3. Hollow fibre

The hollow fibre configuration is similar to tubular configuration, but, as explained earlier, with a smaller diameter of the membranes. This configuration is used in situations where higher pressures are required and the flow of the permeate and feed can be reversed depending on the application. Unlike the tubular configuration, the main advantages are the high density and low retention volume but can be limited by very high pressures or feed rate.

3.6.4.4. Plate and frame

The plate and frame configuration consist of flat membrane sheets placed between plates creating a channel for the permeate and retentate streams. The arrangement of the sheets can vary to alter the flow of the streams and a support plate is placed between two membrane sheets with the active layer facing in the opposite direction of the plate. The main advantage of using the plate and frame over other configurations is that individual sheets can be replaced or removed for cleaning, but this adds to the amount of labour and time it takes to operate.

3.6.5. Membrane Properties

The performance of a membrane process depends to a large extent on the properties of the membrane material which happens to be pore size, surface porosity, structure (e.g., symmetric/asymmetric), hydrophilicity, surface charge and tortuosity (Judd, 2010).

It should be considered that, due to thermodynamic limitations, membrane properties are normally joined together. This means, it is very difficult, maybe impossible, to completely separate one property from another and to control them independently during the synthesis of a membrane. For example, the membrane´s surface chemistry can be changed by coating it with a monomer, but this also changes the surface pore properties (i.e., smaller pore size, increase in roughness, decrease in surface porosity, etc.). Hence, the performance of a membrane cannot be solely determined based on one particular property (Hausmann *et al.,* 2013).

The membrane polarity determines the hydrophilic/hydrophobic behaviour and therefore it is responsible for the wettability/permeability by different feed components. For aqueous feeds, the suitable membrane should be hydrophilic (Zheng *et al.,* 2008).

The properties of retention and rejection may be used interchangeably depending on whether the component is desired or undesired in the retentate stream. Retention (*R*) can be defined in several ways, and one common definition is (Hausmann *et al.,* 2013):

$$R = \frac{c_f - c_p}{c_f} \tag{3.1}$$

where C_f is the concentration of a component in the feed stream and C_p is the concentration of a component in the permeate stream.

Solute retention can be affected by the membrane charge due to electrostatic interactions. It is advised that a neutral membrane, or a membrane of the same particle charge processes should be employed to prevent attraction, which can lead to fouling. Also, the pH of the feed stream should be put into consideration as the membrane charge is influenced by the pH of the fluid in touch with the membrane surface (Rice *et al.,* 2011).

An alternative system that measures membrane performance according to solute passage (*P*) is as follows:

$$P = 1 - R \tag{3.2}$$

The molecular weight cut-off of the membrane is related to the separating ability. Membranes having similar molecular weight cut-off, however, may not have the same retention for a compound since manufacturers use a variety of methods to determine molecular weight cut-

off. Generally, for a given molecular weight cut-off, 90% of the molecules of that molecular weight will be rejected (Hausmann *et al.,* 2013).

Another important property of membrane performance determined by the membrane pores is the porosity of the membrane. The membrane porosity (ε) is a measure for the fraction of membrane volume (V) filled with pores. Generally, the membrane porosity (ε) can be calculated with the following equation:

$$\varepsilon = \frac{V_{pores}}{V_{total}} \tag{3.3}$$

Even though a high porosity is desirable for stable membrane materials, high membrane porosity increases the likelihood to deformation due to pressure, and such compaction decreases the rate of flux. The membrane porosity is not just determined by the membrane area covered by pores, but also by the tortuosity (τ) calculated as (Kallioinen *et al.,* 2007):

$$\tau = \frac{L_{pore}}{\delta} \tag{3.4}$$

Where L_{pore} is the actual pore channel length and δ the membrane thickness.

Another important property of membrane performance is permeate flux. Flux is the amount of permeate that passes through the membrane tangentially, and the term generally is given as a volume per unit membrane per unit time ($L/(h \cdot m^2)$). It determines the membrane area required to process a given amount of feed to a certain concentration in a specific period. The lower the flux, the greater the membrane area required to process the same amount of feed within a certain time than with a higher flux membrane. Flux, therefore, has a direct impact on the economics of an operation, and is used as an indication of membrane fouling and cleaning adequacy. The major factors that affect the flux are trans-membrane pressure (TMP), cross flow velocity (CFV), temperature, and concentration.

3.6.6. Process Configuration

There are two modes of operation for membrane processes: dead-end filtration and the cross-flow mode as shown in Figure 5.

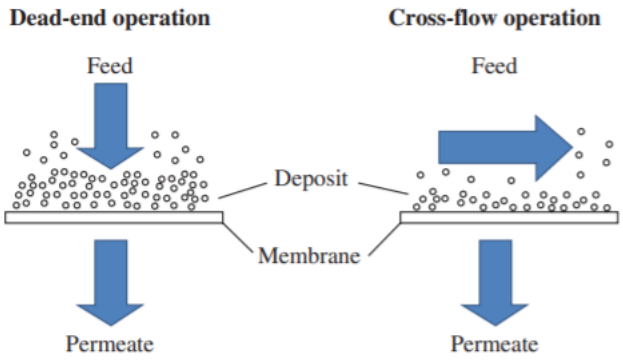


Figure 5. Scheme of (a) dead-end filtration (b) cross-flow filtration (Hausmann et al., 2013)

In the dead-end filtration, the feed is pumped perpendicularly onto the membrane surface and the operation must be run in batches to relieve the concentrated species. For feed containing relatively high solids, the deep end filtration is not suitable as this causes a build-up of solids on the membrane, thereby limiting the filtration (Hausmann *et al.,* 2013).

On the other hand, the cross-flow filtration is mostly used for large-scale process and is sometimes referred as tangential flow filtration. The feed is pumped tangentially along the membrane surface from one end of the membrane module separating the permeate (filtrate) and the retentate (concentrate) as this exits at the other end (Bilad *et al.,* 2014).

Cross-flow filtration can be operated either with constant pressure or with a constant flux. Because of membrane fouling in a constant flux operation, the trans-membrane pressure (TMP) will usually have to be increased with time. For the constant flux operation, the increase of TMP directly results to a membrane permeance loss from its initial condition, because of membrane fouling which will be discussed later (Bilad *et al.,* 2014).

3.6.7. Effects of Operating Parameters

Nowadays, operating parameters also play an important role in membrane separation. The basic parameters such as cross flow velocity, trans-membrane pressure, temperature, pH, and molecular size of solutes are very important during the separation experiments.

- Feed concentration: Concentration in the feed channel is usually specific by each application and adjusting concentration to optimise operation is not possible due to production purposes. The presence of a higher concentration directly affects the viscosity, thus reducing the permeate flux. Moreover, higher solute concentration increases membrane fouling too.

- Temperature: The effect of temperature results in changes of solvent viscosity. Increasing the temperature would lead to an increase in flux because of lower viscosity of the permeating streams for Newtonian fluids. Depending on the feed processed, temperature can affect particle–particle interactions, and thus can potentially lead to fouling phenomena, e.g. due to protein denaturation.

- Cross flow velocity: This is the velocity (measured in m/s) of the feed that enters the membrane module and covers the active layer of the membrane. The CFV has a direct influence on the permeate flux as it affects the solute deposition on the membrane. At high CFV, there is high turbulence, and this disturbs the accumulation of solutes on the membrane surface.

- Trans-Membrane Pressure: TMP has a significant role to play in membrane filtration. This is the pressure difference across the membrane. Membrane filtration at low TMP can alleviate fouling and concentration polarization but has a lower flux. Higher TMP enhances the driving force and hence, promotes flux, but at the same time, increases convective transport of solute towards the membrane and thus fouling.

3.6.8. Concentration Polarization (CP)

This is the accumulation of solutes or particles in a thin liquid layer above the membrane surface, which is an inherent phenomenon of membrane filtration. With the increase in pressure, CP is built up with the accumulation of solutes on the membrane surface which increases resistance to the flow of fluids through the pores and thus reduces the permeate flux.

With the further increase in pressure, the flux will stabilize and reach a state called limiting flux and becomes independent of pressure as it now depends on mass transfer. This happens to differ to the critical flux which is the phase where fouling begins to start and is dependent on the size of the particles in the feed.

In the dead-end filtration, the thickness layer of the CP increases throughout the operation causing the decline of the permeate flux while in the cross-flow filtration, CP occurs immediately after the filtration starts and becomes stable through the filtration.

Since CP is reversible, its effects can be reduced by changing the operating conditions of filtration such as operating at high temperature to favour the dissolution of solutes, lower feed concentration or increasing cross flow velocity to increase turbulence (Hausmann *et al.,* 2013).

3.6.9. Membrane Fouling

Membrane fouling is the deposition of particles on the membrane surface or in the pores of the membrane. This occurs as a result of adsorption, pore blockage, precipitation and cake formation. The main effect of fouling is the decrease in permeate flux and membrane selectivity (Corbatón-Báguena *et al.,* 2018). These effects happen because of the irreversible change in membrane properties caused by physical and chemical interactions between the membrane and foulants (Hausmann *et al.,* 2013). The major forms of fouling are as follows (Wang *et al.,* 2014):

- External Fouling: This occurs when particles, colloids or macromolecules are deposited on the surface of the membrane. It is also referred to as 'fouling layer' on membrane surfaces. Generally, external fouling can be divided into two kinds of fouling layers: cake layer due to the accumulation of retained solids on the membrane, and gel layer, which

is a cross-linked three-dimensional network of the deposited particles, which can be macromolecules, colloids, and inorganic solutes.

- Internal Fouling: This is caused by the adsorption and deposition of solutes and fine particles within the internal structure of membranes, such as the adsorption of foulants to pore-walls and pore narrowing to the point that it can lead to blocking.

As fouling is an inevitable phenomenon, the physical and chemical changes that are developed continue throughout the filtration process. Changes in operation conditions during the filtration process may increase the flux temporarily, but are ineffective in the long run. Therefore, cleaning or replacement of the membrane is thus required to solve the problem.

3.6.10. Membrane Cleaning

The main purpose of cleaning is to avoid the decrease in performance of the membrane by removing the deposited particles. Based on the cleaning techniques, there are two main types: physical and chemical.

- Physical Cleaning: Physical cleaning depends upon mechanical forces. This involves examples as ultrasound waves cleaning, electrical cleaning, air sparging, mechanical cleaning which is rarely used as it involves the use of brushes or sponges, and hydraulic cleaning which comprises of forward flushing or backflushing. Backflushing involves the reversal flow to flush deposits out of the pores and surface of the membrane. It can be carried out during filtration, by using a negative TMP, or during cleaning with water or a cleaning agent. Backflushing during filtration improves permeate flux and reduces the need of cleaning as shown in Figure 6.

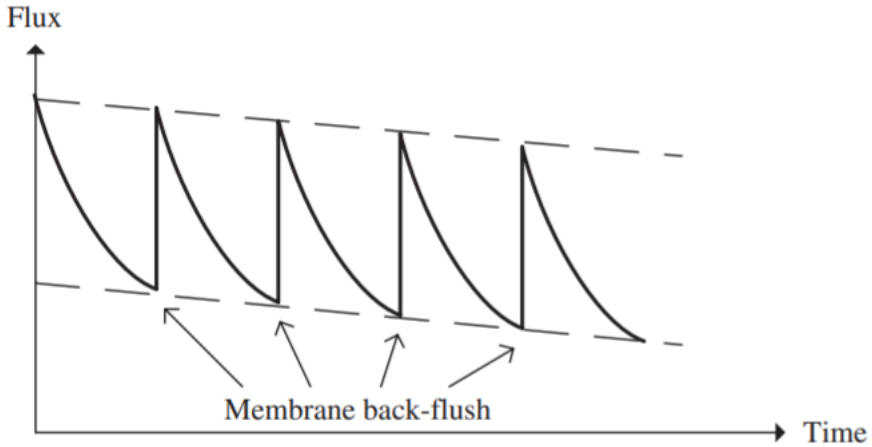


Figure 6. Graphical representation of permeate flux with periodic backflushing during filtration (Hausmann et al., 2013)

Physical cleaning is less effective compared to chemical cleaning. However, it requires no chemical reagents, which makes it less likely to cause membrane degradation/damage (Hausmann *et al.,* 2013).

- Chemical cleaning: Chemical cleaning is the most popular cleaning method. It involves the use of cleaning agents, or detergents, to dissolve or remove deposits and fouling materials from the membrane. Water is the main solvent used for dissolution and dilution of cleaning agents. It is also used for rinsing in between cycles of cleaning agents. The nature of the cleaning agent varies depending on the solute to be removed. Acidic agents are used to remove minerals inorganic deposits, basic agents for organic deposits and disinfectants for biological deposits (Hausmann *et al.,* 2013).

3.6.11. Applications of membranes in the olive oil industry

In the past few years, membrane technology has been researched widely at the laboratory scale level for the recovery of bioactive compounds. Conidi et al. (2019), made a research where solid waste from the olive mill industry, subjected to a solid-liquid extraction with water, was treated by membrane separation techniques by integrating microfiltration, ultrafiltration and nanofiltration systems to obtain high phenolic content fractions. The results showed that the microfiltration system removed the suspended solids and most of the biological active compounds were recovered in the permeate. Ultrafiltration systems showed lower permeate fluxes compared to nanofiltration. Moreover, the nanofiltration membrane tested could retain more than 70% of total phenolic compounds at an optimal operating pressure of 25 bar (Conidi *et al.,* 2019).

The use of membrane technology has proved that it is suitable for the purification and concentration of phenolic compounds in the olive oil industry as it can also be observed in another research where four membranes with different pore sizes were used for the recovery of phenols. The UP005 membrane presented the best results with a low phenolic content rejection, which makes it a promising form of phenolic recovery (Cifuentes-Cabezas *et al.,* 2021).

CHAPTER 4. METHODOLOGY

4.1. PREPARATION OF THE FEED

The two-phase olive mill waste (TPOMW) used for the extraction of phenols was obtained from the Cooperative San Isidro de Segorbe in Castellón, Spain. It was stored at 5ºC to avoid the growth of microorganism that will alter the state and quality of the feed.

A solid-liquid extraction with water as solvent was carried out to extract the phenols from the olive pomace. A mixture of feed and water with ratio of 1:10 was employed, using 300 grams of the olive pomace and 3L of distilled water for each extraction. The optimum operating conditions for the extraction were obtained in a previous work (Moreno Reolid *et al.,* 2020).

4.1.1. Ultrasonic Assisted Extraction (UAE)

The first step of the extraction is the use of the Ultrasonic Assisted Extraction. Previously, along the course of this project, the best conditions for the extraction were selected, and it was used for the duration of the extraction for this master´s thesis. Hence, the extraction was carried at a temperature of 40 ºC, for 45 minutes at a frequency of 37 kHz and amplitude of 100 m using the device shown in Figure 7. The UAE equipment used was from Elma, model Elmasonic P 70 H.

Figure 7. Ultrasonic Assisted Extractor (UAE) (Elma)

To ensure an even distribution of temperature and movement of solid particles, which would avoiding settling, two external impellers were introduced rotating at 240 rpm. Periodically, ice block bags were inserted to control the temperature and avoid the temperature from rising above 40 ºC as the UAE also makes the temperature to slightly rise.

Once the time of extraction has passed, the mixture was sieved to remove the larger solid particles, that would affect the microfiltration process. Finally, the extraction was repeated to obtain 6 litres which will be needed for the ultrafiltration plant.

4.1.2. Centrifugation

The mixture was then separated with the aid of a centrifuge (Figure 8) from Sigma, model Sigma 6-16KS. This separates the small solid particles from the liquid phase and makes it easier for the filtration process. The centrifuge was operated at 9600 rpm for 10 minutes, excluding the time of acceleration and stoppage, in two batches as only six containers were available and were not enough to accommodate the 3L of mixture.

Figure 8. Centrifuge

4.1.3. Vacuum Filtration

After the centrifugation step, some solid particles remained on the surface of the mixture, which will affect the performance of the membrane. This was removed with the use of vacuum filtration using a filter paper of 60 µm and a Laboport KNF vacuum pump.

The filtrate was collected, kept in a plastic container, and stored in the refrigerator at 5^{o}C to conserve the organic content present for each extraction process. The maximum duration of the refrigerated extract was 7 days.

4.2. MEMBRANES USED

Three different membranes, UP005, UP150 and UH050, were used during this research to carry out the experimental test. They are all flat sheet membranes with different pore sizes and permeate flux rates all from the same manufacturer (Microdyn-Nadir) (Table 2). All three membranes were cut and soaked in water before use.

Table 2. Properties of membranes

Membrane	UP005	UP150	UH050
Material	Polyethersulfone (PES)	Polyethersulfone (PES)	Hydrophilic Polyethersulfone (PESH)
MWCO (kDa)	5	150	50
T max (ºC)	95	50	95
Permeability ($Lm^{-2}h^{-1}bar^{-1}$)[a]	> 10	≥ 285	≥ 85

a) Test conditions: clean water, 4 bar, 20 ºC, cross flow operation

4.3. ULTRAFILTRATION PLANTS

Two ultrafiltration plants were used during the project due to their different operating condition capacities. The ultrafiltration plant 1 was used for UP005 and UH050 and the ultrafiltration plant 2 was used for UP150 and UH050 depending on the pressure of the extraction test. The two plants have the same configuration shown in Figure 9. The level of conditions control happens to be a bit different in each plant as the ultrafiltration plant 1 can control the cross flow velocity with a control panel system while the ultrafiltration plant 2 requires turning a valve.

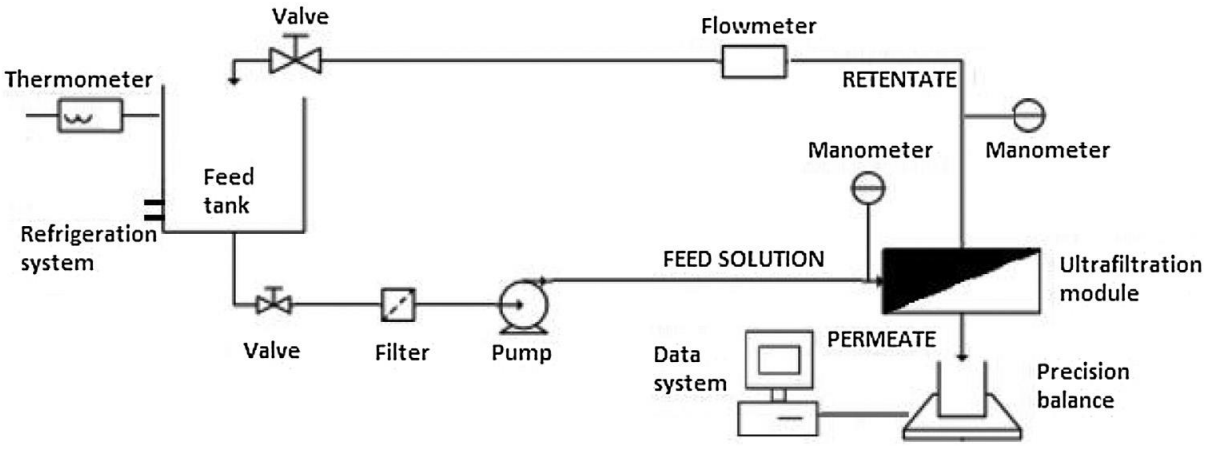


Figure 9. Scheme of ultrafiltration plants (Cifuentes-Cabezas et al., 2021)

4.3.1. Ultrafiltration Plant 1

The ultrafiltration plant is composed of a feed tank of 8L capacity wrapped with a cooling jacket, a pump supplying positive TMP which can supply up to 20 bar, the flat membrane module, two

manometers placed at both ends of the membrane modules and a scale that is connected to a computer that records the permeate flux on an Excel sheet. The membrane module (Figure 10) was designed by the Instituto de Seguridad Industrial, Radiofísica y Medioambiental, ISIRYM (Spain) (Santafé-Moros and Gozálvez-Zafrilla, 2010). The total area of the active surface of the membrane is 0.004563 m^2.

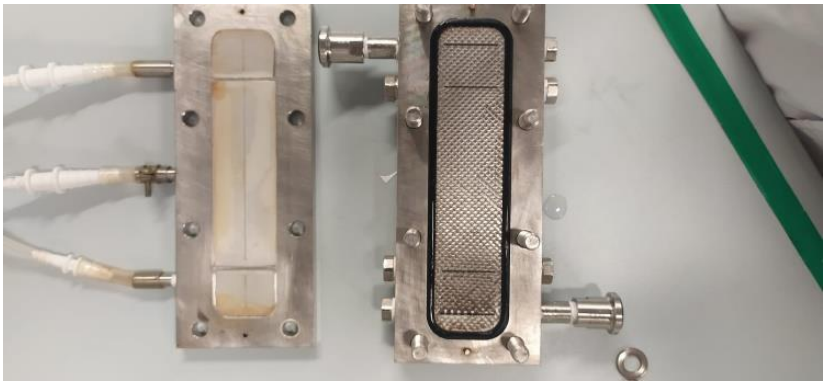


Figure 10. Membrane module for plant 1

The temperature of the system is controlled by the cooling jacket that is connected to the refrigeration unit Frigiterm (Selecta, Spain). The crossflow velocity is controlled with a control panel that is also connected to the pump where the state and pressure difference of the pump can also be monitored. Figure 11 shows a picture of ultrafiltration plant 1.

Figure 11.Ultrafiltration plant 1 setup

4.3.2. Ultrafiltration Plant 2

This plant is similar to the ultrafiltration plant 1 in terms of operation, but this is from Orelis (France). It consists of a feed tank with 8L capacity, a pump supplying positive TMP with a maximum pressure capacity of 4 bar, the flat membrane module, two manometers placed at both ends of the membrane modules and a balance scale that is connected to a computer that records the permeate flux on an Excel sheet. A flat membrane module type Rayflow from Orelis (France) is used with a membrane having an active surface area of 0.01292 m^2 (Figure 12).

Figure 12. Membrane module for plant 2

The temperature is controlled by the same refrigeration unit Frigiterm (Selecta, Spain) used in the other plant. The velocity is adjusted manually and measured with a flowmeter and the TMP with the manometers which are placed at the inlet and outlet of the module. Figure 13 shows a picture of ultrafiltration plant 2.

Figure 13. Ultrafiltration plant 2 setup

4.4 EXPERIMENTAL PROCEDURE

4.4.1. Compaction of membrane

Before using the membrane, it was soaked in distilled water for at least 24 hours. Then, it was inserted in the membrane module and compacted at their respective pressures: 6 bar for UP005, 1.25 bar for UP150 in plant 1, 1.5 bar for UP150 in plant 2, 3 bar for UH050 in plant 1 and 1.8 bar for UH050 in plant 2. When a membrane had to be used in the two plants, a new membrane was cut due to the different surface areas of the membrane modules.

The compaction process involves subjecting the membrane to a pressure higher than required in the next experiments to ensure the membrane adapts to the desired pressure of extraction and does not deform during its use. It was carried out with water to remove any residual preservative the membrane might still have and fill the pores. The compaction process was maintained for at least 3 hours at a cross flow velocity of 1 m/s until a steady permeate flux was obtained.

4.4.2. Initial Permeability

Right after the compaction, the initial permeability was determined. This is a reference value to ensure the membrane was in a good condition after cleaning the membrane immediately after each extraction test. The initial permeability was measured with distilled water at a cross flow velocity of 1 m/s and 4 different TMPs between the minimum pressure allowed in the plants and the respective compaction pressure. The permeate flow was recorded until a stable flow was obtained at each TMP. A graph of the permeate flux versus TMP was plotted to determine the initial permeability of the membrane.

4.4.3. Serial Extraction

Before the extraction tests were carried out with the membranes, a series of extractions from the TPOMW were performed to determine the recovery of phenols. From the first extraction, a sample was taken and stored in the refrigerator at 5^{o}C for a further extraction. The process was repeated for a third extraction process by taking the samples from the second serial extraction.

4.4.4. Experimental Tests

The extraction tests were a continuation of a previous stage of the entire research project (Casas Roncero *et al.,* 2021) where the performance of the UP005 membrane was tested at TMPs of 0.75 and 1.5 bar at crossflow velocities of 1.5, 2.5 and 3.5 m/s. In this research project, the use, and the performance of the membranes UP150 and UH050 were also studied. The tests were stopped when the permeate flux remained stable after 3 hours.

All extraction tests were carried out at three different crossflow velocities (1.5, 2.5 and 3.5 m/s) and TMPs ranging from 0.75 to 5.5 bar. The concentration of the feed was kept constant by the recirculation of the permeate to the feed tank. Table 3 shows the TMPs, and velocities tested on each membrane during the extraction process.

Table 3. Conditions of extraction tests on membranes

Cross Flow Velocity (m/s)	TMP (bar)	Membrane
1.5	0.75	UP150, UH050
	1.5	UH050
	2.5	UP005
	3.5	UP005
	4.5	UP005
2.5	1.5	UH050
	2.5	UP005, UH050
	3.5	UP005
3.5	2.5	UP005
	3.5	UP005
	4.5	UP005
	5.5	UP005

Each test required a temperature of 20ºC, therefore, the feed had to be heated by an external electric coil due to the storage of the feed at 5ºC. When the required temperature was reached, distilled water was removed to avoid dilution of the feed. The TMP and cross velocity were set according to the operating conditions selected for each particular run and monitored constantly to prevent any deviation that could affect the results.

The test lasted for at least 3 hours until a steady permeate flux was obtained taking permeate samples of 50 mL in a falcon tube after 1.5h and 3h. This was stored in the freezer along with a sample of each feed before the extraction for the characterization process. At the end of the extraction, if enough feed was left, it was stored back in the refrigerator for another extraction test.

4.5 CLEANING OF MEMBRANES

4.5.1. Cleaning Protocol

After every extraction test, the membrane module was cleaned to recover the membrane performance. The cleaning process was considered acceptable when the permeability was at least 90% of the initial permeability. Most of the cleaning processes were carried out at 1.5 m/s and 1 bar for 1 hour usually at 35ºC, but with different conditions when the permeability could not be recovered, which will be discussed in section 5.9.

Following the cleaning protocol reported in Luján-Facundo et al., 2015 which resulted in a high cleaning efficiency, the cleaning agent adopted at the start of the project was P3-Ultrasil 115 from Ecolab at a concentration of 0.7% and 35ºC (Luján-Facundo *et al.,* 2015). After some extraction tests, a lower concentration of 0.5% was used, which would be less harmful for the membrane and be more eco-friendly. This concentration was able to recover an acceptable permeability, but not after every extraction test.

The membrane UP150 faced the biggest challenges when recovering the permeability. The membrane was cleaned with various cleaning agents starting with P3-Ultrasil 115 at 0.5% concentration and then using a concentration of 0.7%. Due to the inability to recover the permeability, other cleaning agents like P3-Ultrasil 110 at 0.7% concentration and sodium hypochlorite solution with a concentration of 200 ppm were used.

Finally, for the membrane UH050 in the ultrafiltration plant 2, the P3-Ultrasil 115 with 0.5% concentration at 35ºC was not able to recover the initial membrane permeability while that of 0.7% recovered a higher permeability, but still lower than the initial permeability. Then, an Ultrasil-115 solution of 1% was used and achieved a permeability above the initial permeability. When the membrane UH050 was used in the ultrafiltration plant 1, a P3-Ultrasil concentration of 0.7% at 35ºC was enough for the recovery of the permeability.

4.5.2. Membrane Rinsing

The membrane was rinsed with tap water after each experiment and after the cleaning process to remove any component that remains in the membrane module or plant that would affect the permeability. The plant was run in an open system for 15 minutes, and the water channelled to the drainage, so the water did not recirculate. After the cleaning process, the pH of the water after 15 minutes of rinsing was measured to ensure all the cleaning agent was out of the system. Finally, distilled water was used to rinse the plant to remove the tap water.

4.5.3. Permeability Recovery

The last step of the cleaning process was the measurement of the recovery of the permeability which must achieve at least 90% of the initial permeability. Distilled water was passed through the membrane at a TMP of 1.5 bar for the UP005, and 1 bar for both the UP150 and UH050 with a cross flow velocity of 1 m/s and a temperature of 20ºC.

4.6 CHARACTERIZATION OF SAMPLES

The feed sample and the permeate samples (1.5h and 3h) were characterized to analyse the performance of the membrane under the various conditions. The following characterization processes were carried out:

4.6.1. Total Solids

To measure the total solid content from each sample, a known volume of the sample was inserted into a bottle of known weight and was placed in an oven for at least 24 hours at 105ºC. After the time, the weight of the dry bottle was taken, and the weight difference determined.

4.6.2. Chemical Oxygen Demand (COD)

This test was done to determine the amount of oxygen dissolved available for the oxidation of chemical organic materials present in the feed and permeate samples. This could be used to determine the organic content of the samples. The kit UN3316 (Merck Chemicals) was used reporting a concentration in mg/L. 1 ml of the sample was inserted into the tube and heated at 148^{o}C for 2 hours. In the case of the feed, due to its high organic content, a dilution of 1:2 was done to ensure the results were within the range limits of the spectrophotometer. After the 2 hours, the samples were allowed to cool for 30 minutes, shaken, and then measured.

4.6.3. Phenolic Content (Folin-Ciocalteu Method)

The Folin-Ciocalteu method was used to determine the phenolic content of the samples collected. The method is based on chemical reduction of the yellow reagent using a mixture of tungsten and molybdenum oxides that produces a blue colour with broad light adsorption at 765 nm. A sample of 0.2 ml (quantity changes with dilution) was added to a tube containing 6.8 ml of distilled water to make it 7 ml. A quantity of 0.5 ml Folin-Ciocalteu was added and was agitated for 3 minutes with the Ultrasound equipment. Then, 1 ml of Na_2CO_3 (20%, m/v) was added, immediately shaken, and kept in the dark for 1 hour. After this time, the absorbance was measured at 765 nm. It was quantified with a standard from a tyrosol dissolution, and the phenolic content was determined in mg of tyrosol/L.

4.6.4. Colour

The colour was determined by measuring the absorbance at 436, 525 and 620 nm using a DR600 spectrophotometer (Hach Lange, Germany). This was calculated following equation 4.3 according to Döpkens et al., 2001:

$$Colour = \frac{A_{426}^2 + A_{525}^2 + A_{620}^2}{A_{426} + A_{525} + A_{620}} \tag{4.3}$$

4.6.5. Conductivity

The conductivity shows the concentration of ions present in the samples and was measured with a conductivity meter EC-Meter GLP 31+ from Crison, Spain.

4.6.6. pH

The pH records the acidity level of the samples, and this was measured with a pH meter GLP 21+ from Crison, Spain.

4.6.7. Phenolic profile

This is one of the main characterization steps as the main goal of this project is the recovery and purification of biophenols from TPOMW. This process determines the various phenolic compounds present in the wet olive pomace and the ones present in the permeates. The phenolic compounds present in the feed and permeates were determined with a technique based on Liquid Chromatography coupled to Mass Spectrometry (LC-MS).

The equipment comprised of a 1260 Infinity II LC system connected to a quadrupole-time-of-flight (qTOF) mass analyzer (Agilent Technologies, USA). 4 µL of each sample was injected and separated throughout a Zorbax Extend C18 column (4.6 x 100 mm, 1.8 µm particle size) (Agilent Technologies, USA), at 40°C. The compounds were eluted using water as phase A and acetonitrile as phase B, by means of a gradient where they were both acidified with 0.5% of acetic acid (v/v). The analysis time lasted for 24 minutes, and a post time of 3 minutes was used for equilibrating the column. The flow rate of 0.8 mL/minute.

The mass spectrometer worked with a negative polarity. The main conditions to be optimized for the ionization source were drying gas temperature and flow (200°C and 8 L/min respectively), nebulizer pressure (30 psi) and capillary voltage (3500 V). A calibration solution was used to carry out the ion mass corrections which produced the m/z values of 112.9856, 966.0007 and 1033.9881 as references.

To explore the chromatograms, a software called MassHunter (Agilent, USA) with a Qualitative and Quantitative modes was used. An area was produced when the peaks were integrated during quantification and this was interpolated with the corresponding external calibration curve of caffeic acid, hydroxytyrosol, luteolin, p-coumaric acid or oleuropein.

CHAPTER 5. RESULTS AND DISCUSSION

5.1 RECOVERY OF PHENOLIC CONTENT

This research thesis started with the recovery of the phenolic content from serial extractions from two-phase olive mill waste (TPOMW) and this section shows the results. Each extraction cycle was characterized and the results showing the concentration of phenols obtained from each cycle appear in Figure 14.

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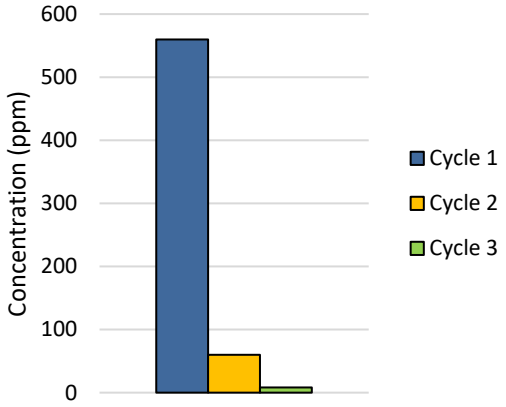

Figure 14. Phenolic recovery

If all the phenolic concentrations were added, and the recovery was calculated, a recovery of 89 % will be obtained from the first cycle which proves that ultrasound assisted extraction with water is a suitable technique for phenol recovery as reported in section 3.5. It should be a bit lower since there will still be a small quantity of phenols in the last residue, but this was so small that it could be neglected. The second and third cycles achieved a lower recovery of phenols by a factor of almost 10 in each cycle, and following this trend, a fourth cycle will not be necessary as a negligible quantity of phenols will be extracted. These results shows that only one extraction cycle was necessary as high quantity of phenols were recovered from the first cycle.

Similar behaviours could be seen in the characterization of the solid contents and COD as high concentrations were recovered in the first cycle (Figure 15a and 15b). The colour content (Figure 15c) was the only parameter that showed lower recoveries than the other parameters in the first cycle with 53% and higher recoveries than the other parameters in the second and third cycles with values of 30.51% and 15.21%, respectively, but since the recovery of colour is not an objective, the results were satisfactory.

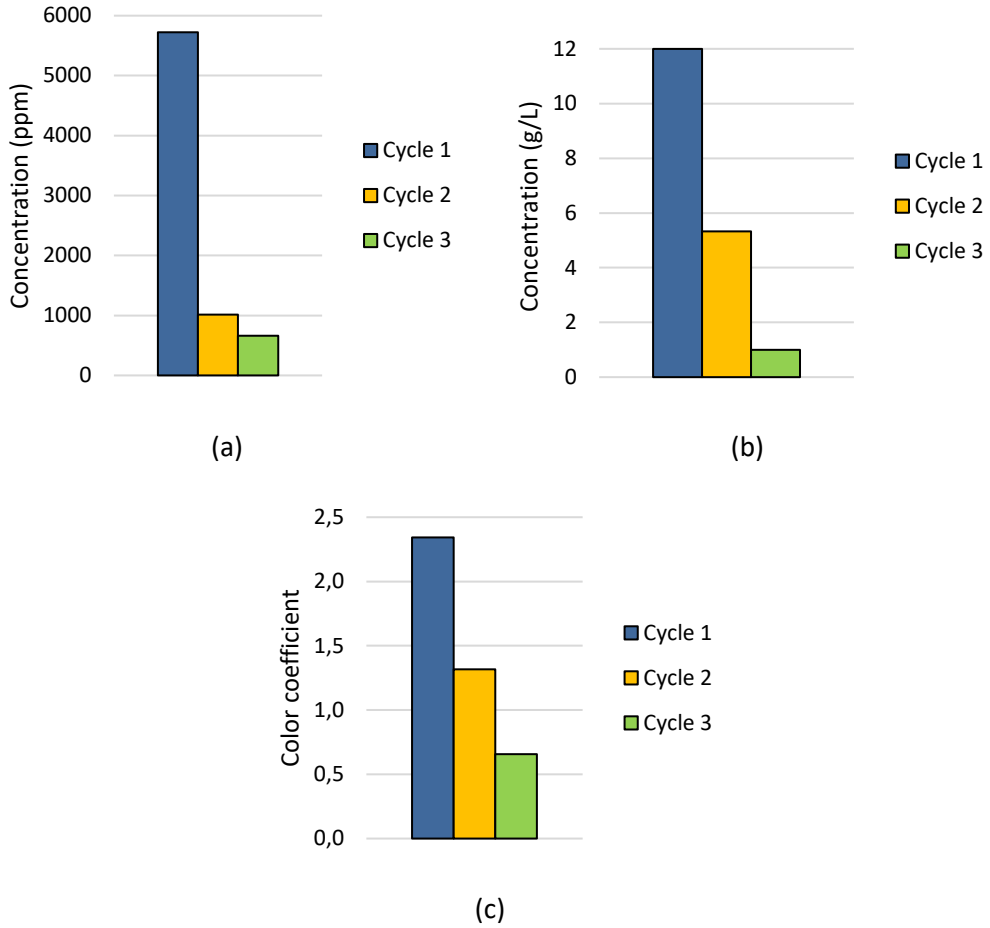

Figure 15. Recovery of (a) COD (b) Total solids (c) Colour

5.2 CHARACTERIZATION OF FEED

The feed from the solid-liquid extraction was characterized. This is important to further determine the rejections of the subsequent membrane process. An average value of the characterizations from the fifteen extractions done during this project were taken and shown in Table 4.

Table 4. Characterization of feed

Parameter	Value	Standard Deviation
Colour	2.41	0.11
Total Solids (g/L)	5.2	1.0
COD (mg O_2/L)	10,800	1500
Phenolic Content (mg of tyrosol/L)	810	66
pH	5.1	0.2
Conductivity (µS/cm)	1,490	79

It was observed that the feed possesses a dark colour with a high level of COD and total solids which was responsible for the concentration polarization and fouling during the subsequent ultrafiltration process.

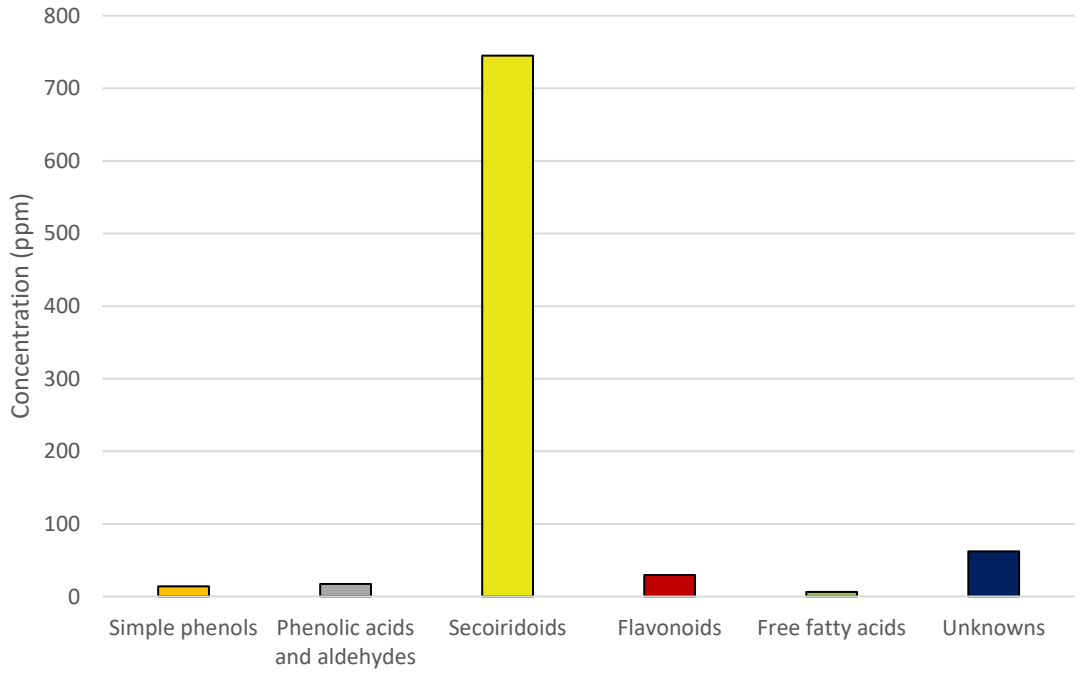

Figure 16. Families of phenolic compounds present in feed

The results from the LC-MS produced six major families of compounds present in the extracts, with four being phenolic compounds (simple phenols, secoiridoids, flavonoids, and phenolic acids and aldehydes). Free fatty acids and a family of unknown compounds were also present (Figure 16). The most abundant group was the secoiridoid family, which accounted for 92% of

the total phenolic concentration present in the feed. The percentage of unknown compounds was 7.7%, while the free fatty acids were found in trace quantities with the values found in Table 5.

Table 5. Families of phenolic compounds found in the feed

Family	Concentration (ppm)	Standard deviation
Simple phenols	14,0	1,8
Phenolic acids and aldehydes	17,3	1,4
Secoiridoids	745	59
Flavonoids	29,6	4,0
Free fatty acids	6,3	0,7
Unknowns	62,21	0,22
TOTAL	810	66

5.3 COMPACTION OF MEMBRANES

This section shows the compaction results of the membranes used in the ultrafiltration plants. As mentioned in Chapter 4, this was done to condition the membrane to the pressures used during the extraction process and to remove any conservative still present.

5.3.1. Compaction of UP005

The UP005 membrane was compacted at 6 bar and 1 m/s due to the high-pressure conditions expected on this membrane. Figure 17 shows an increase in the permeate flux behaviour with time reaching an average permeate flux of 79.49 L/m^2·h. The increase in permeate flux with time was due to the removal of the conservatives present in the membrane and because the pores of the membranes are so small, it would take some time to remove, therefore, the gradual increase of the permeate flux.

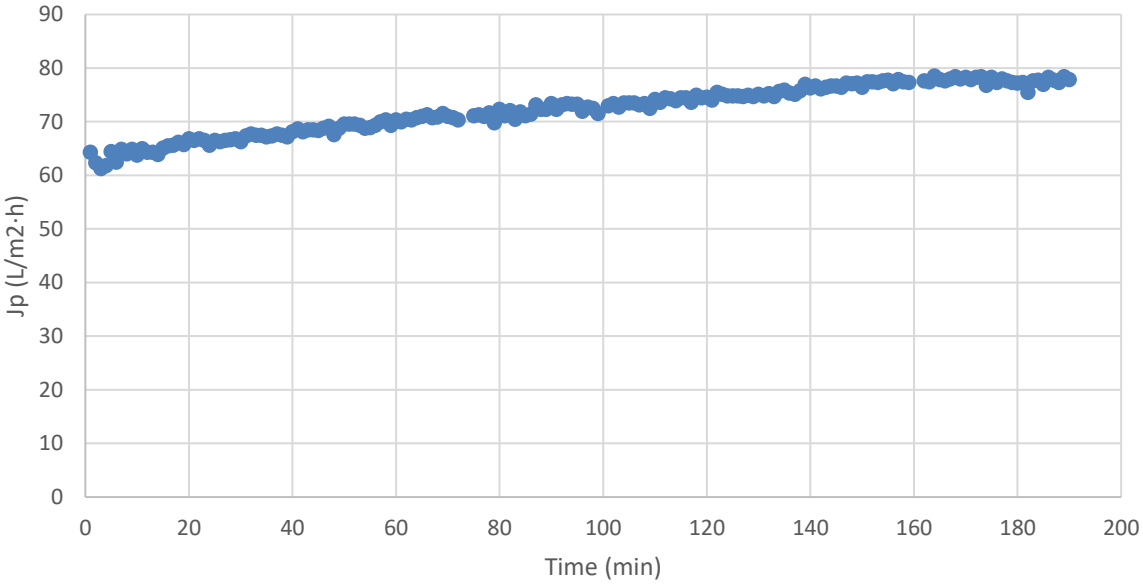

Figure 17. Compaction of membrane UP005[a]

a) Area of UP005 membrane is 0.004563 m^2

5.3.2. Compaction of UP150

The UP150 was initially compacted in the ultrafiltration plant 1 at a pressure of 1.25 bar and 2 m/s and the results are shown in Figure 18. Unlike the UP005, the permeate flux reduces with time giving a higher average permeate flux of 580.45 $L/m^2 \cdot h$ due to the bigger pores of the membrane. Due to the inability to achieve a suitable initial permeability curve, discussed in section 5.4, and the large permeate flux obtained from the compaction process, a new membrane was compacted in the ultrafiltration plant 2.

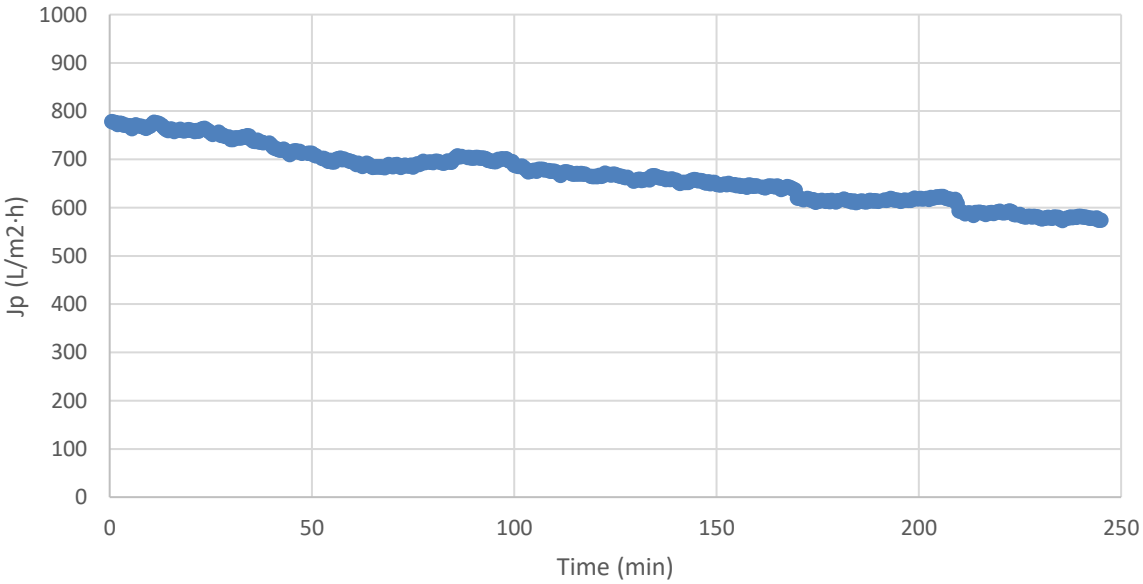

Figure 18. Compaction of UP150 in ultrafiltration plant 1

In the ultrafiltration plant 2, a new membrane UP150 cut out was compacted at a pressure of 1.5 bar and 1 m/s. The curve obtained (Figure 19) happens to be a bit different compared to the other plant showing a much larger drop in permeate flux which is thought to be dirt present in the plant causing fouling as the permeate flux reduces gradually until it becomes more stable giving an average permeate flux of 284.73 L/m^2·h. The results from the ultrafiltration plant 2 were then used, as the initial permeability was able to be achieved, and ultrafiltration tests able to be carried out.

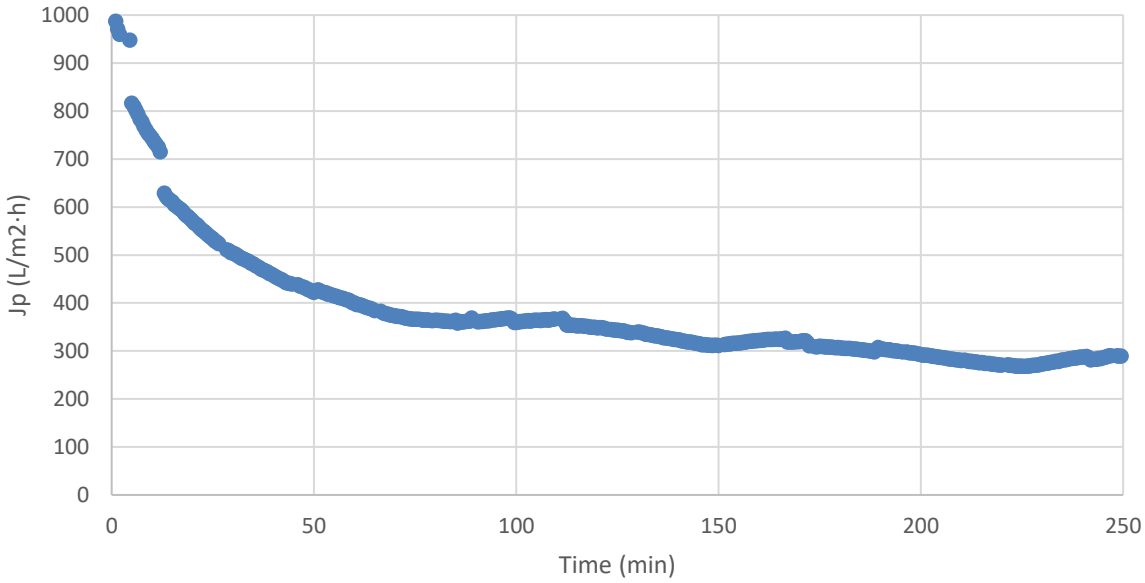

Figure 19. Compaction of UP150 in ultrafiltration plant 2

5.3.3. Compaction of UH050

For lower pressure conditions, the ultrafiltration plant 2 was used and the membrane UH050 compacted a 1.8 bar and 2 m/s (Figure 20). The permeate flux curve gradually reduces until a steady curve was reached having an average value of 468.37 L/m^2·h.

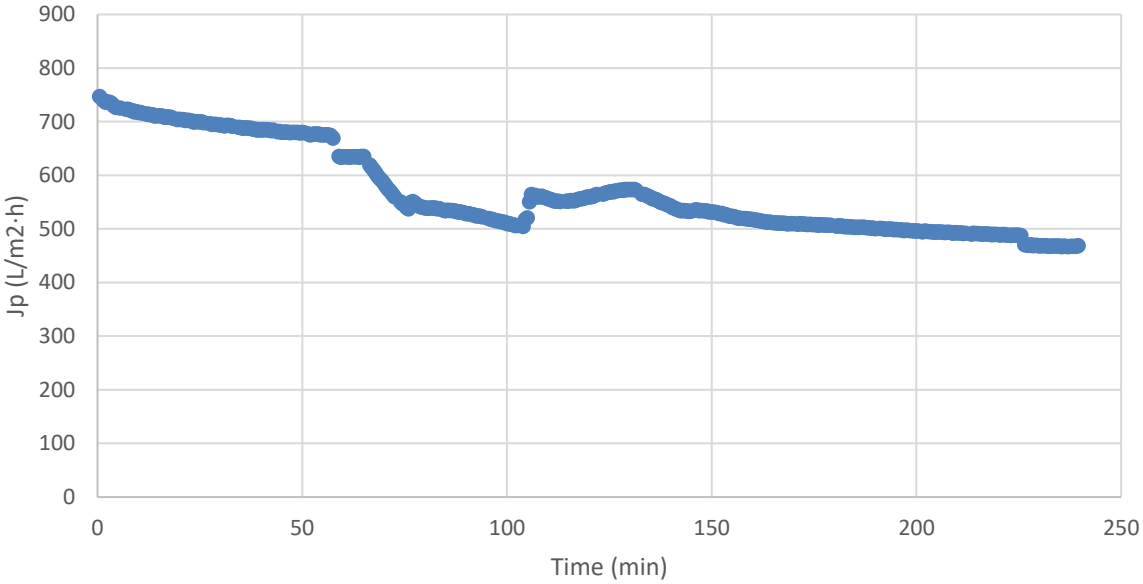

Figure 20. Compaction of UH050 in ultrafiltration plant 2

Due to the inability of the ultrafiltration plant 2 to operate at a velocity of 2.5 m/s without leakage, a new cut out of the UH050 membrane was done and compacted in the ultrafiltration plant 1 at 3 bar and 1 m/s giving an average permeate flux of 580.45 $L/m^2 \cdot h$ and a curve shown in Figure 21.

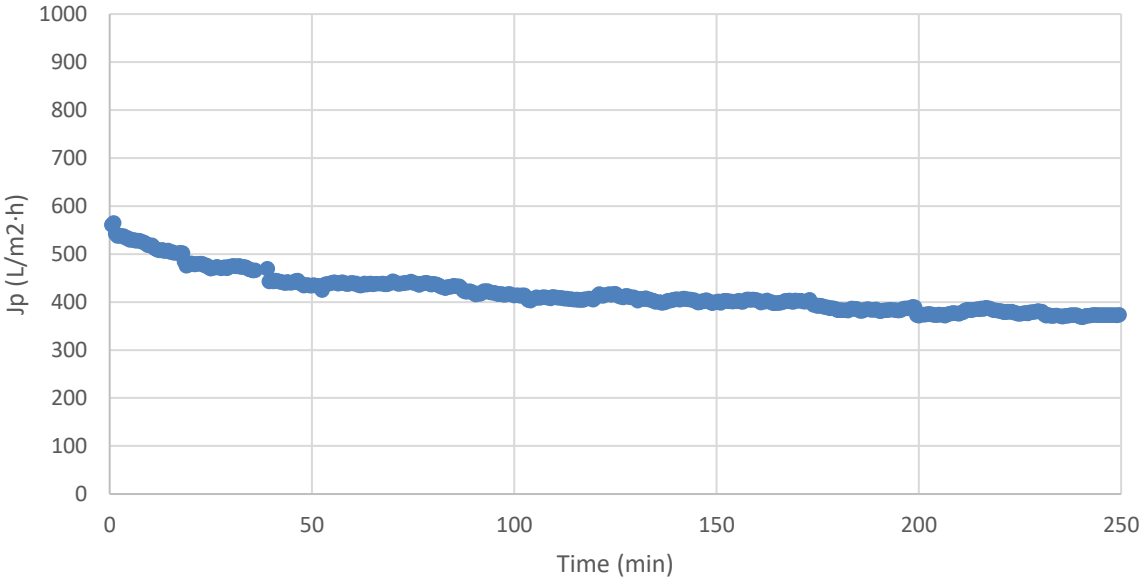

Figure 21. Compaction of UH050 in ultrafiltration plant 1

5.4 INITIAL PERMEABILITY

The results of the initial permeability of the membranes are discussed in this section. Four TMP points within the compaction range and minimum operating pressure were selected and an average of the stable permeate flux was determined at a crossflow velocity of 1 m/s. Then, the average permeate flux was plotted against their respective TMPs giving a straight line. The slope of each straight line was determined, and the constant value is taken as the initial permeability of the membrane, according to Darcy's law (Cifuentes-Cabezas *et al.,* 2021).

5.4.1. Initial permeability of UP005

The initial permeability for the UP005 is shown in Figure 22. As expected, the permeability was low compared to other membranes in this research thesis due to the smaller size of the pores which results to a lower permeate flux. The value obtained was similar to that obtained from the research preceding this research which was 10.33 L/m^2·h·bar and was higher than the permeability provided by the manufacturer of >10 L/m^2·h·bar, and this confirmed that the membrane was in a good condition for the extraction ultrafiltration tests (Casas Roncero *et al.,* 2021).

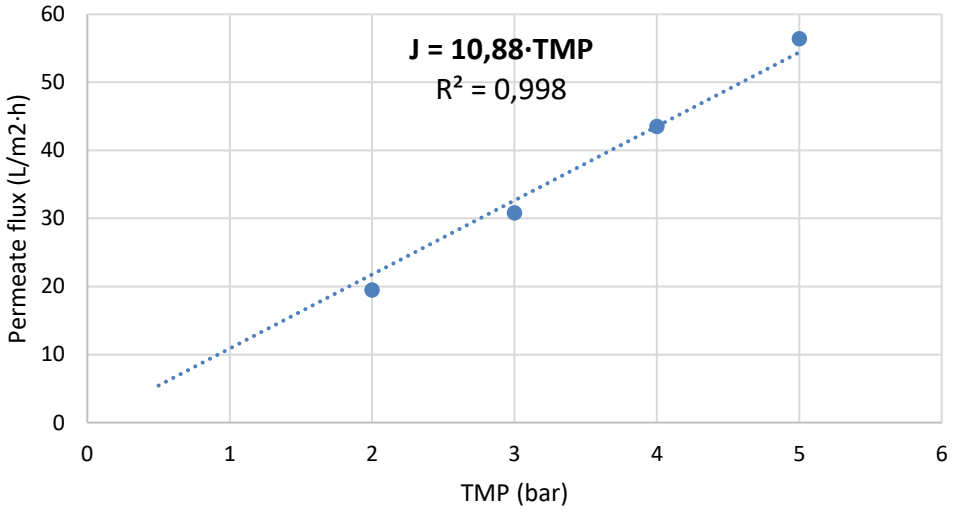

Figure 22. Initial permeability for UP005

5.4.2. Initial permeability of UP150

The initial permeability of the UP150 was much higher compared to the UP005. The permeability was first carried out with the ultrafiltration plant 1 but failed to provide a suitable straight curve. The plant has less sensitivity at pressures lower than 1 bar, and this made it difficult for the membrane to operate effectively due to the large pore size. Hence, a new cut out of the membrane was done and mounted in the ultrafiltration plant 2. This gave a better curve (Figure 23), but despite being the permeability lower than what is provided by the manufacturer (≥285 L/m^2·h·bar), it was used for the ultrafiltration tests. After a series of cleaning processes with

varying conditions and cleaning solutions (reported in section 5.8.2), the membrane was discarded due to the difficulty in recovering the permeability.

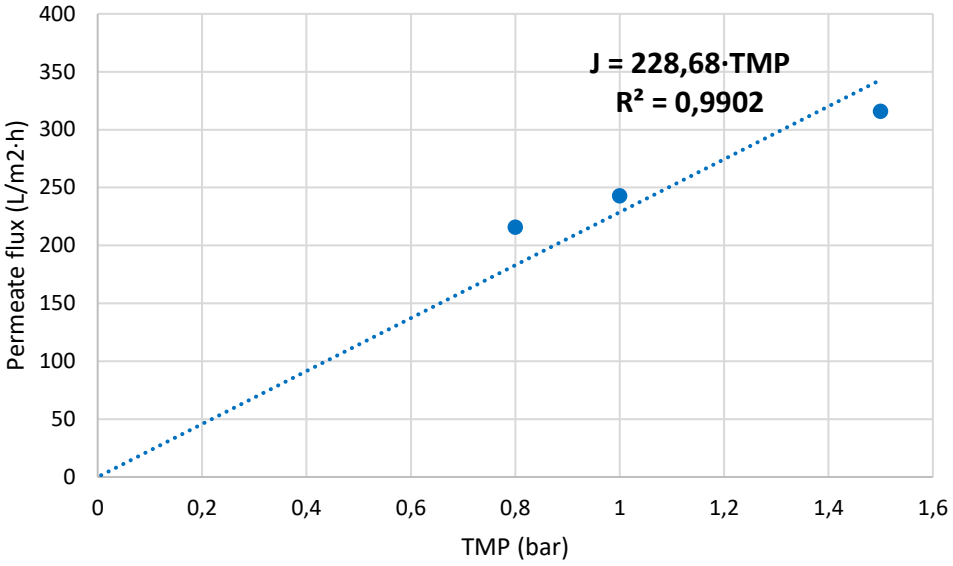

Figure 23. Initial permeabilities of UP150

5.4.3. Initial permeability of UH050

The initial permeability was carried out in the ultrafiltration plant 2. This gave a straight line shown in Figure 24 with a permeability higher than what was provided by the manufacturer (≥85 $L/m^2 \cdot h \cdot bar$), which was close to that obtained in another research with a similar membrane (191.75 $L/m^2 \cdot h \cdot bar$) (Cabezas-Cifuentes *et al.,* 2021).

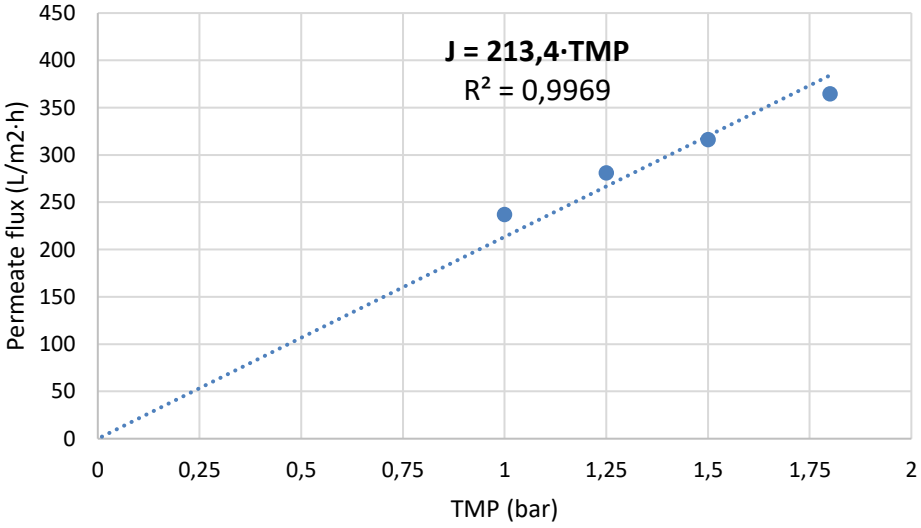

Figure 24. Initial permeabilities of UH050

In summary, the initial permeabilities of the membranes are arranged in Table 6. These results show that there is a direct impact of the pore size on the permeability. The values obtained experimentally fall within the range provided by the manufacturer except for the UP150 membrane.

Table 6. Summary of initial permeabilities

Membrane	Initial permeability ($L/m^2 \cdot h \cdot bar$)
UP150	228.68
UH050	213.40
UP005	10.88

5.5 ULTRAFILTRATION RESULTS

The ultrafiltration tests carried out with the UP005 membrane were a continuation of the previous research on which this thesis was based (Casas Roncero *et al.,* 2021) and these appear in Table 3.

In the case of the UP150, only one ultrafiltration test was carried out due to the low permeate flux obtained and the inability to recover the permeability when cleaning. The membrane was then changed to UH050 and the ultrafiltration tests in Table 3 were carried out. For the UH050, the two ultrafiltration plants were used due to their respective pressure capacity.

5.5.1. UP005 Membrane

Figures 25, 26 and 27 show how the TMPs and cross velocities affect the permeate flux with points taken every 1 minute. For all the CFVs, it can be observed that an increase in TMP will result to a higher permeate flux. This proves that the most important condition that affects the permeate flux for this membrane was the TMP as mentioned in Section 3.6.7. The permeate flux slightly reduced with time, which indicates that the fouling was not very pronounced.

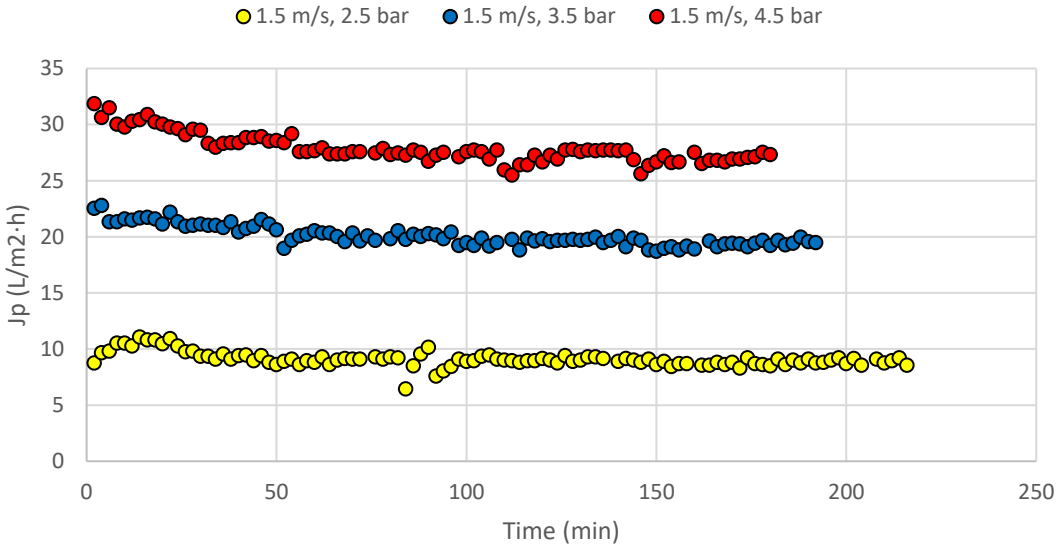

Figure 25. Permeate flux vs time for UP005 membrane at 1.5 m/s

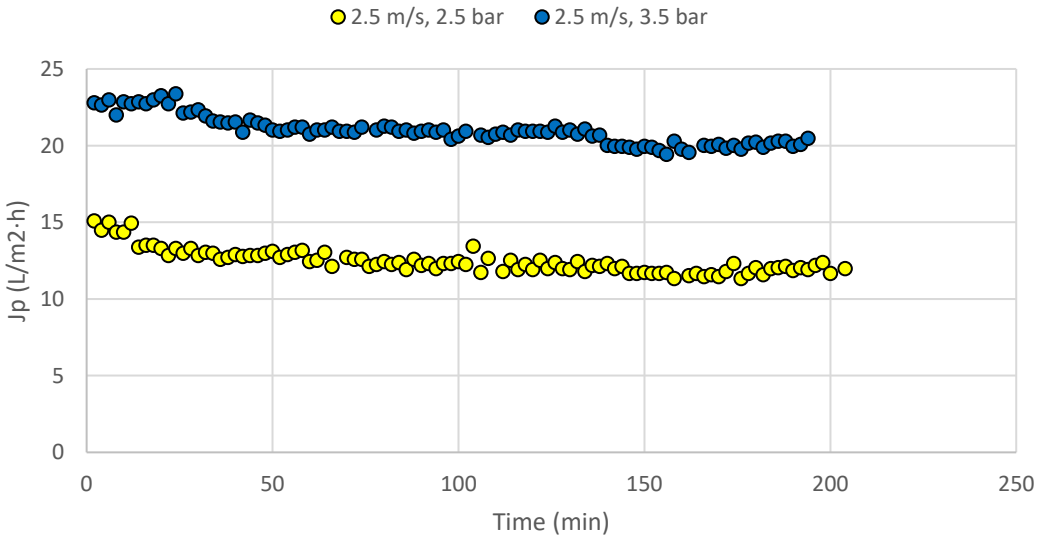

Figure 26. Permeate flux vs time for UP005 membrane at 2.5 m/s

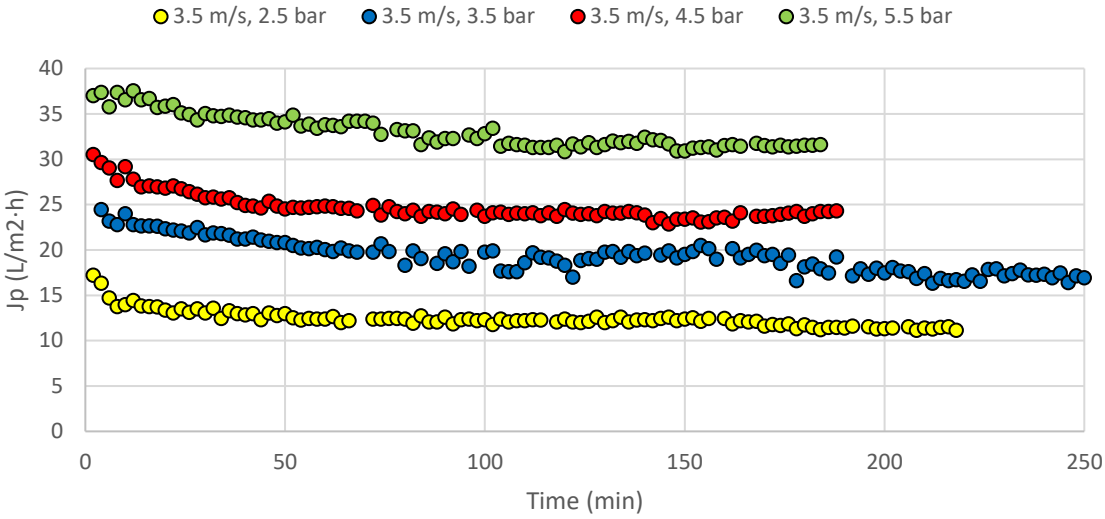

Figure 27. Permeate flux vs time for UP005 membrane at 3.5 m/s

Figure 28 shows the steady state permeate flux of each ultrafiltration test plotted against their respective TMPs. The line in grey colour shows the results obtained from the project preceding this master´s thesis (Casas Roncero *et al.,* 2021). From this graph, it can be observed that almost similar permeate fluxes were obtained at the same TMPs with an approximately 3 L/m^2·h in difference for all the CFVs tested. Therefore, the variation in cross flow velocity does not have a big impact on the change in permeate flux compared to the TMPs.

The result from this project shows a continuation in the curves increase. It was decided not to increase more the TMP, because the phenolic content rejection was very high and further increase in the TMP would produce higher rejections (seen in Section 5.6.1).

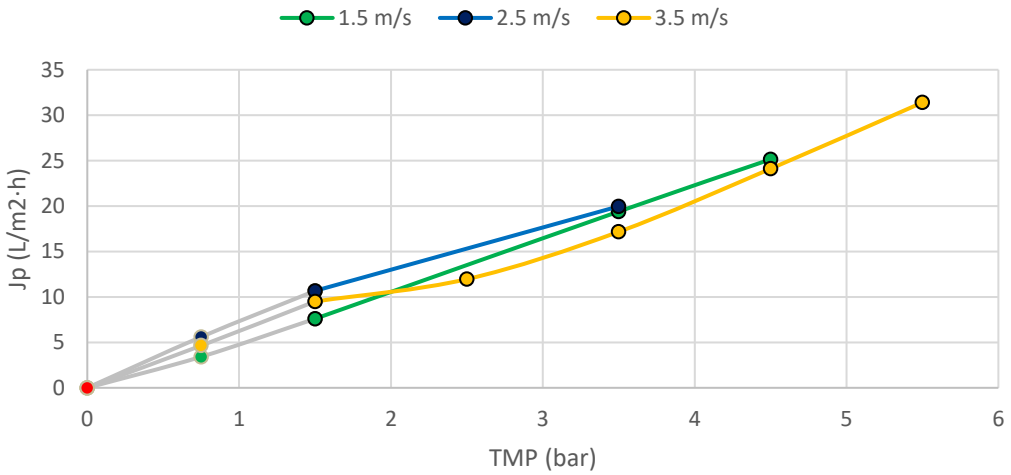

Figure 28. Steady state permeate flux vs TMP for UP005 membrane at the three crossflow velocities

5.5.2. UP150 membrane

Figure 29 shows a permeate flux that reduces with time and a steady permeate flux was achieved at an average of 26.71 L/m^2·h. Considering the large pore size of this membrane, a higher permeation rate was expected. The low values could be because of the size of the molecules present in the feed having a similar size as the pores which could block them and also the presence of some dirt in the plant, thereby increasing fouling. Concentration polarization was generally involved in the first steps of the filtration process and was responsible for the sharp decline in permeate flux. The gradual slow flux decrease at the later stages was caused by the accumulation of foulant molecules on the membrane surface, with the formation and compaction of a cake layer on the membrane surface.

As seen in Mondal et al., 2012, the decrease in permeate flux can be connected to concentration polarization and fouling phenomenon (Mondal *et al.,* 2012).

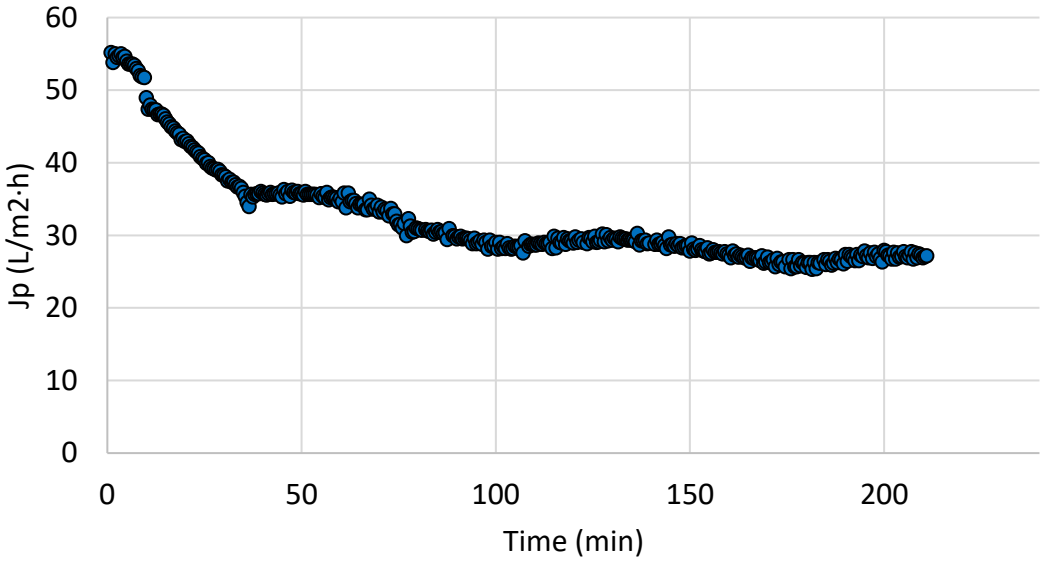

Figure 29. Permeate flux vs time for UP150 membrane at 1.5 m/s, 0.75 bar

5.5.3. UH050 membrane

The permeate flux of the ultrafiltration test carried out in the ultrafiltration plant 2 appears in Figure 30. The graph shows that the permeate flux at 0.75 bar was higher than when operated at 1.5 bar. A similar behaviour happened in the research made by Cifuentes-Cabezas et al., 2021, as the limiting flux in the membrane was reached faster with higher pressures. At this point, a gel layer is formed on the surface of the membrane that serves as an extra filtration layer that causes a decrease in permeate flux with increase in TMP as the gel layer becomes thicker due to compaction (Cifuentes-Cabezas *et al.,* 2021).

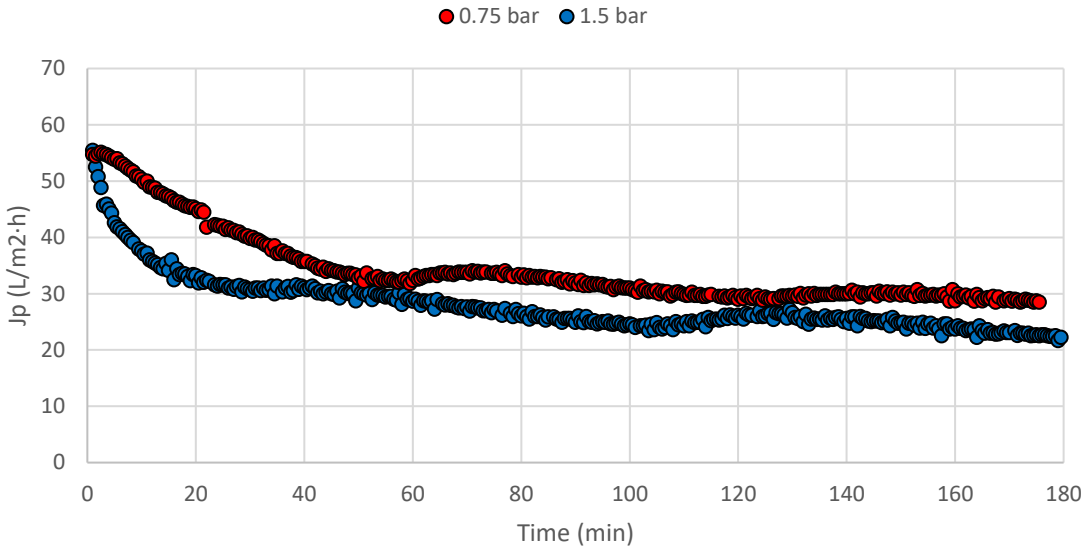

Figure 30. Permeate flux vs time for UH050 membrane at 1.5 m/s

It was decided at this point to stop the ultrafiltration tests at 1.5 m/s, as it was predicted that increasing the pressure will not produce any increase in permeate flux as the limiting flux was reached. Therefore, 2.5 m/s was the next cross flow velocity to be tested. The ultrafiltration test at 1.5 m/s and 1.5 bar was the last test performed in the ultrafiltration plant 2, as it was unable to operate at a cross flow velocity of 2.5 m/s without leakage.

A new membrane was cut-out, soaked and compacted in the ultrafiltration plant 1 in preparation for the next extraction tests. Two extraction tests were able to be carried out due to time constrictions; 2.5 m/s, 1.5 bar and 2.5 m/s, 2.5 bar with the permeate flux appearing in Figure 31.

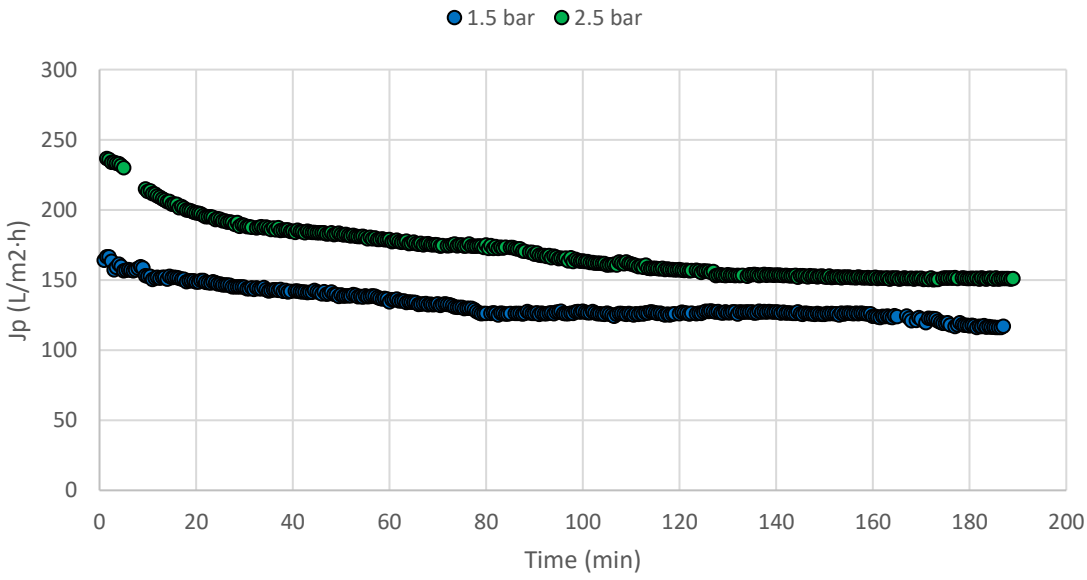

Figure 31. Permeate flux vs time for UH050 membrane at 2.5 m/s

Compared to the ultrafiltration tests in the ultrafiltration plant 2, the CFV of 2.5 m/s results show that an increase in TMP results to an increase in permeate flux. Moreover, as it was thought that the dirt present in the ultrafiltration plant 2 affected the results of the UP150, it could have equally contributed to the low permeate flux caused by fouling in the UH050 explaining why higher values were obtained in the ultrafiltration plant 1. This also affected the cleaning process in the ultrafiltration plant 2 as tougher conditions were required to recover the permeability of the membrane with a failure of the permeability after the ultrafiltration test of 1.5 m/s, 1.5 bar.

For this reason, it was believed that the permeate fluxes at this CFV could have been higher. Therefore, the permeate fluxes were normalized with the flux of water at their respective pressures and ultrafiltration plants as shown in Figure 32.

The tests were stopped at 2.5 m/s due to time constriction during this master´s thesis because it was predicted that the permeate flux will further increase with an increase in TMP before reaching the plateau, which could also be further investigated.

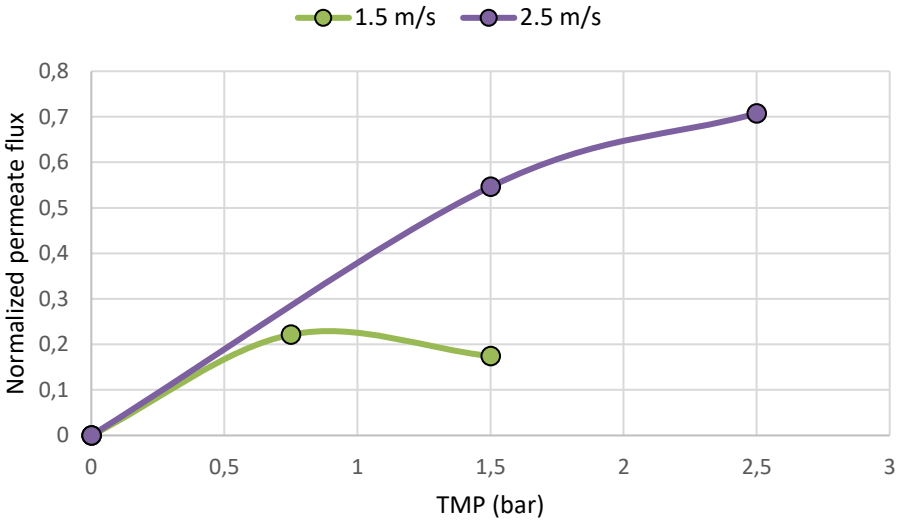

Figure 32. Steady state permeate flux vs TMP for UH050 membrane at two crossflow velocities

5.6 MEMBRANE REJECTION

This section shows the rejection results of the colour, total solid contents, COD and phenolic content of all the solid-liquid extractions and permeates obtained from the extraction test. To calculate this, equation 3.1 in section 3.6.5 was used as the results reported were an average of the values obtained from the characterization of the permeate after 1.5 and 3 hours.

5.6.1. UP005 membrane

From the results obtained for the UP005 (Figure 33), it was observed that the colour happens to be the most rejected parameter at all CFVs and TMPs with a rejection above 95%. Compared to the dark brownish colour of the feed extract, the permeate presented a light yellowish colour at all conditions.

Figure 33 also shows a high rejection of the total solids which was expected as the MWCO of the UP005 was low. The rejection was generally seen to increase with the increase in TMP in all CFVs due to the greater fouling of the membrane.

At 1.5 m/s, there was not much difference in the rejection of COD at various TMPs as it falls within 62 – 65% rejection. When the CFV was increased to 2.5 m/s and 3.5 m/s respectively, it was observed that an increase in the TMP resulted to an increase of COD rejection as it increased from 56 – 72% rejection for both CFVs except for 3.5 m/s, 5.5 bar where it fell to 64.6% rejection. Generally, there is no significant variation in the COD rejection as there is only a 16% variation between the lowest and highest rejections.

Finally, higher rejection values of phenolic content were seen at higher TMPs for each CFV (73 – 89% at 1.5 m/s, 77 – 81% at 2.5 m/s and 72 – 89% at 3.5 m/s) except for 3.5 bar and 4.5 bar at 3.5 m/s were both rejections were almost similar (79% and 78% respectively). The rise in fouling was responsible for the increase in the phenolic content rejection as this happens at high TMPs, where the highest rejections occurred at 1.5 m/s, 4.5 bar and 3.5 m/s, 5.5 bar with around 89% rejection. This also happened in another research where this behaviour was assigned to the fact that the concentration polarization and fouling effect was more severe at higher TMPs, creating additional layers on the membrane surface, thereby increasing the rejection coefficient (Giacobbo *et al.,* 2017). It was also observed that the change in CFV produced almost similar rejections at the same TMPs with the highest variation at 1.5 m/s, 4.5 bar and 3.5 m/s, 4.5 bar varying from 89% to 78%.

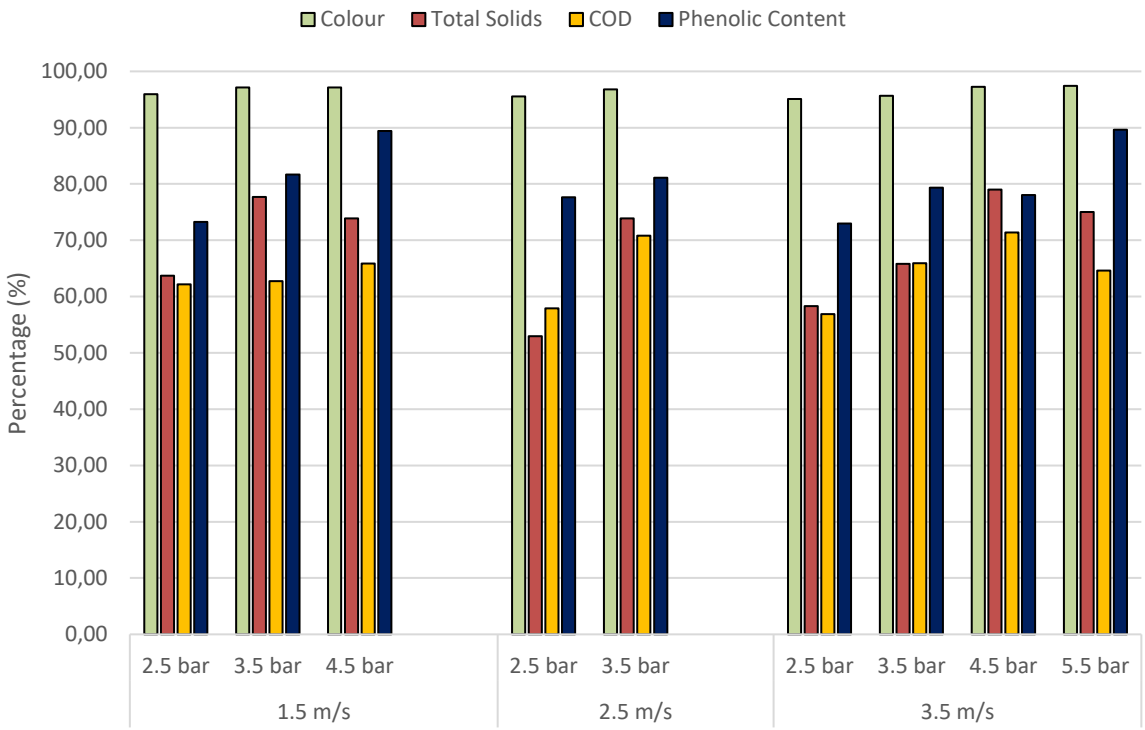

Figure 33. Rejection of colour, total solids, COD, and phenolic content for the UP005 membrane

5.6.2. UP150 membrane

Figure 34 shows the characterization of the only extraction test carried out with the UP150 at 1.5 m/s, 0.75 bar. A high rejection of the colour was obtained giving a permeate with a yellowish pigmentation. The rejection of the total solids and COD where not so high and a similar rejection coefficient was obtained for the phenolic content. The low TMP used and the high MWCO of the membrane could be responsible for the lower rejection in comparison with the UP005 membrane.

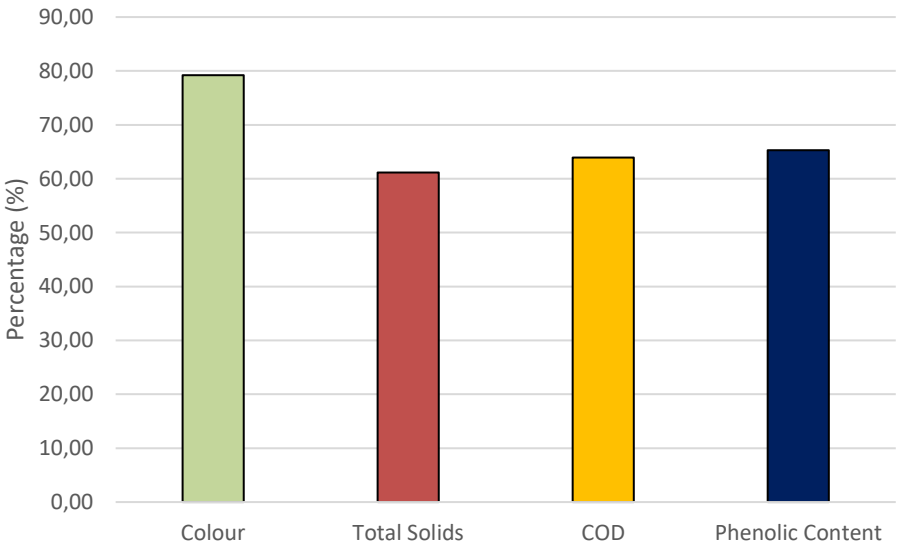

Figure 34. Rejection of colour, total solids, COD, and phenolic content for the UP150 membrane

5.6.3. UH050 membrane

The rejection results from the characterization of the ultrafiltration tests from the UH050 membrane are reported in Figure 35. Generally, in all the runs it was observed that the rejection coefficient of the colour, total solids, COD and phenolic content increased with a rise in CFV and TMP. The results from 2.5 m/s and 2.5 bar gave quite different results after an extraction of 1.5 hours and 3 hours and they were reported separately, with the latter having the highest rejection of all tests.

The colour of the permeates appeared light brownish with the permeate of the 2.5 m/s, 2.5 bar (3 hours) appearing with a yellowish colour as a result of the higher colour rejection. This permeate also had the highest rejection of COD, total solids, and phenolic content as a slight reduction in the permeate flux could be observed between the collection of the two permeates in Figure 29. At lower values of CFVs and TMPs, low levels of rejection were obtained for the COD, total solids, and phenolic content. The rejection increases with the increase in TMPs because at this point there is an accumulation of solutes on the membrane surface caused by concentration polarization and fouling which creates a cake layer that acts as an additional filtration layer. The increase is even higher with the rise in CFV. The rise in rejection of the COD

due to the increase of CFV could be related to the reduction in solute concentration on the surface of the membrane (Cifuentes-Cabezas *et al.,* 2021).

At 1.5 bar, similar results were obtained at 1.5 m/s and 2.5 m/s for the total solids, COD and phenolic content rejections with a difference of 3-4%, being again the rejection higher at the higher CFV.

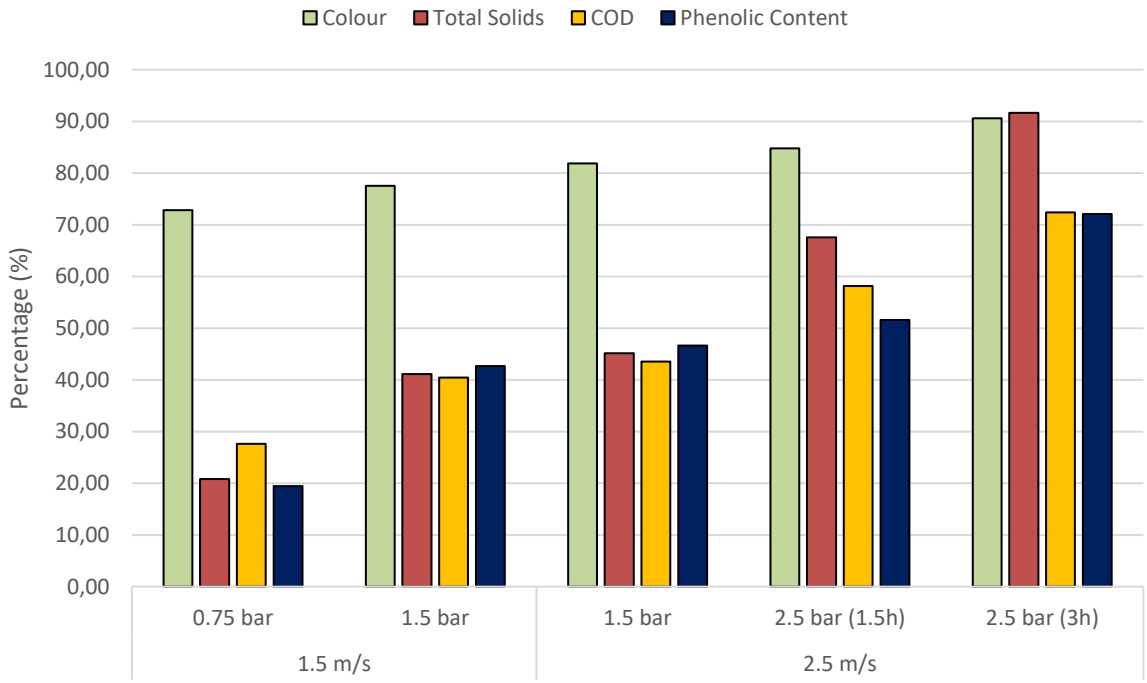

Figure 35. Rejection of colour, total solids, COD, and phenolic content for the UH050 membrane

5.6.4. Rejection of Phenolic Content

This section shows the phenolic content rejection of each family present in the feed as shown in section 5.2. It is important to know the rejection of each individual phenolic compound to understand the performance of the various membranes under different conditions for the recovery of the different families of phenolic compounds.

5.6.4.1. UP005 Membrane

Figure 36 shows the phenolic rejection of membrane UP005. In general, high rejections of the unknown compounds with a 55 – 68% rejection and flavonoid compounds with a 29 - 40% rejection were obtained. Rejection of secoiridoids ranged from 25 – 39% with low rejections of phenolic acids and aldehydes, and simple phenols at all TMPs and CFVs, being lower than 23%. At high TMPs of 4.5 bar and 5.5 bar and the highest crossflow velocity tested (3.5 m/s), a drastic reduction in rejection can be seen in all families which means that the high TMPs at 3.5 m/s favours the penetration of these compounds due to higher turbulence that decreases membrane fouling at this high cross flow velocity. The reduction was less notable for simple phenols and phenolic acids and aldehydes as their rejection was low at all TMPs. This was logical,

as these molecules show smaller size in relation to the other compounds present in the feed. These values were satisfactory as these families include interesting compounds like ferulic acid, caffeic acid, tyrosol or hydroxytyrosol. The most favourable condition is at 3.5 m/s, 5.5 bar only producing rejections of 5.6% of simple phenols and 1.7% of phenolic acids and aldehydes.

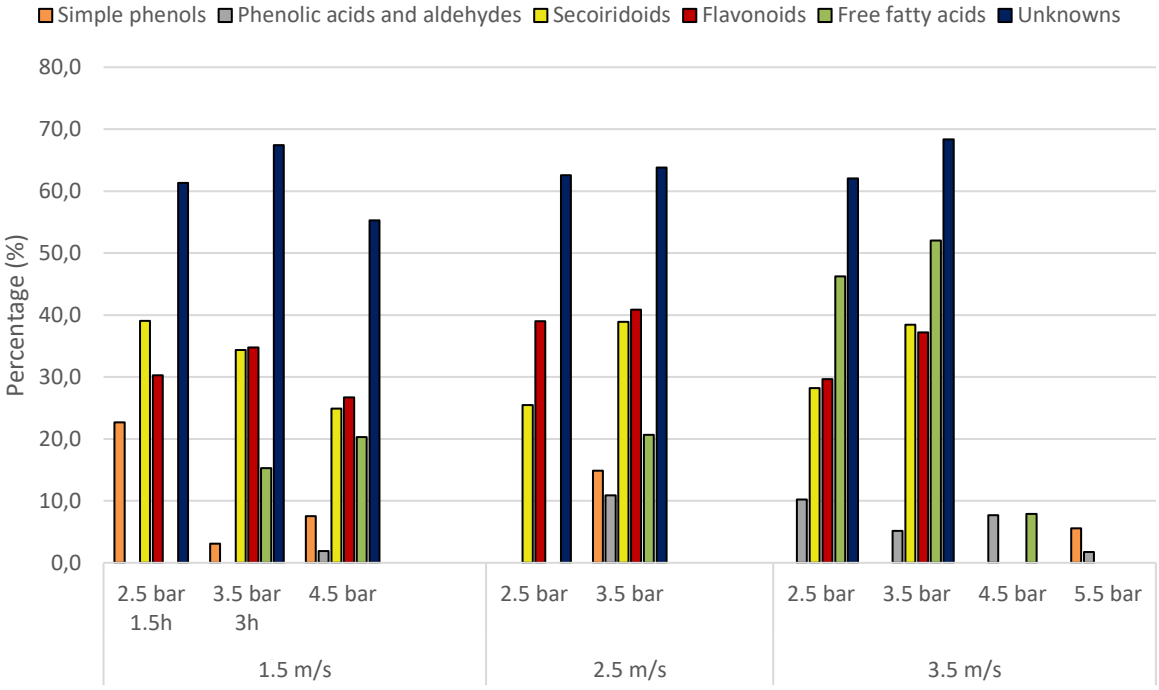

Figure 36. Rejection of phenolic compounds for the UP005 membrane

5.6.4.2. UP150 Membrane

The families of phenolic compounds rejected by the UP150 membrane appear in Figure 37. Free fatty acids was the family with the highest rejection (60%), followed by phenolic acids and aldehydes (37%). Flavonoids and secoiridoids had a lower rejection with 34% and 29% respectively, and simple phenols experienced the least rejection (9.5%).

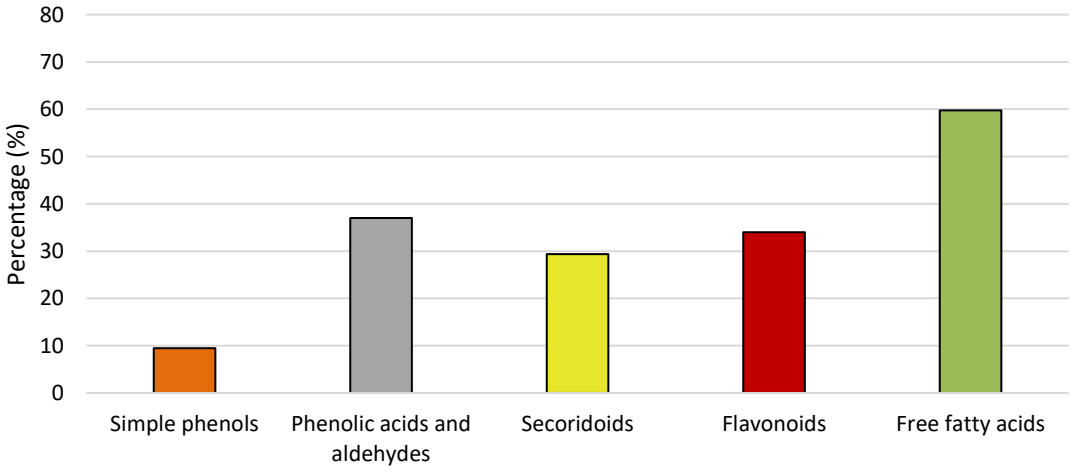

Figure 37. Rejection of phenolic compounds for the UP150 membrane at 1.5 m/s, 0.75 bar

5.6.4.3. UH050 Membrane

The ultrafiltration test performed at 2.5 m/s and 2.5 bar produced different results for the samples that were taken at 1.5 and 3 hours, which are shown in Figure 38. It was observed that at the beginning of the ultrafiltration test, the rejections were extremely low but increased with time, except for free fatty acids, whose rejection was high from the beginning (67 and 71% at 1.5 and 3 hours, respectively). This occurred as the fouling of the membrane increased with time, thereby, increasing the rejections.

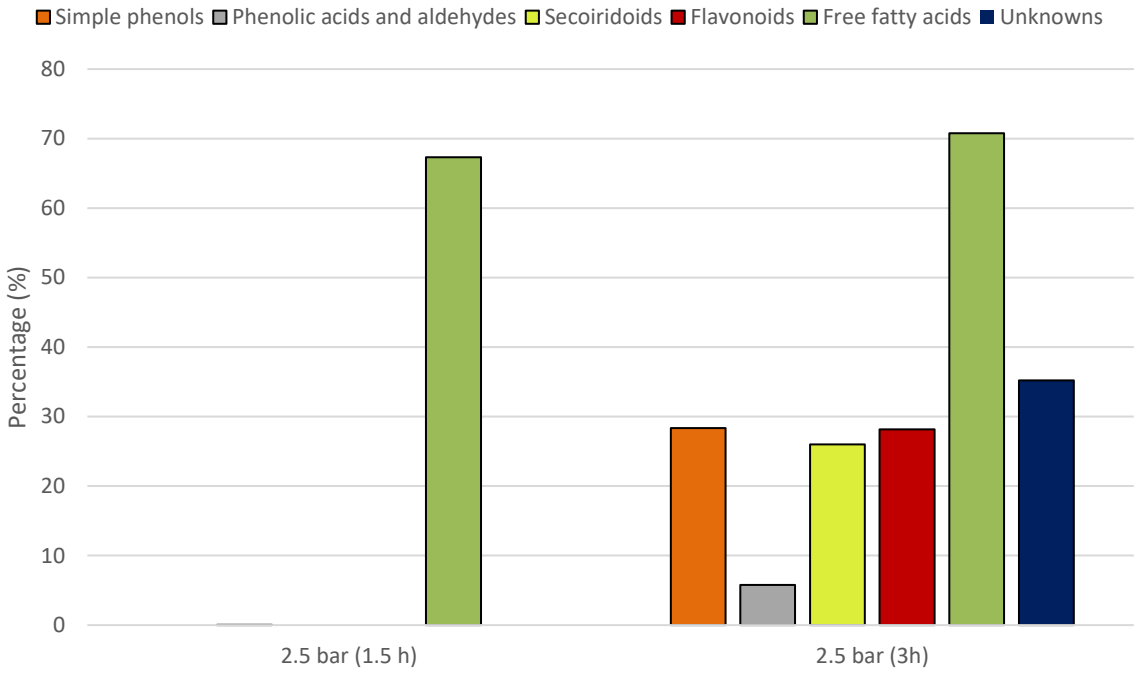

Figure 38. Rejection of phenolic compounds for the UH050 membrane at 2.5 m/s, 2.5 bar

In Figure 39, the difference in rejection at 1.5 bar and 2.5 bar after 3 hours were compared. In general, rejections increased with increase in TMP due to greater membrane fouling. However, the rejections were not very high, except for the free fatty acids having the highest increase with TMP, from 32 – 71%. The simple phenols followed with an increase from 0% rejection at 1.5 bar to 28% rejection at 2.5 bar, while other families had a slight increase in the rejection.

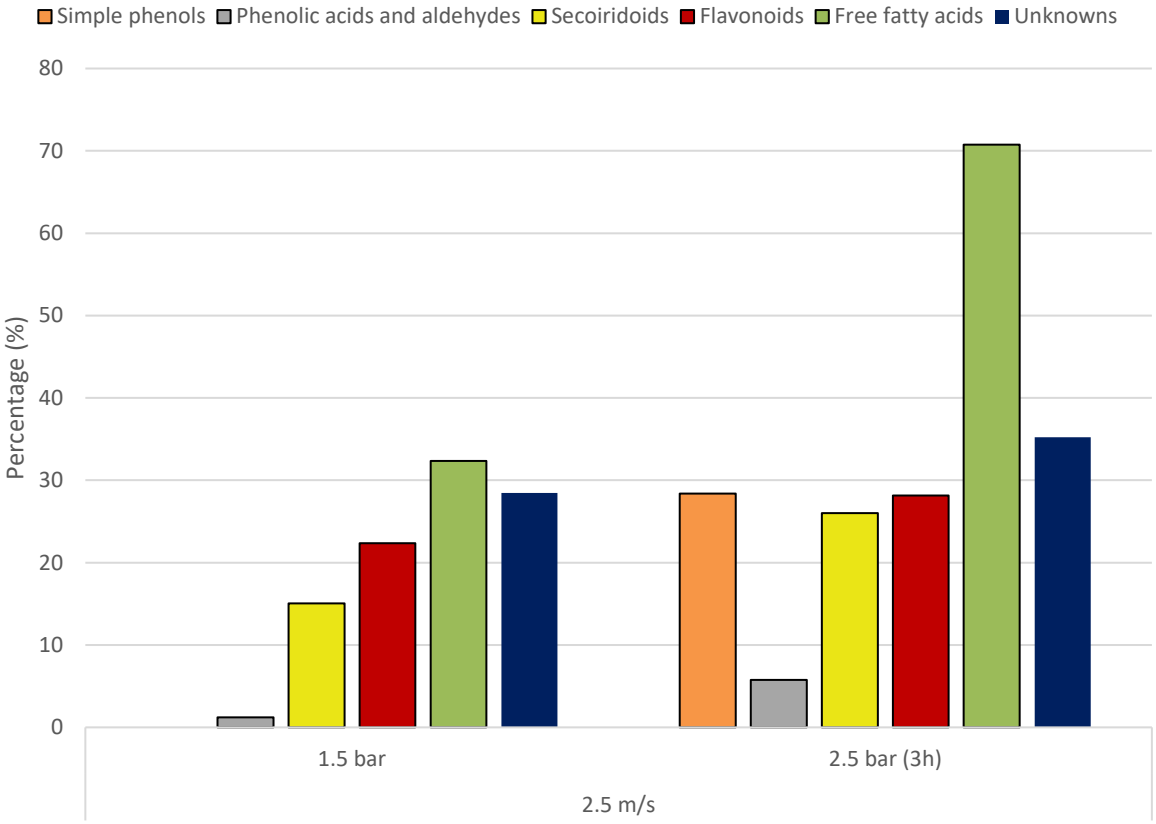

Figure 39. Rejection of phenolic compounds for the UH050 membrane

5.6.5 Comparison of membranes

The highest permeate flux was obtained with the UH050 membrane at the conditions of 2.5 m/s and 2.5 bar producing 150.96 L/m^2·h. Lower values were obtained from the UP005, as expected, due to its lower MWCO, having a range of 8.24 L/m^2·h at 1.5 m/s, 2.5 bar to 31.51 L/m^2·h at 3.5 m/s, 5.5 bar. The UP150 membrane produced a low permeate flux at 1.5 m/s, 0.75 bar (26.71 L/m^2·h) considering that this membrane has the biggest MWCO and initial permeability, what indicates that this membrane suffered severe fouling.

The rejection of colour in all membranes was seen to be high. It was seen that the UP005 had the biggest rejection due to the smaller pore size as all the ultrafiltration tests with this membrane had a colour rejection greater than 90%. Only the UH050 membrane when operated at 2.5 m/s, 2.5 bar after 3 hours was able to produce a colour rejection of 90% as other tests with the UH050 and UP150 only produced a colour rejection within 72 – 84%.

The UP005 also showed a higher rejection of the total solids compared to other membranes, as even when operated at lower CFVs and TMPs, the rejection was higher than when other membranes were used except for the condition of 2.5 m/s, 2.5 bar at 3 hours in the UH050 membrane which produced a slightly higher rejection.

When the membranes were operated at their lowest CFVs and TMPs during this master thesis, the UP005 gave the highest rejection of the COD, which was expected as it had the lowest MWCO amongst the membranes used, while the UH050 gave much lower values for COD

rejected. The biggest influence of CFV and TMP on the rejections for all membranes was in the UH050 when increased from 1.5 m/s, 0.75 bar to 2.5 m/s, 2.5 bar as the rejection went from 28 to 58 – 72% rejection depending on the time of the permeate taken.

For the phenolic compound rejection, UP005 membrane produced the highest rejection compared to the UP150 and UH050. In the UP005 and UH050, it was observed that increasing the TMP at all CFVs would result in an increase of the phenolic content rejection which was attributed to the higher concentration at the membrane surface and greater fouling at higher TMPs. High rejections were also seen in the UP150 membrane having a low permeate flux as discussed in section 5.5.2, which could be caused by the pores been blocked by compounds having similar size as the pores, which contributed to the low permeate flux and high rejections.

In terms of phenolic families rejected, the UP005 at 3.5 m/s, 4.5 and 5.5 bar produced the lowest rejections compared to the UP150 and UH050 membranes. In all membranes, low rejections of the simple phenols and phenolic acids and aldehydes were obtained, with rejections lower than 30%.

5.7 SELECTION OF THE BEST CONDITIONS FOR THE ULTRAFILTRATION PROCESS

After comparing the performance of the membranes, in this section, the best membrane and operating conditions are determined, which was one of the objectives of this master`s thesis. The best membrane was expected to have low phenolic compounds rejection, high COD and total solids rejection and high permeate flux.

Considering the permeate flux, the membrane with the highest permeate flux would be the most suitable, which directly affects the performance of the membrane in terms of production, in order to obtain a certain volume of product. The UP150 was discarded due to the low permeate flux (26.71 $L/m^2 \cdot h$) despite having the highest MWCO compared to other membranes. For the other membranes, the best conditions to obtain their respective highest permeate flux were 3.5 m/s, 5.5 bar for UP005 (31.51 $L/m^2 \cdot h$) and 2.5 m/s, 2.5 bar for UH050 (150.96 $L/m^2 \cdot h$) which would be more suitable due to highest flux obtained under milder conditions, that require less energy consumption.

The rejections at these two conditions for the membranes produced similar values. The UH050 produced better rejection results at 2.5 m/s, 2.5 bar compared to the UP005 at 3.5 m/s, 5.5 bar as it showed a higher rejection of total solids (91% with UH050 compared to 75% with UP005), a higher rejection of COD (72% with UH050 compared to 65% with UP005) and a lower rejection of phenolic content (72% with UH050 compared to 89% with UP005). Theoretically, low COD rejections and high phenolic content rejection is not suitable, as this means the permeate is similar to the feed extract, thereby, loosing lots of polyphenols. For this reason, from the rejections, the UH050 membrane at 2.5 m/s, 2.5 bar is a better option. Meanwhile, regarding the phenolic family rejection, from Figure 36 and Figure 39, it could be observed that lower rejections in all families were obtained from the UP005 at 3.5 m/s, 5.5 bar compared to the rejections from the UH050 at 2.5 m/s, 2.5 bar, which rejected 26% of secoiridoids, the most abundant phenols present in the extract.

In summary, the much lower rejection of phenolic content would have made the UP005 at 3.5 m/s, 5.5 bar to be the best choice, but considering the much lower permeate flux, lower rejection of COD and lower rejection of total solids compared to the UH050 at 2.5 m/s, 2.5 bar, the best condition for the ultrafiltration process was decided to be 2.5 m/s, 2.5 bar with the UH050 membrane.

Table 7. Permeate composition at 2.5 m/s, 2.5 bar with UH050 membrane

Colour	0.22
Total Solids (g/L)	0.42
COD (mg O_2/L)	3325
pH	5.14
Conductivity (µS/cm)	756
Phenolic Content	*(ppm)*
Simple Phenols	14.6
Phenolic Acids and Aldehydes	28.2
Secoiridoids	683.4
Flavonoids	25.1
Free Fatty Acids	4.5
Unknowns	44.8

5.8 PERMEABILITY RECOVERY

As mentioned in section 4.5.1, the protocol for cleaning the membranes was with the use of P3-Ultrasil 115 with 0.7% (v/v) for 1 hour at 35 °C, which was satisfactory to recover the permeability of the membranes by removing the fouling particles. In cases where the permeability could not be recovered under these conditions, the cleaning process was repeated by changing the parameters such as temperature or concentration.

5.8.1. Recovery of permeability in UP005 membrane

Figure 40 shows the permeabilities recovered after every extraction test taken with the UP005 membrane, where the red straight line represents the minimum permeability that must be reached to have a satisfactory recovery. As shown in Figure 20, the initial permeability of the UP005 membrane is 10.88 L/m^2·h, and as mentioned in Section 4.5.1, a permeability above 90

% of the initial permeability (9.79 L/m^2·h) will be considered satisfactory after each extraction test.

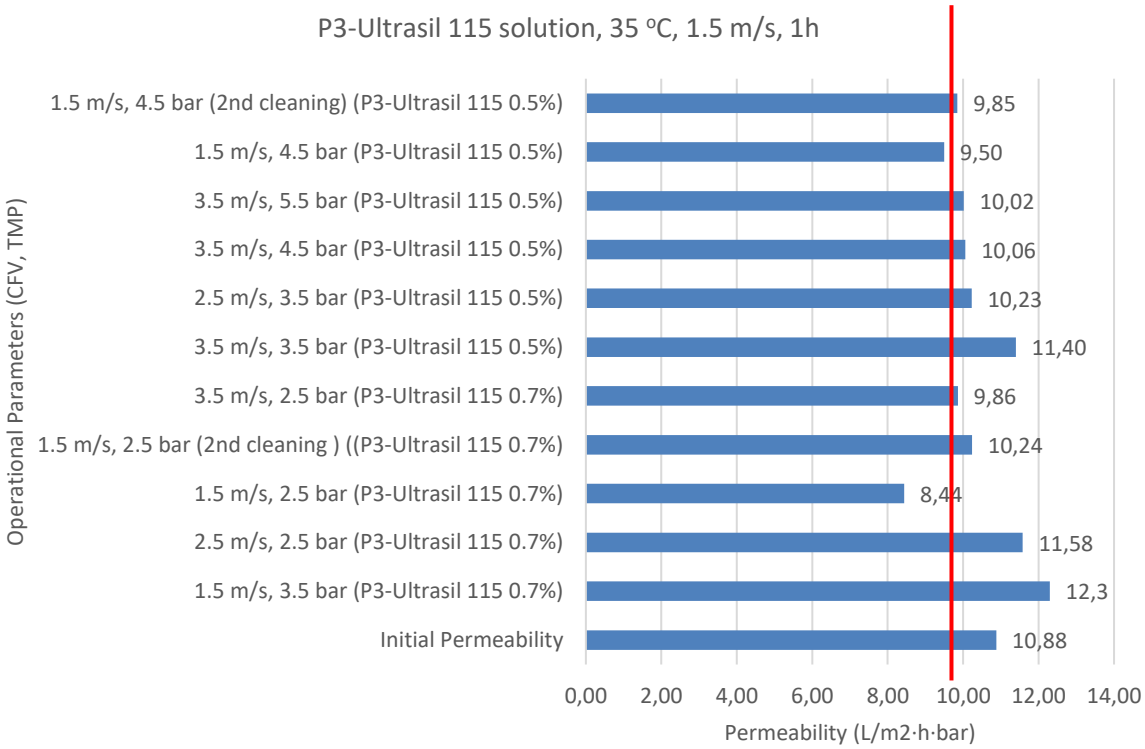

Figure 40. Permeabilities recovered after each ultrafiltration test for UP005 membrane

A concentration of 0.7% of P3-Ultrasil 115 solution at 35ºC was used for the first five extraction tests (from test 1.5 m/s, 3.5 bar to 3.5 m/s, 2.5 bar in Figure 40). This concentration was suitable to recover the permeability except for the extraction test at 1.5 m/s, 2.5 bar which had to be repeated at 40ºC to achieve the required permeability recovered. By the time the permeability recovered for the extraction test at 3.5 m/s, 2.5 bar, the P3-Ultrasil 115 solution had been used for a fifth time and had a darker colour compared to the initial state. This could have contributed to the lower permeability recovered of this cleaning process compared to the previous and it was decided to discard this P3-Ultrasil 115 solution and a new one prepared for further cleanings.

At this stage, a lower concentration of P3-Ultrasil 115 of 0.5 % was prepared considering that it would be less harmful to the membrane and the environment. The decrease in concentration did not affect the permeability recovery as high values were obtained from extraction tests at 3.5 m/s, 3.5 bar to 3.5 m/s, 5.5 bar in Figure 40.

During the permeability recovery of the extraction tests of 1.5 m/s and 4.5 bar, the cleaning protocol failed to recover the permeability. The solution of 0.5% concentration of P3-Ultrasil 115 which was already on its fifth cycle and with a darker colour produced a permeability lower than the 90% allowed. The cleaning was repeated with a new solution concentration of 0.5% while increasing the temperature to 40ºC to aid dissolution and recovered a permeability slightly higher than the minimum allowed.

It was noticed that when the extraction tests were of lower cross velocity and high TMP, a previously used P3-Ultrasil 115 at 0.5 and 0.7% concentrations did not properly clean the membrane and produced low permeabilities, hence, the cleaning had to be repeated at a higher temperatures (40°C) to recover the permeability. This is because, at low cross velocity and high TMP, there is lower turbulence and higher convective transport of solute molecules towards the membrane surface, which encourages the formation of a cake layer which will require a tougher cleaning solution to recover the permeability.

5.8.2. Recovery of permeability in UP150 membrane

As mentioned in section 4.5.1, the recovery of the permeability of the UP150 membrane was challenging. The membrane was first cleaned with P3-Ultrasil 115 at 0.5% concentration at 35°C after the ultrafiltration test, which failed to recover the permeability. The cleaning process was repeated with a concentration of 0.7%, but equally failed despite increasing the temperature to 40°C. A new cleaning solution was prepared with P3-Ultrasil 110 at 0.7% concentration and the membrane was cleaned at 45°C, but this was also unable to recover the permeability despite having a higher permeability compared to the other cleaning agents.

It was believed that the dirt present in the ultrafiltration plant 2 affected the cleaning process, so, a solution of sodium hypochlorite with 200ppm concentration was used to clean the membrane at 40°C which, finally, was able to recover the permeability. Figure 41 shows the results from the permeability recovered from the cleaning agents used.

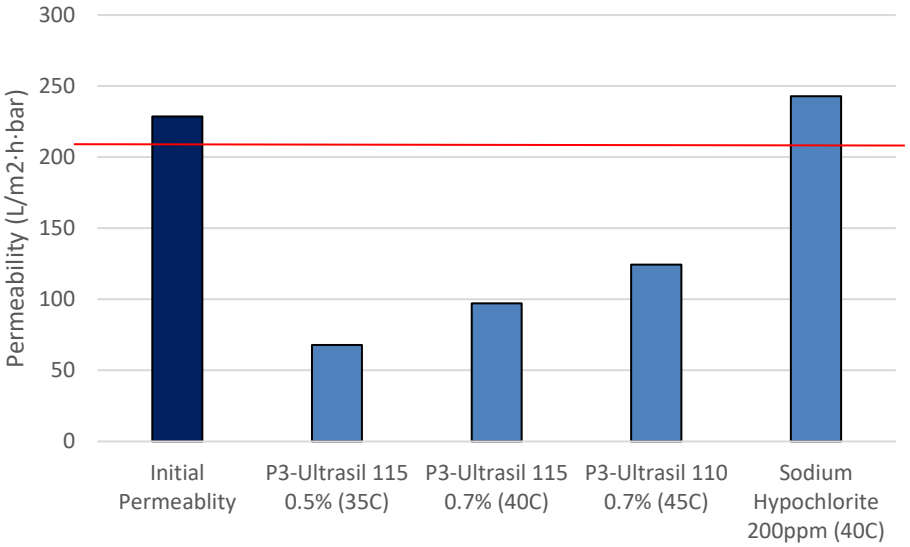

Figure 41. Permeabilities recovered after cleaning for UP150 membrane

5.8.3. Recovery of permeability in UH050 membrane

The recovery of the permeability of UH050 membrane also faced some difficulties in the ultrafiltration plant 2. Two sets of experiments were carried out in this plant setup and multiple cleanings with different conditions had to be employed to recover the permeability, which appear in Figure 42 and Figure 43. After the first ultrafiltration test of 1.5 m/s, 0.75 bar, a P3-

Ultrasil 115 solution with 0.5% concentration at 35ºC was used. This produced a very low recovery, therefore, a concentration of 0.7% was used at 45ºC, which produced a slightly higher recovery, but still far from the minimum recovery required (90% of the initial permeability). It was decided to increase the concentration of the P3-Ultrasil 115 to 1% at 45ºC. This solution was able to recover the permeability, which allowed for the second ultrafiltration test to be carried out.

The cleaning of the membrane after the ultrafiltration test of 1.5 m/s, 1.5 bar also produced some challenges. Based on the previous cleaning conditions, a solution of P3-Ultrasil 115 of 0.7% at 45ºC was used with the intention of protecting the membrane from the exposure of high alkaline solutions. This cleaning process failed to recover the permeability, so it was decided to increase the concentration and cross flow velocity to 1% and 1.5 m/s respectively at 45ºC. A slightly higher recovery was obtained, but still lower than 90% of the initial permeability. Finally, another cycle of cleaning was done with the same solution but this time, increasing the cross velocity to 2.5 m/s which produced a lower permeability. The cleaning processes were stopped at this stage because the plant could not be operated at the conditions for the subsequent ultrafiltration tests due to leakage in the ultrafiltration plant.

The cleaning of the membrane UH050 in the ultrafiltration plant 1 was done with the P3-Ultrasil 115 with a concentration of 0.7% for 1 hour and 35ºC, producing a very high permeability recovery after the extraction tests at 2.5 m/s and 1.5 bar and 2.5 bar were carried out.

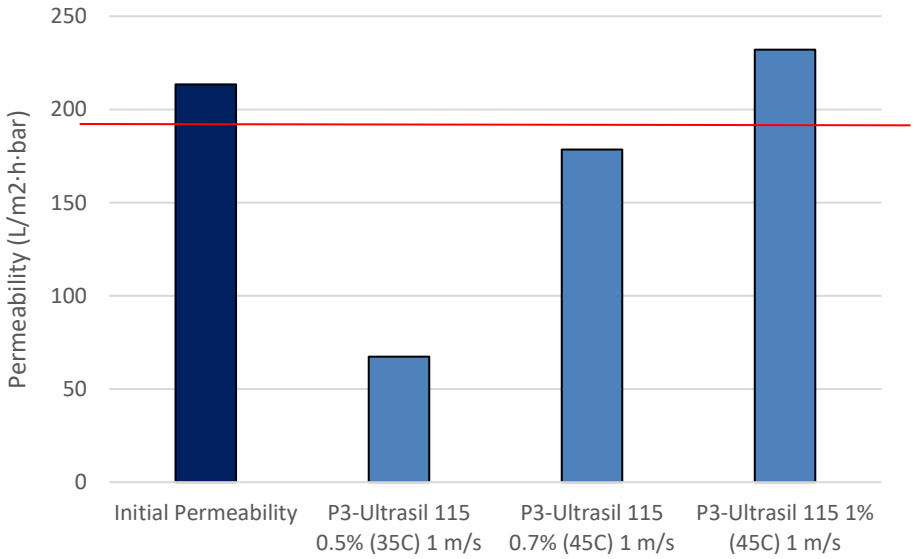

Figure 42. Permeabilities recovered during cleaning for ultrafiltration at 1.5 m/s, 0.75 bar for UH050

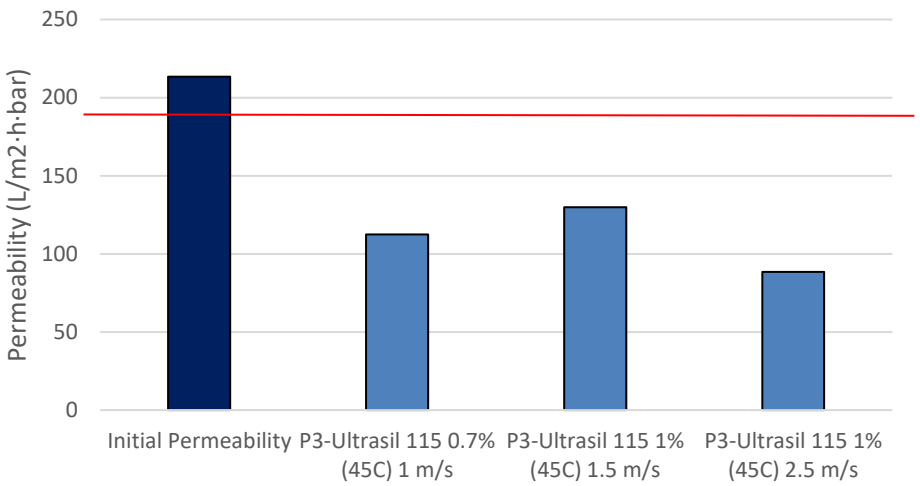

Figure 43. Permeabilities recovered during cleaning for ultrafiltration at 1.5 m/s, 1.5 bar for UH050

CHAPTER 6. CONCLUSIONS

From the results obtained from the solid-liquid extraction and ultrafiltration tests, the following conclusions can be deducted:

- High recoveries of phenolic content and COD can be obtained from one solid-liquid extraction cycle. From the first cycle, 89% of phenolic content, 77% of COD, 65% of total solids and 54% of the colour were recovered, which makes one cycle suitable since the goal was to recover the phenolic content. This shows that a second cycle was not required.

- From the ultrafiltration tests, it was observed that the UH050 produced the highest permeate flux at 2.5 m/s, 2.5 bar (150.96 $L/m^2 \cdot h$), while the UP005 produced 31.51 $L/m^2 \cdot h$ at the highest CFV and TMP (3.5 m/s, 5.5 bar) and the UP150 produced a low permeate flux (26.71 $L/m^2 \cdot h$) in relation to its MWCO at 1.5 m/s, 0.75 bar.

- The colour is the most rejected parameter in all ultrafiltration tests. The UP005 produced the highest rejection with a variation of 95 – 97%, the UH050 rejection varied from 72 – 90% and the ultrafiltration tests with the UP150 rejected 79%.

- The total solids rejection was more affected by the TMP than by the CFV and it was seen to increase with the increase in TMP at all CFVs. The UP005 rejection varied from 53 – 79%, the UH050 from 21 – 91% and the UP150 around 61%.

- In the case of the COD, the UH050 experienced the highest influence of CFV and TMP when increased from 1.5 m/s, 0.75 bar to 2.5 m/s, 2.5 bar as the rejection varied from 28% to 58-72% rejection depending on the time the permeate was collected. The UP005 gave the highest rejection at the highest CFV and TMP and it varied from 57 – 72% while the UP150 produced a 64% rejection.

- The UP005 produced the highest phenolic content rejection of all membranes (73 - 90%) as the UH050 varied from 19 – 72%, while the UP150 produced 65.29% rejection. It was observed that increasing the TMP at all CFVs resulted in an increase of phenolic content rejection with the UP005 and UH050 due to the fouling factor.

- Four families of phenolic compounds (simple phenols, phenolic acids and aldehydes, flavonoids and secoiridoids), free fatty acids and an unknown compounds were identified with the LC-MS, being secoiridoids the most abundant family accounting for 92%. Generally, low amounts of phenolic acids and aldehydes, and simple phenols were rejected in all ultrafiltration tests, with the lowest rejections obtained with the UP005

at 3.5 m/s, 4.5 and 5.5 bar. The most rejected family with the UP005 membrane is the unknown compounds except at 3.5 m/s, 4.5 and 5.5 bar, while the most rejected family with the UP150 and UH050 was the free fatty acids.

- The cleaning protocol of 0.7% (v/v) P3-Ultrasil 115 at 35 ^{o}C, for 1 hour and 1.5 m/s was effective for the UH050 membrane at 2.5 m/s, 1.5 bar and 2.5 bar. This condition failed at 1.5 m/s, 0.75 bar and 1.5 bar, as the permeability could only be recovered with a solution of 1% (v/v) P3-Ultrasil 115 and 45^{o}C for the condition of 1.5 m/s, 0.75 bar, while all the cleaning protocols employed failed at 1.5 m/s, 1.5 bar. Reducing the concentration of the cleaning agent from 0.7% to 0.5% (v/v) showed to be effective for the UP005 membrane at high CFVs for five successive cycles of cleaning reusing the same solution. However, at low CFV and high TMP the cleaning had to be done at a higher temperature of 40^{o}C. The permeability of the UP150 could not be recovered under any condition with P3-Ultrasil 115 or P3-Ultrasil 110, but a solution of sodium hypochlorite (200 ppm) recovered the permeability, what is indicative of the severe fouling suffered by this membrane.

- Considering the highest permeate flux, the high rejection of total solids and COD, and the low rejection of total phenolic compounds and phenolic families, the best results were obtained with the UH050 membrane at 2.5 m/s, 2.5 bar.

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DOCUMENT 2. BUDGET

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CHAPTER 1. NEED FOR THE BUDGET

This document shows the information related to this master´s thesis. The experimental procedures and all materials and equipment were taken into consideration. The ¨Recommendations for the preparation of budgets for R&D&I activities (Revision 2018) at UPV¨ was taken into consideration for the calculations. The budget is divided into four sections:

- Labour: The experimental work was carried out by an Erasmus student from the University of Bologna at the Universitat Politècnica de València with the purpose of making a master´s thesis of 18 ECTS for which an internship agreement was signed.

- Fungible Materials: This budget section shows the budget resulting from the materials used in all experimental procedures which cannot be reused and are therefore not depreciable.

- Depreciable Material: The experimental procedures were carried out in the laboratory of the Chemical and Nuclear Engineering Department of the Universitat Politècnica de València, where many of the equipment and materials used, therefore, are also used for other projects.

  In order to obtain the amount of depreciable materials and equipment, straight-line depreciation has been applied, according to Equation 1:

$$Amount = \frac{C \cdot t_{used}}{T_{depreciation}} \tag{1.1}$$

Where *C* is the cost of the equipment, t_{used} is the used time used during the experimental procedures, and $T_{depreciation}$ is the period of depreciation.

- General Budget: The following factors were taken into consideration to obtain the total budget:
  - 2% of direct ancillary costs are considered
  - 25% indirect costs.
  - 13% general cost
  - Industrial profits are not considered
  - The value added tax (V.A.T.) is the same for all materials and equipment used in the project, with a value of 21%.

From this information, the material execution budget is obtained as the sum of the total amounts for labour, fungible material, and depreciable material, taking into account indirect costs. Next, the contract execution budget is calculated, considering overheads. Finally, the V.A.T. percentage is applied, and the base tender budget or total budget of the project is obtained.

CHAPTER 2. BUDGET CONTENT

2.1. LABOUR

Table 1 shows the breakdown of prices for contracted labour.

Table 1. Breakdown of prices for contracted labour

	Quantity (h)	Unit Price (€/Unit)	Amount (€)
Internship	360	4.10	**1,476.00**

The total amount for the labour is **€1,476**.

2.2. FUNGIBLE MATERIAL

Table 2 shows the breakdown of prices for fungible materials used in the project.

Table 2. Breakdown of prices for fungible materials used

Concept	Unit	Quantity	Unit Price (€/Unit)	Amount (€)
Olive Pomace	kg	6	0.00	0.00
Membrane UP005	m^2	0.013	1924.00	25.01
Membrane UP150	m^2	0.026	1924.00	50.02
Membrane UH050	m^2	0.026	1924.00	50.02
Filter paper	units	15	0.19	2.85
Filters (0.45 µm)	Units	50	0.21	10.50
Syringes (5 mL)	Units	10	0.14	1.40
Distilled water	L	1500	0.00	0.00
Pasteur pipette	Units	50	0.12	6.00
Sodium hypochlorite	ml	21.6	0.01	0.24
Na_2CO_3	g	40	0.20	8.00

Concept	Unit	Quantity	Unit Price (€/Unit)	Amount (€)
Folin-Ciocalteu reagent	L	0.1	232.00	23.20
Micropipette tips (1000 µL)	Units	100	0.08	8.00
Micropipette tips (1000 mL)	Units	60	0.12	7.20
COD tubes UN3316	Units	60	3.39	203.4
Spectrophotometer cuvettes (10 mm)	Units	50	0.06	3.00
Nitrile safety gloves	Units	20	0.30	6.00
Vial with cap (1.5 mL)	Units	50	1.09	54.5
Falcon tubes (50 mL)	Units	50	0.34	17.00
Eppendorf tubes (1.5 mL)	Units	20	0.04	0.80
P3 – Ultrasil 110	g	50	0.01	0.50
P3 – Ultrasil 115	g	300	0.01	1.59
Total Fungible Materials Amount				**479.23**

The total amount for the fungible materials is **€479.23**.

2.3. DEPRECIABLE MATERIAL

Table 3 shows the decomposed price table for the depreciable material used in the work.

Table 3. Breakdown of depreciable material

Concept	Cost (€)	Depreciation (years)	Time (d)	Amount (€)
Ultrafiltration plant 1	9,900.00	12	90	203.42
Ultrafiltration plant 2	9,900.00	12	30	67.80
Ultrasound equipment Elmasonic P 70 H	275.00	12	15	0.94
Centrifuge Sigma 6-16KS	10,094.38	12	15	34.57
Refrigerator	373.28	12	120	10.22
Freezer	319.95	12	120	8.76

Concept	Cost (€)	Depreciation (years)	Time (d)	Amount (€)
Plastic funnel	0.83	12	15	0.00
Plastic bucket	2.79	12	15	0.01
Sieve	7.79	12	15	0.40
Plastic dosing shovel	1.74	12	15	0.01
Metal spring clamp	2.23	12	120	0.06
Nylon spring clamp	2.76	12	120	0.08
Micropipette (1000µL)	55.00	12	15	0.19
Micropipette (5mL)	67.76	12	15	0.23
Conductivity meter GLP 31+	114.95	12	15	0.39
pH meter GPL 21+	708.00	12	15	2.42
Spectrophotometer Hach DR 600	9,990.00	12	15	34.21
LC-ESI-qToF-MS equipment	200,600.00	12	5	228.99
Electrical resistance	23.50	12	120	0.64
Flow meter	373,73	12	120	10.24
Manometer	15.05	12	120	0.41
Electric stirrer (2 pcs)	1,768.00	12	15	6.05
Rubber hoses	11.14	12	120	0.31
Oven	2,048.73	12	15	7.02
Plastic jug (2L)	3.51	12	120	0.10
Plastic jug (5L)	5.69	12	120	0.16
Kern precision balance (d=0.1mg) (2 pcs)	1,060.40	12	120	29.05
Kern precision balance (d=0.01g)	798.00	12	20	3.64

Concept	Cost (€)	Depreciation (years)	Time (d)	Amount (€)
Rayflow membrane module	1,562.00	12	120	42.79
Vacuum pump LABOPORT	860.58	12	15	2.95
Computer (2 units)	1,100.00	6	120	30.14
Connecting cables	16.12	6	120	0.44
Stop watch (5 units)	6.89	12	15	0.02
Glass beaker (1L) (2 pcs)	1.20	12	120	0.03
Plastic beaker (1L)	1.05	12	120	0.04
Glass beaker (50mL) (2 pcs)	7.25	12	120	0.19
Glass beaker (600mL) (2 pcs)	8.41	12	120	0.23
Precision stainless steel tweezers	7.33	12	15	0.03
Vacuum filtration equipment	233.83	12	15	0.80
Amber jars (30mL) (50pcs)	37.50	12	15	0.13
Thermoreactor TR300	940.80	12	15	3.22
Spectroquant Photometer Nova 30	4,346.30	12	15	14.87
Metal spatula	3.00	12	15	0.01
Test tube (1L)	14.99	12	15	0.05
Test tube (50 mL)	1.82	12	15	0.01
Water bath	515.00	12	15	1.76
Total Depreciable Materials Amount				**748.03**

The total amount of depreciable equipment used is **€748.03**.

2.4. GENERAL BUDGET

The general budget is presented in three parts. The first, Table 4, shows the breakdown to obtain the material execution budget.

Table 4. Material execution budget

Concept	**Amount (€)**
Labour Amount	1,476.00
Fungible Material Amount	479.23
Depreciable Materials Amount	748.03
Direct Ancillary Costs (2%)	54.07
Indirect Costs (25%)	689.33
Material Execution Budget	**3,446.66**

The total material execution budget is **€3,446.66**.

Table 5 shows the breakdown for obtaining the contract execution budget.

Table 5. Contract execution budget

Concept	**Amount (€)**
Material Execution Budget	3,446.66
General Expenses (13%)	447.68
Industrial Profit (0%)	0.00
Contract Execution Budget	**3,894.34**

The total contract execution budget is **€3,894.34**.

Finally, Table 6 shows the price table of the basic tender budget.

Table 6. Basic Tender budget

Concept	**Amount (€)**
Contract Execution Budget	3,894.34
General Expenses (13%)	506.26
Contract Execution Budget	**4,400.60**

The total budget invested in the master´s thesis is **€4,400.60.**