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Oleuropein rich extract from olive leaves by combining microfiltration, ultrafiltration and nanofiltration

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ABSTRACT

Membrane separation technology, for bioactive compounds separation, gained great attention lately. Our main goal in this work was to produce an oleuropein concentrate. The water extract of olive leaves has been subjected to a screening on the basis of molecular size. First microfiltration process (0.2 μm) allowed large particles removal, a following step of ultrafiltration permitted the removal of molecules larger than 5 kDa, finally a nanofiltration process (300 Da) allowed the concentration of polyphenols mainly oleuropein. Permeate fluxes of ultrafiltration and nanofiltration were investigated and analyzed.

Results revealed that a large portion of phenolic compounds were recovered in the permeate fraction of the UF process. The nanofiltration retentate showed high polyphenol and flavonoid contents. Based on the content of solute in feed and retentate fractions of NF membrane, oleuropein was concentrated approximately 10 times to reach 1685 mg/100 g extract. In addition, this fraction demonstrated high antioxidant capacities monitored by total antioxidant capacity and ferric-reducing ability power. High antibacterial activity was observed against *S. enterica* and *K. pneumonieae* (25 and 28 mm, respectively).

1. Introduction

In recent years, there has been a growing interest in obtaining biologically active compounds from natural sources. The protective effects of diets rich in fruits and vegetables against cardiovascular diseases and certain cancers have been attributed partly to antioxidants contained therein. Olive tree is one of the potential natural antioxidant sources because of its phenolic contents in fruits [1], oil [2] and leaves [3,4].

Olive leaves are regarded as a cheap raw material which can be used as a good source of high-added value bioactive compounds [5].

Several reports have demonstrated that olive leaf extract has the capacity to lower blood pressure in animals and increase blood flow in the coronary arteries, to relieve arrhythmia and prevent intestinal muscle spasms [5–9].

Oleuropein, the major constituent of the secoiridoid family in olive leaves, has been shown to be a potential antioxidant endowed with anti-inflammatory and antithrombotic properties [3,10,11]. Oleuropein is also suggested to support hypotensive and radical scavenging activities and its hydrolysis leads to antimicrobial compounds [3]. Consequently, there is a growing interest in extracting and separating oleuropein from olive leaves because natural active compounds are safer for human health than synthetic chemicals.

In this context, several techniques have been adopted for recovering olive polyphenols. These techniques involve mainly extraction, centrifugation, precipitation and chromatographic procedures. However, in these processes, complexities result from both their cost and operational characteristics and from the toxicity and flammability of organic solvent required in large amounts [12,13].

Recently, there has been an increasing interest in the application of membrane technologies for separation, purification and concentration of bioactive compounds from aqueous solutions. Membrane processes have been investigated for high quality concentration of phenolic compounds due to their low operating temperature and minimal energy consumption [14,15]. This procedure is based on the principle of selective permeation of the solute molecules through either polymeric or inorganic semi-permeable membranes.

Nanofiltration has been successfully employed for concentrating phenolic compounds extracted from natural products [16]. Indeed, Dammak et al. [17] used nanofiltration to concentrate aqueous oleuropein solution. However, there is no information

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available on the concentration of oleuropein from olive leaves using three different membrane filtration types; micro-, ultra- and nano-filtrations.

The aim of this study was to produce a concentrated fraction of oleuropein from extract of olive leaves. The extract was fractionated using a sequence of different membrane operations: the microfiltration (MF) followed by the ultrafiltration (UF). The ultrafiltration permeate was then subjected to a nanofiltration (NF). Different fractions were evaluated in terms of total phenolics content, flavonoids content, antioxidant capacity and HPLC profiles. Antioxidant and antibacterial activities of the nanofiltration retentate were investigated.

2. Materials and methods

2.1. Raw material

Olive leaves (*O. europaea*, Chemlali variety) were collected from Sfax (Tunisia), dried in a tunnel microwave dryer (Adasen, JN-100, China) (1200 W, 70 °C) for 10 min, then milled and stored in darkness at 4 °C until extraction.

2.2. Equipment and processes

The feed solution was prepared from olive leaf powder dispersion in water at a rate of 2.5% and stirring for 1 h at room temperature (30 °C). The obtained mixture was decanted for 4 h and the supernatant was clarified through linen cotton in order to remove large particles.

The fractionation process of olive leaf extract performed through the combination of three membrane operations was presented in Fig. 1. The feed stream was pre-treated in a microfiltration system through 0.2 μm pores size membrane (Microporous membrane TOPER Model, diameter 300 mm, CN). The microfiltration permeate was submitted to a cross-flow ultrafiltration system of molecular weight cut-off (MWCO) 5 kDa (GE Power & Water, ZeeWeed 1500 Minnetonka, MN). The UF membrane can retain colloidal substances, protein and macromolecular pigments in the feed liquid and it permitted to recuperate the larger polyphenols contained in the extract. Finally, the ultrafiltration permeate feed a cross-flow nanofiltration System of MWCO 300 Da (GE Osmonics, HL2540TF, Minnetonka, MN). The cross-flow nanofiltration system retentate delivered a concentrated extract rich in oleuropein. Manometers before and after the MF,UF and NF membranes were used to measure the inlet and the outlet pressure so as to control the trans-membrane pressure (ΔP) which was equal to 1 bar for MF and UF. However, the NF was operated at a trans-membrane pressure of 9 bar. Fig. 2 shows a photo of permeate and retentate samples of the olive leaves extract of each filtration process.

The permeate flux J (L/h m^2) was measured during UF and NF processes and calculated according to the following equation:

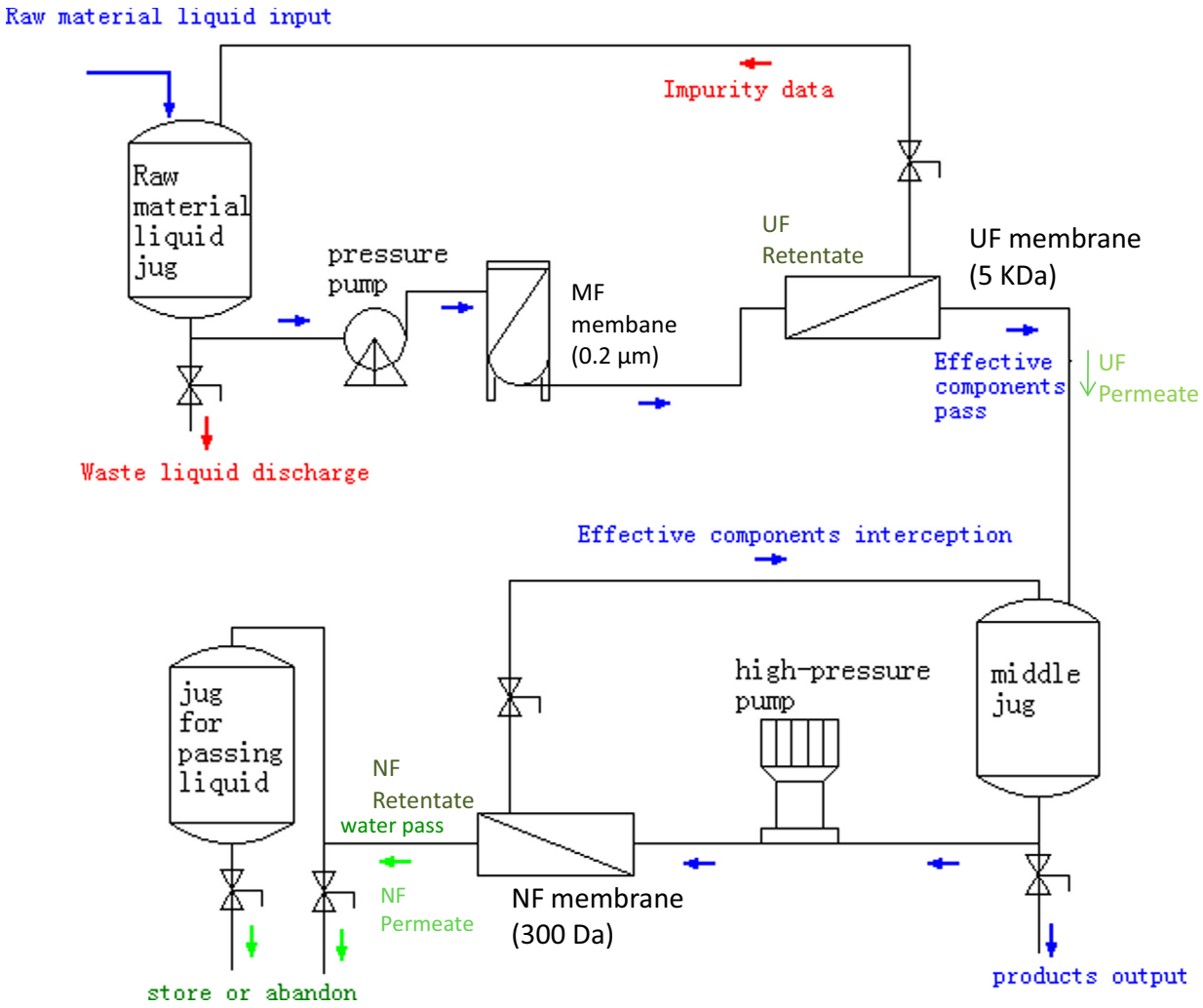

Fig. 1. Experimental set up used to perform filtration process.