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An integrated process for the recovery of high added-value compounds from olive oil using solid support free liquid-liquid extraction and chromatography techniques☆

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ABSTRACT

An integrated extraction and purification process for the direct recovery of high added value compounds from extra virgin olive oil (EVOO) is proposed by using solid support free liquid-liquid extraction and chromatography techniques. Two different extraction methods were developed on a laboratory-scale Centrifugal Partition Extractor (CPE): a sequential strategy consisting of several "extraction-recovery" cycles and a continuous strategy based on stationary phase co-current elution. In both cases, EVOO was used as mobile phase diluted in food grade *n*-hexane (feed mobile phase) and the required biphasic system was obtained by adding ethanol and water as polar solvents. For the sequential process, 17.5 L of feed EVOO containing organic phase (i.e. 7 L of EVOO treated) were extracted yielding 9.5 g of total phenolic fraction corresponding to a productivity of 5.8 g/h/L of CPE column. Regarding the second approach, the co-current process, 2 L of the feed oil phase (containing to 0.8 L of EVOO) were treated at 100 mL/min yielding 1.03 g of total phenolic fraction corresponding to a productivity of 8.9 g/h/L of CPE column. The total phenolic fraction was then fractionated by using stepwise gradient elution Centrifugal Partition Chromatography (CPC). The biphasic solvent systems were composed of *n*-hexane, ethyl acetate, ethanol and water in different proportions (X/Y/2/3, v/v). In a single run of 4 h on a column with a capacity of 1L, 910 mg of oleocanthal, 882 mg of oleacein, 104 mg of hydroxytyrosol were successfully recovered from 5 g of phenolic extract with purities of 85%, 92% and 90%, respectively. CPC fractions were then submitted to orthogonal chromatographic steps (adsorption on silica gel or size exclusion chromatography) leading to the isolation of additional eleven compounds belonging to triterpens, phenolic compounds and secoiridoids. Among them, elenolic acid ethylester was found to be new compound. Thin Layer Chromatography (TLC), Nuclear magnetic Resonance (NMR) and High Performance Liquid Chromatography – Diode Array Detector (HPLC-DAD) were used for monitoring and evaluation purposes throughout the entire procedure.

1. Introduction

Mediterranean diet is an internationally recognized dietary pattern having olive oil as a key component providing the main source of fat [1]. In recent years, several studies revealed that this ancient oil is more than just a source of mono-unsaturated fat (95–98% of the total weigh) but it is also a valuable resource of health-beneficial compounds namely olive oil biophenols or total phenolic fraction (TPF) [2–6]. TPF is a complex mixture of compounds belonging to diverse chemical classes among them simple phenolics, lignans, flavonoids, secoiridoids, and triterpenic acids. The most charac-

Abbreviation: EVOO, extra virgin olive oil; TPF, total phenolic fraction; TPC, total phenolic content.

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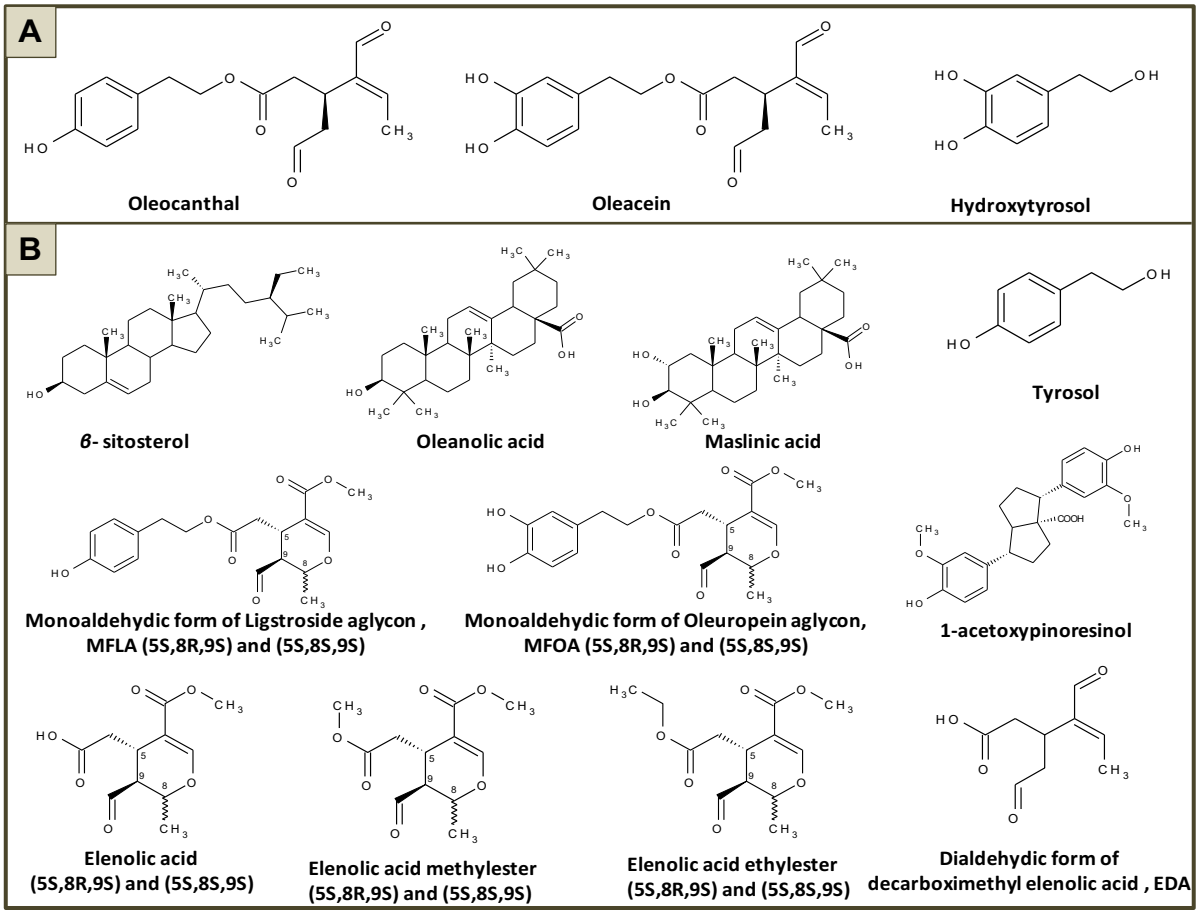

Fig. 1. Chemical structures of the isolated compounds obtained: A) directly from CPC analysis, B) after elaboration of CPC fractions.

teristic TPF constituents are hydroxytyrosol, tyrosol, oleocanthal, oleacein together with oleuropein and ligstroside aglycons (Fig. 1).

Oleocanthal is a secoiridoid which was firstly reported by Montedoro et al. [7,8] and it is also known as (−)-decarboxymethyl ligstroside aglycone or *p*-HPEA-EDA. Many studies report on a strong antioxidant propriety of oleocanthal [9] while an inhibiting effect of the cyclooxygenase enzyme by acting as a non-steroidal anti-inflammatory agent with an ibuprofen-like activity [10,11] has been demonstrated. Lately, oleocanthal has been reported as a potential neuroprotective agent capable of reducing the risk of Alzheimer diseases [12,13].

Oleacein, also known as 3,4-dihydroxyphenylethanol-elenolic acid dialdehyde or 3,4-DHPEA-EDA, has also been found to be a highly antioxidant agent [14]. Moreover, oleacein has been reported as a potent compound for the cardiovascular system by acting as an inhibitor of the angiotensin converting enzyme [15] and by inhibiting neutral endopeptidase [16]. Recently, special interest was accorded to the activity of oleacein for protecting endothelial progenitor cells against angiotensin II-induced cell senescence [17]. Generally, oleacein is much less pharmacologically investigated compared to oleocanthal although it is equally present in high levels in EVOO. Due to the complexity of olive oil biophenols but also the chemical nature of these compounds, their isolation and purification remains a challenging task and the related published information is limited. Towards this effort a new strategy based on the combination at-line of centrifugal partition extraction (CPE) and chromatography (CPC) was employed.

Centrifugal Partition Chromatography (CPC) is a separation technique, developed by Murayama et al. [18] which is based on the partitioning of solutes between two immiscible liquid phases [19–22]. A CPC column consists of a series of partition cells linked by ducts in cascade and arranged in a centrifuge (one axis, two high pressure rotary seals). One liquid (stationary phase), is maintained inside the column by the centrifugal force field generated by column rotation (50–800 times the gravity, depending on the apparatus), while the other one (mobile phase), is pumped through it. Thus, there is no solid chromatographic support and as a consequence, CPC is an excellent alternative to more traditional solid support chromatographic techniques as irreversible adsorption or degradation of samples on solid support are avoided. Additional benefits include total sample recovery, high loading capacity, good selectivity, versatility and high productivities while low solvent consumption [20]. Today, CPC is mainly applied to complex extracts from plants and principally for preparative purification purposes [23–26]. For instance, several natural metabolites belonging to different substances classes (triterpenoids, saponins, phenolic compounds, flavonoids, alkaloids, peptides) have been isolated at gram scale [24,26].

More recently, centrifugal partition extraction (CPE) has been presented as a highly productive support free liquid-liquid technique for the isolation of several natural metabolite classes from plant extracts, such as saponins [27], glucosinolates [28], alkaloids [29], phenolic acids and polyphenols [30,31]. The main difference between CPE and CPC columns relies on the column length (i.e. volume and number of partition cells). For an equivalent column capacity, the CPE rotor contains less partition cells of larger volume the latter being interconnected in series by larger ducts. CPE technique was also studied as a liquid-liquid extractor, and success-