Oleuropein: Methods for extraction, purifying and applying

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ABSTRACT

Oleuropein is one of the most abundant phenolic compounds present in olive leaves, and many studies have shown that this compound has important biological properties (anti-inflammatory, anti-atherogenic, anticancer, antimicrobial and antiviral) and that is why it has been gaining prominence in research. Oleuropein can be extracted from different sources using standard and unconventional methods, and can be recovered and purified (mainly by chromatographic techniques), for later use in several areas. The literature presents information about oleuropein alone in scientific research with different objectives, however, it was observed by the authors that studies that compile the existing information on these secoiridoides are very scarce and, therefore, this review was developed with the aim of providing current information to the scientific community, about the different techniques of extraction and purification of oleuropein, as well as the opportunities for applications and uses of this compound.

Keywords: phenolic compound; extraction; olive leaves; review.

INTRODUCTION

Oleuropein, characterized as a bitter glycoside, is one of the most abundant phenolic compounds in olive leaves, and it is also present in the trunk and the fruit of the olive tree (skin, pulp and stone) (Romero *et al*., 2017). Several studies have indicated that this phenolic compound presented interesting biological and pharmacological properties, in large part attributed to its putative antioxidant and anti-inflammatory effects (Cavaca *et al.*, 2020), antimicrobial properties (Guinda, 2006; Tripoli *et al*., 2005; Bayram *et al*., 2020), anticancer potential (Bonoli *et al.*, 2004; Przychodzen *et al*., 2019); cardioprotection activity (Papachristodoulou *et al*., 2019; Nediani *et al*., 2019); affects lipid metabolism (Fki *et al*., 2020; Ucella, 2001), reduce body weight, alleviated kidney injury, and decreased oxidative stress and inflammatory response and inhibits platelet aggregation (Liu *et al*., 2019) and reduces LDL levels (Hadrich *et al*., 2016).

Its ability to act as a natural antioxidant has also attracted the attention of researchers, as consumers increasingly seek natural products or products that contain natural components in their formulation, inducing the industry to replace synthetic antioxidants with those extracted from plant sources (Mosca *et al*., 2013).

The choice of method of extraction and purification is considered one of the most critical stages of research involving natural products. The efficiency of the extraction process depends on several parameters, such as the type of sample, the compound to be extracted, its location in the plant material (Mustafa & Turner, 2011), besides the type of solvent (Xynos *et al*., 2012), time and temperature of the extraction (Galanakis *et al*., 2010), and the methods

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of recovery and purification. Due to the importance of oleuropein in relation to several applications as well as the increase of research on the characteristics and applications of this compound, this review was elaborated with the objective of compiling the information available in the literature up to the present moment.

Data Sources and Search Strategy

A comprehensive review was conducted by systematically searching in PubMed and Springer Link for all studies that developed an a priori OBS until May 2019. Subject headings related to OBSs (Oleuropein, phenolic compound, olive leaves) and other key terms (extraction, application, purification) were considered. Exclusion criteria were articles that did not meet the inclusion criteria mentioned above; For a writing of this review a total of 113 references were selected which presented the information and data of interest to the authors.

Oleuropein

Oleuropein is the major phenolic constituent of the oil leaf (*Olea europaea L.*) and it is also present in olive oil and fruit, up to 14% of the dry weight (Bonechi *et al.,* 2019). This compound consists of a molecule of elenolic acid linked to the orthodiphenol hydroxytyrosol by an ester bond and to a molecule of glucose by a glycosidic bond (Figure 1). The rupture of the glycosidic bond gives rise to glucose and the oleuropein aglycon that, under an aqueous environment, is rapidly transformed into the dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol (Sivakumar *et al*., 2018) which is also a bitter substance.

The biosynthesis of oleuropein in *Oleaceae* (Figure 2) follow the mevalonic acid pathway, branching leads to biosynthesis of geraniol, 10 hydroxygeranoil, 10 hydroxynerol, and iridoidal. From iridoidal, loganin is biosynthesized, and later deoxyloganic acid, 7 epiloganic acid, and loganic acid are incorporated into ligustroside. Ligustroside is considered as a direct precursor of oleuropein, via 7 ketologanic acid as intermediate. A probable biosynthetic route from deoxyloganic acid, 7 epiloganic acid, 7 ketologanic acid, 8 epikingisidic acid, oleoside 11 methyl ester, 7 β 1 D glucopyranosyl 11 methyl oleoside, and ligustroside to oleuropein (Damtoft *et al*., 1992; Ahamad *et al*., 2019).

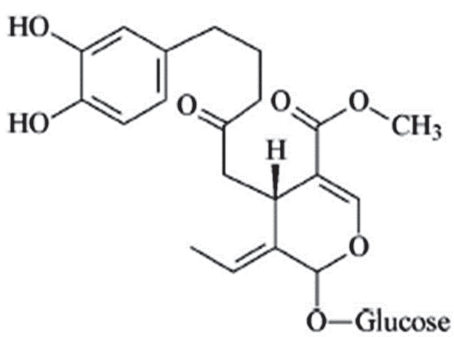

Figure 1: Structure oleuropein (Bonechi *et al*., 2019).

The enzyme involved in this reaction is â-glucosidase that rapidly acts during the crushing and malaxation steps of the olive oil extraction process to modulate the phenolic profile of virgin olive oil, and also contributes to the degradation of oleuropein at the beginning of the brining stage of natural table olives (Ramírez *et al*., 2016). Several factors influence the hydrolysis of oleuropein, such as pH, type of organic acid, time as well as the interaction between these factors (Romero *et al.*, 2020).

Oleuropein occurs in the *Oleaceae, Gentianaceae* and *Cornaleae* family and in lower concentrations in other plants and residues (olive pomace by e.g) (Böhmer-Maas *et al*., 2020) however the highest concentrations are described in olive leaves (60-90 $mg.g^{-1}$ dry base) and young olives (63-105 $mg.g^{-1}$ dry base) (Tayoub *et al*., 2012). Oleuropein has several pharmacological properties, including antioxidant, anti-inflammatory, anti-atherogenic, anti-cancer, antimicrobial and antiviral, and for these reasons, it is commercially available as food supplement in Mediterranean countries (Omar, 2010). In view of all the reported benefits for oleuropein, it is necessary to conduct studies that seek new sources of the compound, as well as methods that provide better extractions and purifications of oleuropein, aiming at different application markets.

Oleuropein extraction methods

The extraction methods used for isolation of biomolecule from olive leaves show risk of residual solvent and less extraction efficiency. Hence, there is a need to develop novel techniques to encapsulate the risks headed with extraction process. Blanching of olive leaves causes deactivation of enzymes, and further exposure to ultrasonic waves enhances mass transfer of solvent and promotes the release of oleuropein. Hot blanching technique shows a significant linear upswing in the concentration of oleuropein when compared to direct extraction techniques (Sucharitha *et al*., 2019).

The extraction of oleuropein can be done in different ways, being classified in conventional and non-conventional (Figure 3), and these are described below.

In blanching, the thermal treatment applied to the olive leaves leads to structural changes of the plant tissue, being responsible for the loosens of the cellulosic networks; thereby, it promotes the leaching of contents from olive leaves. Extraction and isolation of the active moiety from the plant can be easily affected by the processing methods. Hence, suitable conditions are required to maximize the extraction efficiency and to

minimize the processing loss of phenolics. Sucharitha *et al*. (2019) studied the effect of three different techniques on the efficiency of oleuropein extraction. Hot blanching technique showed a significant linear upswing in the concentration of oleuropein when compared to direct extraction techniques.

Conventional

Extraction by Soxhlet

The Soxhlet extraction method is the one most commonly used in laboratories, but it may promote the partial degradation of thermolabile compounds, as well as consuming large amounts of water and energy (Bimakr *et al*., 2012).

When examining different extraction systems and factors such as solvent type, pH and temperature, it was found that the combination of solvents with water showed higher efficiency of extraction (the highest content of oleuropein (13 $mg.g^{-1}$ dry leaf olive) was obtained using 80% ethanol, followed by 20% acetonitrile (10 $mg.g^{-1}$ dry olive leaf powder). With this, the importance of water as a co-solvent to increase the amount of extracted oleuropein was confirmed (Yateem *et al*., 2014).

The elevation of the extraction temperature, showed a significant increase in the oleuropein content; and the increase in pH provided a reduction in extraction yield. In addition, the results showed that Soxhlet was more efficient in extracting oleuropein compared to the cold extraction technique (Yateem *et al*., 2014).

Yasemi *et al*. (2017) also evaluated different extraction methods, including maceration, soxhlet, ultrasound and microchannel, with soxleth extraction showing the greatest recovery among conventional techniques (62%). In addition, different combinations of solvents were also tested, where for soxleth the best combination was ethanol: water (80:20). However, the emerging techniques showed greater extraction yields (values between 80 and 95%) compared to the conventional extraction methods evaluated by these researchers.

Cold Solvent Extraction

In this technique, also known as maceration, the sample is immersed in a non-volatile solvent at room temperature. Extraction can be done using water (Ghomari *et al.*, 2019; Benincasa *et al.*, 2019; Ghelichkhani *et al*., 2019) or alcoholic solvents (Bayraktar *et al*., 2019; Kabbash *et al.*, 2019; Ghomari *et al*., 2019). Ethanol, methanol, ethyl acetate, water, hexane, diethyl ether and acetone have been used as solvents. Methanol seems to be a very effective solvent, yet its use may result in undesirable toxic residues in the final extracts (Lamprou *et al*., 2020).

It is a time-consuming process, which may lead to incomplete extraction, besides facing the probability of contamination when using solvents containing large amounts of water (Melecchi *et al*., 2002; Miranda & Cuéllar, 2001).

Coppa *et al*. (2017) were able to obtain a lyophilized extract from olive leaves, which contained approximately

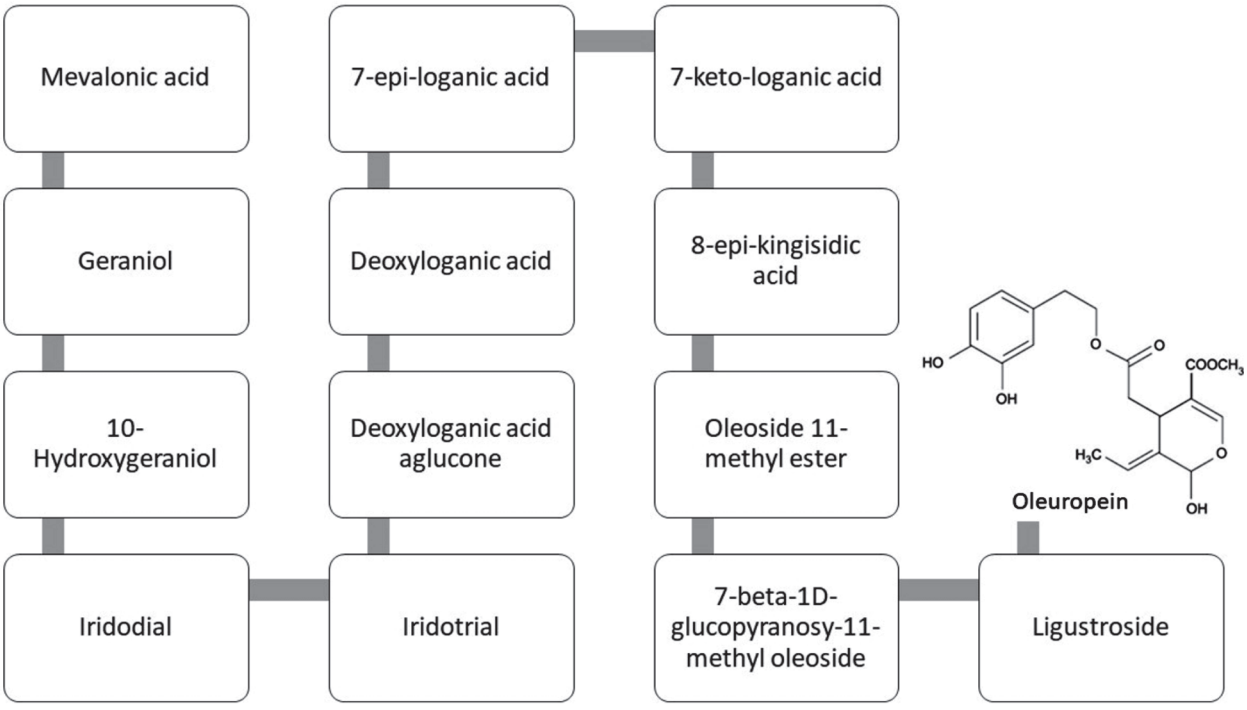

Figure 2: Biosynthesis of oleuropein in *Oleaceae.*

18 g. 100 g^{-1}. These authors used maceration at 25 °C for 2 h with a mixture of ethanol and water (70:30 v / v) and 1% acetic acid, and observed that the highest oleuropein content was reached with an olive leaf: solvent ratio equal to 1: 3, while the proportions 1: 8 and 1: 6 were also tested, with or without acetic acid present.

Cho *et al*., (2020) determined optimal conditions for olive leaf extraction by examining the effect of extraction solvent (water, aqueous methanol, aqueous ethanol, and aqueous acetone) on selected extract properties. The highest extraction efficiency of 20.41% was obtained using 90 vol% methanol.

Non-conventional methods

The production of bioactive compounds from olive leaves requires treatments capable of breaking the lignocellulosic structure. Current research focuses on use of inexpensive, quick, and not harmful to the environment treatments, searching a more simplified large-scale operation approach (Lama-Muñoz *et al.*, 2019). Non-conventional treatments such as ultrasound, supercritical fluid, pressurized fluid, microwave assisted, microchannels and membranes are highlighted in this scenario.

Ultrasound assisted extraction

Recently, advances in applied chemistry have led to possible new emerging industrial techniques like ultrasound-assisted extraction (UAE). This technology is a promising candidate as a green treatment solution for olive leaves utilization in a biorefinery. However, this application goes through prior optimization of technique and operating conditions (Lama-Muñoz *et al.*, 2019).

Ultrasound-assisted extraction has the advantage of reducing the extraction time of the component of interest, mainly due to the physical and chemical effects promoted by cavitation, a phenomenon that accelerates the chemical reactions (Soria & Villamiel, 2010). This method has been described by several authors (Shirzad *et al*., 2017; Cifá *et al.*, 2018; Irakli *et al*., 2018; Giacometti *et al*., 2018) with the aim of extracting oleuropein from olive leaves.

In this phenomenon, the formation and collapse of cavitation bubbles occur, creating discrete areas of high pressure and temperature, which contribute to the extraction of the compounds (Bendicho *et al*., 2012). In addition, the ultrasound system allows for a reduction in the volume of chemical solvents, as it is a simple and effective process (Charpe & Rathod, 2012). A disadvantage of this process is the possible damage that can be caused by free radicals (Fellows, 2009). When used in plants, the ultrasonic process favors the dilation and hydration of the plant material and causes the enlargement of the pores of the cell wall. This leads to an increase in swelling and mass transfer ratio and, eventually, to cell wall breakdown, and in this case causes increased solvent penetration (Toma *et al*., 2001; Chung *et al*., 2010).

Zun-Qiu *et al*. (2015) obtained 13.52% pure oleuropein from olive leaves by means of ultrasound-assisted extraction using a solution of 80% methanol-water (v/v). The same authors obtained oleuropein with a purity of 96.54% and with purification efficiency of 78.49%, using

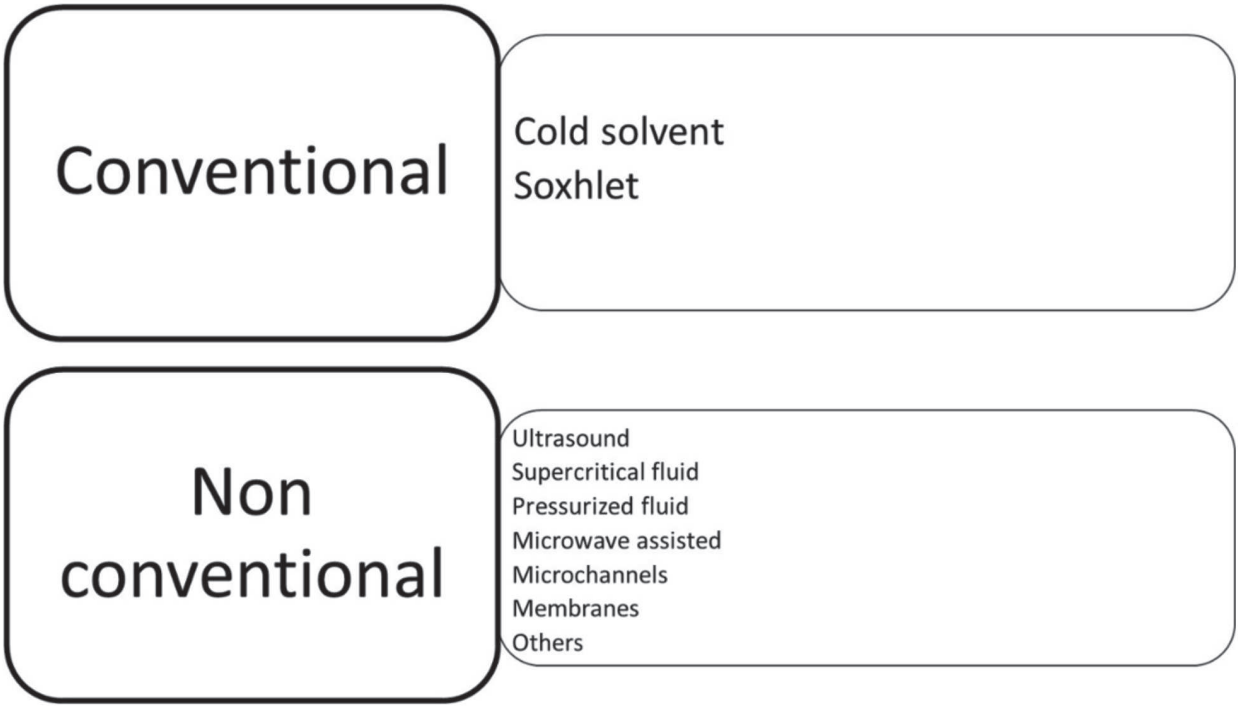

Figure 3: Main methods of extracting oleuropein.

silica gel column chromatography. Their study aimed to investigate the changes in oleuropein and total phenolic content over a year, as well as evaluating the antioxidant activity of oleuropein in vitro. The authors obtained different levels of oleuropein in 29 varietals cultivated in a region of China that is important for olive cultivation, but both had similar trends throughout the year. Samples harvested in January showed a higher content of oleuropein, and the lowest value was observed in samples collected in July. During the period of differentiation of flower buds and fruit development, a reduction in oleuropein content was observed.

Khemakhem *et al*. (2017) observed that the extraction kinetics of oleuropein from olive leaves improved with increasing temperature, using ultrasound-assisted extraction and water as solvent. This process also enhanced the initial extraction rate, with approximately 88% oleuropein extracted in the first minute of the experiment lasting 10 min, reaching 6.57 ± 0.18 g.100g^{-1} at 50 °C.

Lama-Muñoz *et al*. (2019) studied the optimization of extraction of oleuropein from olive leaves through an investigation of the influence of key factors of ultrasound-assisted extraction using an experimental central composite design, in comparison with conventional Soxhlet extraction. The highest extraction efficiency and antioxidant capacity were obtained under optimal increment of temperature and amplitude conditions (40 °C and 30%, respectively).

Del Mar-Contreras *et al*. (2020) obtained an extract with both high level of oleuropein was achieved using the following operational parameters: solid-to-liquid ratio, 5.9%; ethanol concentration, 47%; extraction time, 50 min.

Extraction with supercritical fluid

Substances in the supercritical state have intermediate properties between the properties of a gas and a liquid. With diffusion coefficient, low viscosity and absence of surface tension, referring to the characteristics of a gas, supercritical fluids have a greater diffusion capacity than liquid ones, thus allowing a fast and efficient transfer of mass and ease of penetration into a solid matrix. The density of these fluids is close to that of a liquid, providing good solvent power (Taylor, 1996; Luque De Castro, 2004). The technique of supercritical fluid extraction is commonly used for thermally sensitive analytes, since one of its advantages over conventional methods is the use of low temperatures. Other advantages are low energy consumption and low levels of degradation of chemical compounds; moreover, products of higher quality are obtained, in addition to high selectivity by polar analytes. In this technique, operational changes during extraction are allowed, facilitating the recovery of specific compounds (Reverchon & De Marco, 2006).

The most commonly used solvent for extraction in supercritical media is carbon dioxide (CO_2), which is inert, non-flammable and does not harm the environment (Jarvis & Morgan, 1997) however, this substance has an apolar nature, making it impossible to use in extracting polar compounds (Duarte, 2011). Also, because high pressure is used, expensive equipment is needed, increasing the cost of the final product (Herrero *et al*., 2006).

The extraction of oleuropein from olive leaves using method supercritical fluid has been described by some authors (Xynos *et al.*, 2012; Bastante *et al*., 2018; Baldino *et al*., 2018). In the development of a green extraction procedure with supercritical fluid to produce extracts enriched with oleuropein from olive leaves, Xynos *et al*. (2012*)* acquired 30% of this compound in the dry extract, using 20% of ethanol as co-solvent and supercritical CO_2 at 300 bar. This 30% was equivalent to the recovery of 5.1% of the original content of oleuropein in olive leaves.

Extraction with pressurized fluid

Extraction with pressurized fluid, also known as pressurized solvent extraction or accelerated solvent extraction, allows extraction in a closed and inert environment, under pressures not exceeding 200 bar and temperatures between 25 and 200 ºC. In this process, the pressurized solvents remain in the liquid state, even in temperatures above boiling point, allowing extraction at high temperatures. Thus, inert solvents, such as ethanol and water, which are used in the extraction of some phenolic compounds at low temperatures, can be very efficient at high temperatures applied in the extraction with pressurized fluid. This technique allows rapid extraction and reduction of solvent consumption, and has therefore been successfully used in the extraction of several classes of compounds in plants (Santos *et al*., 2012; Jaski *et al.*, 2019).

Xynos *et al*. (2014) applied pressurized liquid extraction to optimize extraction yield of the oleuropein content of leaves of *O. europea*, and to analyze the antioxidant activity of the extracts. The maximum content of oleuropein in leaf extract was 26.1%, and this content was influenced mainly by three factors, in order of statistical significance: ethanol concentration - with positive effect; temperature - with negative effect; and extraction cycles - with positive effect. The highest yield was 46.64%, and the antioxidant activity was not statistically significant when correlated with the phenolic content of leaf extracts.

The pressurized liquid extraction (PLE) is an alternative extraction technique to supercritical fluid extraction (SFE), investigated by Rosa *et al*., (2019) and proved adequate for the extraction of phenolic compounds from the olive leaf. The highest total yield using PLE, with ethanol:water, 10.3 MPa, 60 °C and 110 min was of 30.91%. The PLE

extract obtained with ethanol at 60 °C presented the highest concentration of oleuropein (73.65 mg.g^{-1} extract).

Microwave-assisted extraction

In microwave-assisted extraction (MAE), the microwave energy heats the solvents that are in contact with solid samples in order to allow the compounds of interest to be shared (Luque De Castro, 2004). This technique fulfills the requirements of green chemistry, because it uses less solvent, has a low cost of extraction with an increase in production and, as a main advantage, it reduces the time of extraction. The reduced extraction time is attributed to the difference between conventional heating and microwave heating (Wang *et al*., 2005). Three steps are involved in this type of extraction: the separation of solutes present in the active sites of plant matrices by increasing temperature and pressure; diffusion of the solvent through the solid sample; and the release of the solutes into the solvent (Alupului *et al*., 2012).

The extraction of oleuropein from olive leaves using the microwave method was described by da Rosa *et al*. (2019) three different extraction techniques were used and compared to the extract of phenolic compounds from olive leaves using a mixture of water or ethanol and different temperatures. MAE at a higher temperature (86 °C) was more efficient in terms of TP yield, with short extraction time (3 min). MAE performed with water as a solvent was effective in disrupting the olive leaf cells thereby promoting the release of the compounds. Under this condition, the TP yield was increased by 82% when compared to maceration.

Japón-Luján *et al*. (2006) used microwave-assisted extraction in olive leaves with a mixture of ethanol and water (80:20), and obtained 2.3% of oleuropein in the extract.

Sahin *et al*. (2017) used microwave-assisted extraction without solvents and obtained a maximum yield of oleuropein of 0.060 ppm in leaves of *O. europea*, with the following optimized conditions: 250 W of irradiation power, 2 min extraction time and 5 g amount of sample. Water was used as pre-treatment to improve the extraction process.

Microfluidic system (microchannels)

Microchannel devices are currently widely used for the most varied applications in the food industry. In these systems, lower molecular distance leads to greater diffusion, increasing the possibility of mass transfer (Hisamoto *et al*., 2001). One of the major differences between microchannels and large-scale devices is the increased surface / volume ratio. Accordingly, miniaturization can be an effective way of increasing heat and mass transfer rate. This type of process can reduce the volume of solvents, the process time and the cost of mass production (Faryadi *et al.*, 2014; Wang *et al*., 2005)

In a study with the use of microfluidic devices for the extraction of oleuropein, using ethyl acetate in aqueous phase, Naleini *et al*. (2015) obtained a 68.7% yield of oleuropein. This was measured in relation to the content in the feed extract of the microfluidic device under optimized aqueous phase pH conditions, temperature, flow rate and residence time. In this research, the microchannel method was compared to a conventional method, and the authors observed higher yield in extraction, simplicity of operation, and an economical and environmentally friendly procedure, all of which were undoubted advantages.

Yasemi *et al.* (2017) evaluated the efficiency of the method of microchannels for extracting oleuropein, were obtained a yield of 96.29% of oleuropein using this system with optimized operating parameters; this value was much higher than the yields found through maceration, Soxhlet and ultrasound-assisted methods.

Heydarid *et al.*, (2018) studied the optimization of oleuropein extraction from organic extracts using a microfluidic device and response surface methodology. The maximum extraction yield was obtained under the following conditions: deionized water as extractant phase, temperature of 40 ºC, flow rate ratio of 0.16 and residence time of 0.1010 min. The extraction yield of 70.93% was obtained under the above conditions with relative standard deviation of 2.0 %.

Extraction with membranes

In recent years, membrane technologies for the separation, purification and concentration of bioactive compounds from aqueous solutions have gained great attention and have showed an increasing potential for application (Khemakhem *et al*., 2017; Ranieri *et al*., 2018; Piacentini *et al.*, 2019). This technique is based on the principle of selective permeation of solute molecules by means of a semipermeable polymer or inorganic support, namely, membranes. These structures provide a high quality of phenolic compound concentration due to low operating temperature, besides providing low energy consumption (Cassano *et al*., 2013; Kim *et al*., 2015).

Khemakhem *et al*. (2017) obtained an extract rich in oleuropein (1685 mg.100g^{-1} extract in the retained nanofiltration material) from olive leaves combining microfiltration, ultrafiltration and nanofiltration. In this study, a water extract was prepared and then subjected to molecular size-based sorting. Microfiltration (0.2 ìm) was initially performed to remove large particles, followed by ultrafiltration to remove molecules larger than 5 kDa and, at the end nanofiltration (300 Da), to provide the concentration of polyphenols, mainly oleuropein.

Others methods

Differents oleuropein extraction techniques are emerging due to the need to obtain and recover this compounds that are present in residues from the production of olives. Optimization of infrared-assisted extraction was conducted by Abi-Khattar *et al.,* (2019) using Response Surface Methodology (RSM) in order to intensify polyphenol recovery from olive leaves. The extraction efficiency using Ired-Irrad®, a newly-patented infrared apparatus (IR), was compared to water bath (WB) conventional extraction. Under optimal conditions, as suggested by the model and confirmed experimentally, the total phenolic content yield was enhanced by more than 30% using IR as contrasted to WB, which even required 27% more ethanol consumption. High performance liquid chromatography analyses quantified the oleuropein in 18%.

Lamprou *et al.* (2020) studied, a new cost effective process for the extraction of phenolic compounds from olive leaves was examined by acid hydrolysis (H_2SO_4). The resultant extract, after hydrolysis, contained an optimum amount of oleuropein of 43.2 $mg.g^{-1}$ (dry weight basis).

A plausible alternative to conventional extractants to be used in liquid–liquid extraction us ionic liquids (ILs). These are called "green solvents," because of their physicochemical properties, ease of recovery, and adaptation to specific applications by cation and anion selection. Imidazolium-based ILs are the most studied, because they have a remarkable ability to extract both polar and nonpolar compounds (Visser *et al.*, 2003). They also have a wide range of molecules that can be solubilized, given their ability to change their degree of hydrophobicity. In addition, they have high conductivity and low viscosities (Belhocine *et al.*, 2011) belongs to this class of ILs. On the other hand, phosphonium-based ILs, have high chemical and thermal stability at temperatures under 250°C.

Julio *et al*. (2019) proposed to use liquid–liquid extraction to obtain oleuropein from model solutions. Considering the organic phase, two conventional solvents, namely, n-butyl acetate and ethyl acetate and three ionic liquids, were used. The effects of stirring time on the extraction percentages (%E) of oleuropein, together with variations of distribution coefficient (Dc) with different aqueous–organic (A/O) volumetric ratios, were analyzed. A mathematical model to calculate the liquid–liquid extraction was presented. Experimental results showed that [P6,6,6,14] [DCA] was the solvent with the highest extraction efficiency.

The content of these leaves varies according to many factors such as climatic conditions, moisture content, age and variety of the plant, agricultural practices, and the extraction procedures used. Thus, extraction is an important and determinative step in the analysis and the use of the cellular bioactive compounds contained in these leaves.Therefore, the identification of the appropriate extraction methods is a limiting step to increase the yield of these compounds and to provide a more efficient purification (Ghomari *et al.*, 2019).

The extraction of phenolic compounds, such as oleuropein, influenced by several experimental parameters depending on these techniques, such as time, temperature and solvent, and with a view to a suitable choice among them, the table 1 shows the advantages and disadvantages of applying each technique.

Methods of purification of oleuropein

The technique usually applied for the purification of extracts with oleuropein is column chromatography. This process occurs by the separation of two phases (solid and liquid), based on the adsorption capacity and solubility, where the dynamic equilibrium is established between the concentration of the solute in the two phases. The target compound is eluted in a stationary phase with a mixture of suitable solvents. This type of chromatography is simple, efficient and inexpensive. At the end of the purification the obtained fractions must be collected according to their chromatographic profile (Skoog, 1992).

Column chromatography on silica gel using a mixture of methanol and ethyl acetate (1:13, v / v) as eluent was applied by Zun-Qiu *et al.* (2015) to separate and obtain pure oleuropein from olive leaves. In this study, 13.52% of oleuropein was obtained with a purity of 96.54%, and purification efficiency of 78.49%.

Dang, Nie & Liang (2010) purified crude extracts of oleuropein by column chromatography, using as the stationary phase sephadex LH-20 and as the mobile phase 50% ethanol. The purity of oleuropein obtained was 82.9%. Flavonoids and oleuropein were selectively purified from leaves and O. europaea with LSA-21 resin by Li *et al.* (2011), in a study which evaluated the performance and separability of eight macroporous resins (D101, DM130, HPD450, LSA -21, LSA-40, 07C, LSD001 and HPD600). In this study, LSA-21 resin had better adsorption properties, and the content of total flavonoids and oleuropein in the final purified products increased 13, 2 and 7.5-fold in 87.9% and 85.6% recovery yields, respectively.

Andreadou *et al.* (2006) purified extracts of olive leaves to isolate oleuropein by column chromatography, using as the mobile phase a solution composed of CH_2Cl_2: methanol (98: 2), and Si 60 Merck (15-40 mm) as the stationary phase. Purified oleuropein was obtained in glycosidic form, free of aglycone forms, and with purity of 95%.

Table 1: Advantages and disadvantages of oleuropein extraction methods

Extraction methods	Advantages	Disadvantages	References
Cold Solvent	Does not degrade the target substance. Simple and low-cost process.	Delayed process, which favors incomplete extraction. Another problem is the likelihood of contamination by using solvents containing large amounts of water.	Melecchi *et al.* (2002); Miranda & Cuéllar (2001)
Soxhlet	Extraction of high efficiency, with the sample always in contact with the solvent, having its constant renewal. A simple process that requires no filtration after the extraction is finished.	It can promote the partial degradation of thermolabile compounds, as well as the high consumption of water and energy.	Bimakr *et al.* (2012); Fellows (2009)
Ultrasound assisted	Reducing the time and volume of chemical solvents, being a simple and effective process.	Possible damage can be caused by free radicals.	Jarvis & Morgan (1997); Herrero *et al.* (2006)
Supercritical fluid	Use of low temperatures, and can be used for thermally sensitive analytes. Other advantages are the low energy consumption, low levels of degradation of chemical compounds. In this technique, operational changes during extraction are allowed, facilitating the recovery of specific compounds. In addition, the most commonly used solvent is carbon dioxide (CO_2), which is inert, non-flammable and does not harm the environment.	Carbon dioxide (CO_2) is apolar in nature, which, having four non-ligand pairs of electrons, is able to dissolve weakly polar substances or with some polarity at pressures greater than 250 bar but does not dissolve high molecular weight compounds such as amino acids, proteins, sugars, polysaccharides, inorganic salts and flavonoids. Considering the use of high pressures, expensive equipment is required, increasing the cost of the final product.	Reverchon & De Marco (2006)
Pressurized fluid	Allows for the fast extraction and reduction of solvent consumption. Therefore, inert solvents such as ethanol and water, which are used in the extraction of some phenolic compounds at low temperatures, can be very efficient at high temperatures applied in the extraction with pressurized fluid.	Higher operating cost	Santos *et al.* (2012)
Microwave-assisted	This technique meets the requirements of the perspective of green chemistry, because it uses less solvent, low extraction cost with increased production and has as main advantage the reduction of extraction time. The reduced extraction time is attributed to the difference between conventional heating and microwave heating.	Not applicable	Wang *et al.* (2005)
Microfluidic system (microchannels)	One of the major differences between microchannels and large-scale devices is the increased surface/volume ratio. Accordingly, miniaturization can be an effective way of increasing heat and mass transfer rate. This type of process can reduce the volume of solvents, process time and the cost of mass production.	They may present physical effects such as capillarity and surface tension, and therefore require a more complex form of system modeling. Microfabrication techniques still require cost reductions and the development of new materials supplying applications in the most diverse areas.	Wang *et al.* (2005); Faryadi *et al.* (2014)

To be continued

Continuation Table 1

Extraction methods	Advantages	Disadvantages	References
Membranes	They provide high quality phenolic compound concentration considering the low operating temperature, in addition to providing low energy consumption.	When the solution to be filtered contains solutes that can not pass through the membrane occurs fouling due to the accumulation of retained solutes, which provides additional resistance to mass transfer. Constant removal of these solutes from the surface of the membrane is required.	Cassano *et al.* (2013); Kim *et al.* (2015)

The adsorption process in the macroporous resins is reversible owing to the difference in the polarity, pore size, and specific surface area of the resins; the adsorption and desorption of the effective components vary. In practical applications, the resin is required to adsorb large amounts of the target components, and the desorption rate is required to be high to ensure the maximum recovery of the effective components. Therefore, Li *et al*. (2019) study simultaneous purification and separation of oleuropein, were 11 macroporous resins were preliminarily screened by static adsorption and desorption experiments. By comparing the ability of 11 macroporous resins to adsorb and desorb oleuropein, HPD-100B was selected as the ideal resin for the separation and enrichment of the component. The static adsorption equilibria of oleuropein on the HPD-100B resin were well fitted to the Langmuir isotherm model at various temperatures. Optimal separation conditions were achieved by the following kinetic and dynamic adsorption/desorption experiments: 60 BV loading amount of adsorption at 25°C with a flow rate of 3 BV/h, followed by desorption with a gradient elution of 20% ethanol (7 BV) and 40% ethanol (9 BV) at 25°C and 3 BV/h. The purity of oleuropein obtained under the optimized conditions increased by 8.2-fold compared with that of the initial extract. The recovery of oleuropein were 89.8%. This method demonstrates a practical approach for bulk extraction and purification of oleuropein in an economical way.

Khemakhem *et al*. (2017) studied the combination of different technologies such as micro, ultra and nanofiltration for separation and concentration of oleuropein. They concluded that the separation of compounds by membrane constitutes an attractive alternative to conventional processes such as by chromatographic column, showing that these technologies offer unique separation capacity, possibilities for scale expansion and low energy consumption. The authors even concentrated the oleuropein content up to 50 times, reaching an extract with 265.23 mg $100g^{-1}$ of oleuropein.

Applications of oleuropein

A number of studies have been carried out with leaf extracts and olive fruit for technological and functional purposes, but few have been developed with pure oleuropein alone. There are reports of the use of this compound in food preservation as a natural antioxidant (Dua *et al*., 2015; Al-Rimawi *et al*., 2017), in sanitizing formulations (Dominciano *et al*., 2016) and as a functional component in dairy products (Zoidou *et al*., 2017). The effects of the enzyme-linked immunosorbent assay on the treatment of diseases (Esmailidehaj *et al*., 2016; Shi *et al*., 2017) have also been investigated. In addition, oleuropein

has the potential for use in cosmetics (Perugini *et al*., 2008). Other studies have demonstrated the antimicrobial properties of oleuropein (Bisignano *et al*., 1999; Furneri *et al*., 2002). Figure 4 summarizes the main applications of oleuropein as a coadjuvant in technological and therapeutic processes.

Food preservation and antioxidant potential

Oxidative processes in foods cause deterioration and consequently induce loss of quality. In a study on the application of oleuropein extracts, an improvement in the lipid oxidative stability and storage quality of Tabaq-Maz (fried ribs popular in India) was observed (Dua *et al*., 2015). Also, protective effects of oleuropein were observed against lipid oxidation in hamburgers stored at 4 ° C, suggesting the commercial use of oleuropein as a natural preservative in meat foods (Al-Rimawi *et al*., 2017). The antioxidant activity was higher than the original extract obtained from olive leaves (Khemakhem *et al*., 2017), demonstrating the potential of oleuropein to prevent oxidation of food. Zun-Qiu *et al*. (2015) extracted and purified oleuropein from olive leaves, and found that oleuropein is more effective than butyl hydroxy toluene (BHT) for application in food and human health, after a comprehensive assessment of antioxidant activity in vitro. A high oleuropein extract added to extra-virgin olive oils and refined olive oil improved the oxidative stability with an increased induction period, which is the period necessary for the products formed in the oxidation to be detected (Coppa *et al*., 2017).

Functional component in dairy products

Oleuropein is a bioactive with beneficial properties for human health, because it presents antioxidant, antimicrobial, hypoglycemic and anti-inflammatory activities, among others (Fernández-Bolaños *et al*., 2006; Guinda, 2006).

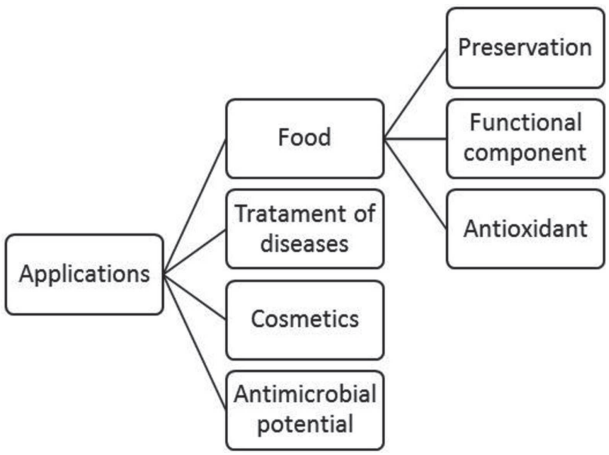

Figure 4: Main applications of oleuropein.

As milk and yogurt are good matrices for the development of various functional foods and popular dairy products with high nutritional value, Dominciano *et al*. (2016) added oleuropein to these foods to increase health benefits upon ingestion. In this study, the researchers reported that oleuropein was stable at all stages of preparation and during the period of validity of both products, without degradation occurring during the heat treatment of the milk and without hydrolysis by the acids produced in the fermentation process of yogurt. In addition, oleuropein was not metabolized by lactic acid bacteria, and it did not inhibit the growth of these microorganisms. Furthermore, it remained stable in the final products and did not alter the sensorial characteristics of the products. In a study by Zoidou *et al.* (2017), milk and yogurt added with oleuropein exhibited acceptable taste, color and texture, similar to conventional. In this study, oleurpein was stable during heat treatment, fermentation and storage.

Treatment of diseases

Several studies have reported positive medicinal effects of the use of oleuropein, as it can act against oxidative stress (Esmailidehaj *et al*., 2016; Hadrich *et al*., 2016; Shi *et al*., 2017; Sun *et al*., 2017; Fki *et al*., 2020) in the reduction of body weight, total cholesterol and triglycerides (Andreadou, 2006; Hadrich *et al*., 2016) as a possible preventive component of diabetes (Wainstein *et al*., 2012; Del Ben *et al*., 2019) in combating hypertension (Sun *et al*., 2017) in the treatment of interstitial hemorrhage (CVA), hepatitis B and cancer (Seçme *et al*., 2016; Shi *et al*., 2017; Zhao *et al*., 2009; Barzegar *et al*., 2019; Nassir *et al*., 2019; Zhang & Zhang 2019), in nonalcoholic fatty liver disease (Santini *et al*., 2020) in protecting against Alzheimer's, Parkinson's and hemorrhagic cystitis (Sherif *et al*., 2016) as a preventive/palliative treatment of lupus nephritis (Castejon *et al*., 2019) and in preventing myocarditis (Grossi *et al*., 2013; Pasban-Aliabadi *et al*., 2013). A relevant aspect is that much of the ingested oleuropein is absorbed into the human organism (Vissers *et al*., 2002).

Cosmetics

In a study by Perugini *et al*. (2008), an emulsion and an emulsifier containing oleuropein were prepared to evaluate their cosmetic properties against UVB (Ultra Violet Radiation)-induced erythema. These materials were applied in volunteers before exposure to UVB irradiation to investigate their protective activity, and after UVB-induced erythema, for the analysis of the lenitive effect. The results demonstrated that formulations containing oleuropein exhibit lenitive efficacy with reduced damage caused by UV.

Antimicrobial potential

Polystyrene and stainless steel are widely used in food industry equipment and utensils. It is important to study the formation of biofilm on the surface of these materials, since they are difficult to sterilize and failures can lead to contamination in food (Dominciano *et al*., 2016).

Oleuropein presented a higher antimicrobial effect for multispecies biofilms formed on surfaces of polystyrene microplates, with greater reduction in the association of *Listeria monocytogenes*, *Escherichia coli* and *Staphylococcus aureus* (91 and 49%, respectively). It increased the efficiency of peracetic acid (APA), a commercial sanitizer, in the inactivation of *Listeria monocytogenes* biofilms on stainless steel surfaces (Dominciano *et al*., 2016).

Oleuropein, in concentrations of 20 to 320 mg. L^{-1}, showed an inhibitory effect on the growth of *Mycoplasma hominis, Mycoplasma fermentans, Mycoplasma pneumoniae* and *Mycoplasma pirum*, in a study by Furneri *et al*. (2002).

In a study to verify the in vitro antimicrobial activity of oleuropein against pathogenic bacteria in humans, Bisignano *et al*. (1999) observed that oleuropein inhibited the growth of *Salmonella typhi*, *Vibrio parahaemolyticus* and *S. aureus*, with MIC values between 62.5 and 500 ìg. mL-1 for ATCC strains and between 31.25 and 250 ìg.mL^{-1} for clinical isolates. For *Haemophilus influenzae* and *Moraxella catarrhalis*, oleuropein was ineffective at the concentrations tested (0.015 to 500 ìg.mL^{-1}).

In the supplementation of olive leaves in the diet of chickens, after slaughtering the thighs and drumsticks of the chickens that received olive leaves showed greater microbiological stability than the control, in which 5 g/kg prevented the growth of *Staphylococcus aureus*, psychrotrophic and mesophilic aerobes and 10 g/kg prevented the growth of *Enterococcus spp*., lactic acid bacteria, thermotolerant and total coliforms, *Pseudomonas, Clostridium perfringens* and *Escherichia coli* ($p < 0.05$). The results indicate the feasibility of using olive leaves as a food supplement, improving the microbiological quality of chicken meat. The advantages of using olive leaves were granted due to the presence of polyphenols and oleuropein (Marangoni *et al*., 2015).

CONCLUSIONS

Oleuropein is a bitter glycoside, present in all parts of the olive tree, but mainly in the leaves, and studies indicate that this phenolic compound has important anti-inflammatory, antimicrobial and antiviral activities, among others. Its ability to act as a natural antioxidant has also attracted the attention of researchers, as consumers increasingly seek natural products or those that contain natural constituents in their formulation, prompting the food and cosmetics industry to consider replacing synthetic antioxidants with those extracted from vegetable sources. This phenol can be extracted by techniques involving the use of cold solvents, Soxhlet, ultrasound, supercritical fluids, pressurized fluid, microwave, microfluidic system and filtration. Extraction of phenolic compounds such as oleuropein is influenced by several experimental parameters depending on these techniques, such as time, temperature and type of solvent. Studies aiming at obtaining this compound have been conducted, seeking the optimization of the process as well as the search for "clean" technologies that use non-toxic solvents and present a low cost.

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