Extraction of oleuropein from olive leaves and applicability in foods

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REVIEW ARTICLE

Abstract

Oleuropein is a phenolic compound found in all parts of the olive tree (*Olea europaea* L.), although at higher levels in the leaves. The health benefits associated with the consumption of oleuropein include the prevention of cardiac diseases, improvement in lipid metabolism, and decrease in obesity-related disturbs, among others. In addition, several studies have shown that oleuropein presents antimicrobial, antioxidant, and inflammatory properties. The scientific interest in the methods for the extraction of oleuropein from olive leaves has markedly increased in recent years, aiming to extend its application in foods, cosmetics, and drugs. In this review, the extraction procedures available in the literature are described according to their advantages and disadvantages that directly affect the extraction yield. The applicability of oleuropein in food products is also discussed.

Keywords: antioxidants; extraction techniques; food applications; phenolic compounds; oleuropein

Introduction

Oleuropein (Figure 1) is a phenolic compound mostly found in olive (*Olea europaea* L.) leaves (Aouidi et al., 2012), being classified as the ester formed by 3,4-dihydroxyphenyl ethanol (hydroxytyrosol) and the glucoside of elenolic acid (Tan *et al*., 2003). The compound was firstly detected in olives in 1908 and described by Bourquelot and Vintilesco as a green thin solid with a melting point of 89°C (Leonardis *et al*., 2008). Oleuropein is almost absent in olive oil due to its high solubility in water and enzymatic degradation during oil extraction (Paiva-Martins and Pinto, 2008). Olive leaves may contain 60–90 mg/g of oleuropein in dry mass (Ansari *et al*., 2011). However, the oleuropein content depends on several factors, including olive variety, plant region, season, olive maturation during harvesting, and the type of olive processing (Al-Rimawi *et al*., 2014; Hassen *et al*., 2015). During the maturation of olives, oleuropein is biotransformed through hydrolysis into hydroxytyrosol, also known as 3,4-dihydroxyphenyl ethanol, which is also reported as a potential antioxidant agent found in olive oil (Leonardis *et al*., 2008). In this process, the ester bond linking hydroxytyrosol to the glucoside of elenolic acid is hydrolyzed, and hydroxytyrosol is released along with the elenolic acid glucoside (Hassen *et al*., 2015). Natively, hydroxytyrosol is rarely available freely, except for olives in the advanced maturation stage, due to the oleuropein hydrolysis. The faster the olive maturation, the higher is its content in hydroxytyrosol (Artajo *et al*., 2007).

Extracts from leaves rich in biophenols have protective effects in foods with a high content of unsaturated fat, also providing health benefits to the consumers (Jimenez *et al*., 2011; Nunes *et al*., 2016). Several studies have revealed that oleuropein is effective against bacterial cells (Dominciano *et al*., 2016; Tripoli *et al*., 2005), virus (Micol *et al*., 2005), and inflammatory processes (Visioli *et al*., 2002), among others. Moreover, oleuropein can avoid cardiac diseases, improve lipid metabolism, minimize

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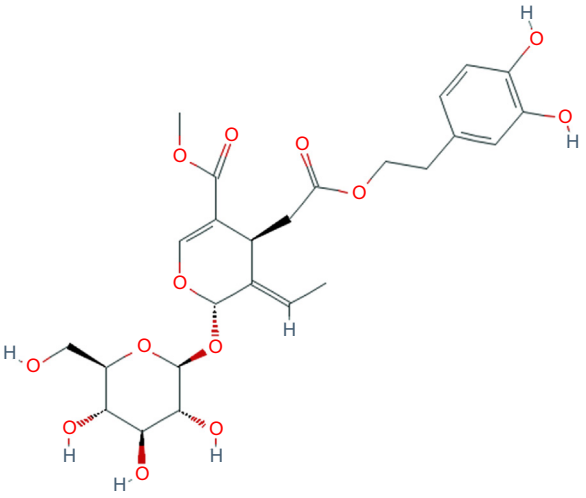

Figure 1. Chemical structure of oleuropein (National Center for Biotechnology Information 2020).

troubles related to obesity, and protect enzymes and cells from oxidizing reactions (Japón-Luján and Luque De Castro, 2007). It was demonstrated that oleuropein and other compounds found in the olive leaves acted as antioxidant agents, preventing the formation of free radicals (Al-Rimawi *et al.*, 2017a, 2017b) due to their efficacy in chelating metals, such as copper and iron, and catalyzing reactions of free radical production such as lipid oxidation (Mokhtar *et al.*, 2014). The antioxidant activity is credited for the presence of the *o*-diphenolic group in the oleuropein molecule (Hayes *et al.*, 2011). Oleuropein and its derivative hydroxytyrosol have demonstrated synergistic effects with vitamin C and tocopherol (vitamin E) (Japón-Luján and Luque De Castro, 2007). In corroboration with potential food applications, the human absorption of oleuropein-aglycone, hydroxytyrosol, and tyrosol is up to 60% (Vissers *et al.*, 2002), being rapidly distributed among several organs and tissues, including the heart, brain, and liver (Bazoti *et al.*, 2010). These properties have stimulated the development of suitable and efficient methods to extract oleuropein from olive leaves, each one having advantages and disadvantages that directly affect the extraction yield. Sampling, solvent composition, time, and extraction temperature are the main parameters affecting the efficiency of the extraction methods for oleuropein (Mustafa and Turner, 2011; Xynos *et al.*, 2012). The aim of this review was to describe and critically evaluate the oleuropein extraction procedures available in the literature and discuss the applicability of this compound in food products.

Methods for Extraction of Oleuropein from Olive Leaves

An overview of the most applied procedures for the extraction of oleuropein from olive leaves is illustrated in Figure 2. The most commonly used techniques involve conventional solid–liquid extraction and ultrasound-assisted extraction. Moreover, emerging technologies including microwave-assisted, supercritical fluid, pressurized liquid and membrane separation techniques have attracted considerable research interest regarding their potential use for oleuropein extraction.

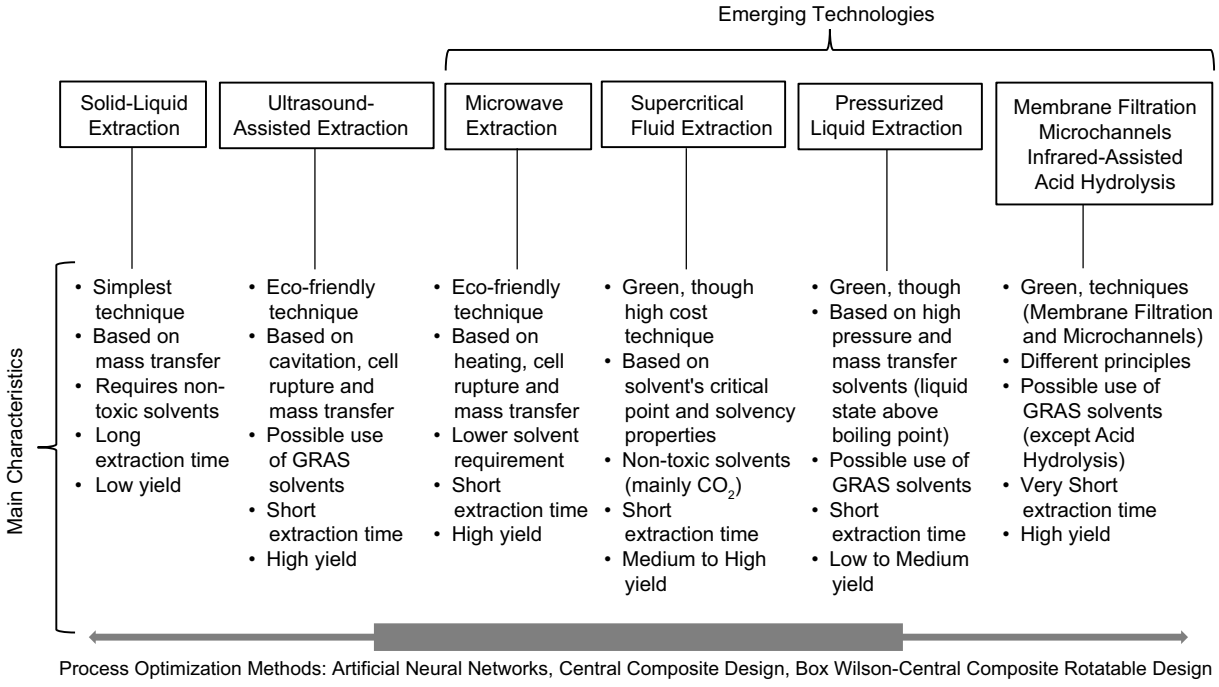

Figure 2. The main characteristics of the methods used for extraction of oleuropein from olive leaves.

Solid–liquid extraction

Solid–liquid extraction is considered as one of the simplest techniques for the isolation of bioactive compounds, based on the mass transfer between the solvent and the solid sample during a suitable extraction time, with or without mixing or heating (Coppa *et al*., 2017; Japón-Luján and Luque De Castro, 2006). This method is widely employed for the extraction of oleuropein, although it requires a high-quality solvent and a long extraction period when compared to other methods (Prado *et al*., 2013). Moreover, it is crucial to extract oleuropein from olive leaves using nontoxic solvents, since this phenolic compound is mainly used in food products for human consumption (Coppa *et al*., 2017). Table 1 presents the main outcomes from the studies available in the literature on the solid–liquid extraction methods for oleuropein and their respective yields. The Soxhlet technique and the cold solvent method are the most commonly used solid–liquid procedures for oleuropein extraction (Otero *et al*., 2020). Sahin *et al*. (2011) carried out the extraction of phenolic compounds from vegetable sources with the Soxhlet method using methanol (100%) and supercritical fluid, with the former technique providing better yields (37.8 mg/g of oleuropein). However, a mixture of ethanol, isopropanol, and water (50:25:25) provided a lower yield (18.5 mg/g) of oleuropein (Uzel, 2018). Recently, Lama-Muñoz *et al*. (2019, 2020) extracted oleuropein using the Soxhlet procedure from olive leaves employing a mixture of ethanol and water (60:40), reaching 65.6 to 122.3 mg/g of oleuropein.

The cold solvent extraction, also known as maceration, is frequently applied for the extraction of bioactive compounds from solid vegetable matrices (Rahmanian *et al*., 2015). However, the main drawbacks involved in the oleuropein extraction by maceration are the requirement of long extraction time and the low yield (Ávila *et al*., 2007; Maran *et al*., 2013). Ansari *et al*. (2011) extracted oleuropein from olive leaves by maceration at 60°C with different solvents and extraction times. Deionized water as the solvent and 4 h of extraction time resulted in the best yield (13 mg/g) as longer times caused oleuropein oxidation. Benincasa *et al*. (2019) used distilled water and obtained a slightly higher yield (19.3 mg/g) of oleuropein, when compared with the values reported by Ansari *et al*. (2011). A different approach was studied by Jimenez *et al*. (2011), who scalded the olive leaves at 95°C for 4.5 min, followed by cooling with cold water before drying at 45°C for 18 h. About 760 g of dried leaves were grounded and macerated in ethanol: water solution (1:1 v/v) for 24 h at room temperature. Under these conditions, a high concentration (290 mg/kg) of oleuropein was obtained in the final extracts. Coppa *et al*. (2017) macerated olive leaves with ethanol and water (70:30) at 25°C for 120 min, obtaining a yield of 180 mg/g in the freeze-dried extract. Compared with the previously mentioned finding, Deng *et al*. (2017) observed a lower yield (5.2 mg/g) using methanol and water (80:20) at 50°C during a longer extraction time (282 min).

Stamatopoulos *et al*. (2012) also extracted oleuropein using ethanol and water solution (7:3 v: v) under agitation for 5 min. However, the authors performed a steam bleaching as a pretreatment to facilitate extraction of the phenolic compounds from olive leaves. As a result, the oleuropein content in the extracts and its antioxidant

Table 1. Experimental conditions and yields of solid–liquid extraction of oleuropein from olive leaves.

Solid–liquid extraction method	Experimental conditions			Yield (mg/g)	Reference
	Temperature (°C)	Solvent (proportion)	Time (minutes)		
Soxhlet extraction	50	Methanol (100%)	NR	37.8	Sahin *et al*. (2011)
Soxhlet extraction	160	Ethanol: isopropanol: water (50:25:25)	120	18.5	Uzel (2018)
Soxhlet extraction	NR	Ethanol: water (60:40)	240	65.6	Lama-Muñoz *et al*. (2019)
Soxhlet extraction	NR	Ethanol: water (60:40)	240	122.3	Lama-Muñoz *et al*. (2020)
Maceration	60	Deionized water (100%)	4	13	Ansari *et al*. (2011)
Maceration	RT	Ethanol: water (50:50)	1440	290.0	Jimenez *et al*. (2011)
Maceration[1]	40	Ethanol: water (70:30)	60	9.2	Stamatopoulos *et al*. (2012)
Maceration[1,2]	40–85	Ethanol: water (70:30)	30	103.1	Stamatopoulos *et al*. (2013)
Maceration	25	Ethanol: water (70:30)	120	180.0[3]	Coppa *et al*. (2017)
Maceration	50	Methanol: water (80:20)	282	5.2	Deng *et al*. (2017)
Maceration	60	Distilled water (100%)	NR	19.3	Benincasa *et al*. (2019)

[1]Steam bleaching was performed for 10 min, as a pretreatment of the olive leaves.
[2]Performed using multiple extraction stages.
[3]Relative to the freeze-dried extract.
NR: not reported; RT: room temperature.

potential were 35 and 13 times superior, respectively, when compared to extracts without the pretreatment. Although this procedure provides shorter extraction times and simpler techniques, the authors pointed out that pretreatment for over 10 min can degrade the oleuropein. Subsequently, Stamatopoulos *et al*. (2013) isolated oleuropein from olive leaves by multiple extraction stages optimized by a whitening technique, in which leaves were submitted to three extraction stages performed with 60 min duration time per stage, using different ethanol solutions (20, 40, 55, 70, 80, and 90%, v/v) and temperatures of 40, 60, 65, 70, and 85°C. At each stage, samples were collected every 10 min, filtered, and characterized in terms of phenolic composition by the Folin–Ciocalteau assay. The best conditions were 30 min of extraction at 85°C using solvent ethanol 70%, reaching an oleuropein yield of 23 times higher (103.1 mg of oleuropein/g of dried mass) than the value (4.6 mg of oleuropein/g of dried mass) obtained in the conventional method (48 h at 40°C). However, according to the authors, it is economically preferable to conduct the extraction at 40°C since the yield was only 17% greater than when using a temperature of 85°C.

Ultrasound-assisted extraction

In the food industry, ultrasound is a technique widely used for the extraction of vitamins A, D, and E; antioxidant compounds; flavonoids; phenols; polysaccharides; alkaloids; and natural compounds used as additives in several food products (Yang and Zhang, 2008). The extraction process using ultrasound equipment is a simple procedure that consists in placing the solid of interest in contact with the solvent in a glass-made vessel inside the apparatus, allowing easy handling and control of the time and temperature desired (García-Salas *et al*., 2010; Klejdus *et al*., 2009). The equipment produces the cavitation phenomenon, which will cause mechanical stress on cells, a consequent of increase of their dilatation, leading to cell rupture and hydration of the material, and facilitating the mass transfer between the solid material and the extractor solvent (Esclapez *et al*., 2011). It is also possible to substitute the organic solvent for generally recognized as safe (GRAS) products to improve the extraction (Vilkhu *et al*., 2008). According to Shirsath *et al*. (2012), the device frequency is a crucial parameter for the extraction, as low frequencies of about 20 kHz are more effective on plant materials, as olive leaves. Due to the effects promoted by cavitation, the bubbles can implode more easily than those generated at high frequency, thus facilitating the release of the substances of interest (Esclapez *et al*., 2011). The ultrasound-assisted extraction of oleuropein has been performed either using ultrasonic bath or homogenizer (probe) devices, as presented in Table 2. By using methanol as the extraction solvent, Jerman *et al*. (2010) obtained 12.3 mg/g of oleuropein in a ultrasound bath for 20 min. Achat *et al*. (2012) obtained a much higher yield of oleuropein (414.3 mg/g) after maceration of olive in ultrasound bath at 16°C for 45 min, which is comparable to solid–liquid extraction yields. The highest yield (812.9 mg/g) was described by Yasemi *et al*. (2017), who extracted oleuropein in methanol: water (80:20) using an ultrasound bath of 25 kHz. In contrast, lower yields of 134.5 and 7.0 mg/g using ultrasound bath were reported by Ismaili *et al*. (2016) and Deng *et al*. (2017), respectively. Other applications of ultrasound bath extraction methods yielded 27.3 mg/g (Cifà *et al*., 2018) and 106.5 mg/g (Irakli *et al*., 2018).

Table 2. Experimental conditions and yields of ultrasound-assisted extraction of oleuropein from olive leaves.

Device (frequency, kHz)	Experimental conditions			Yield (mg/g)	Reference
	Temperature (°C)	Solvent (proportion)	Time (minutes)		
UB (30)	44	Methanol (100%)	20	12.2	Jerman *et al*. (2010)
UB (25)	16	Olive oil (10%)	45	414.3	Achat *et al*. (2012)
UB (NR)	NR	Acetonitrile: tetrahydrofuran (50:50)	30	134.5	Ismaili *et al*. (2016)
UB (NR)	47	Methanol: water (80:20)	30	7.0	Deng *et al*. (2017)
UB (80)	25	Methanol: water (80:20)	10	812.9	Yasemi *et al*. (2017)
UB (35)	25	Ethanol: water (70:30)	120	27.3	Cifà *et al*. (2018)
UB (37)	60	Acetone: water (50:50)	10	106.5	Irakli *et al*. (2018)
UH (20)	50	Ethanol: water (75:25)	3	76.7	Xie *et al*. (2015)
UH (20)	60	Ethanol: water (80:20)	5	11.4	Giacometti *et al*. (2018)
UH (40)	20	Ethanol: water (60:40)	17.91	69.9	Lama-Muñoz *et al*. (2019)

UB: ultrasonic bath; UH: ultrasonic homogenizer (probe); NR: not reported.

Regarding the extraction using ultrasonic homogenizers, a yield of 76.7 mg/g was obtained by Xie *et al*. (2015) using ethanol and water (75:25) in a very short time (3 min). Other solvent combinations in ultrasound-assisted extractions with probes included ethanol and water at 80:20 (Giacometti *et al*., 2018) and 60:40 (Lama-Muñoz *et al*., 2019), although their respective yields (11.4 and 69.9) were lower than those previously mentioned.

Extraction by emerging technologies

In recent years, other nonconventional technologies have been proposed for the extraction of oleuropein, including microfluid systems (microchannels) and infrared-assisted methods (Table 3). In particular, microchannels have gained extensive attention because of higher yields of oleuropein achieved, low operational costs, and low environmental impact of the procedure (Yasemi *et al*., 2017). In addition, intense research activities are currently being addressed to include special statistical approaches for the optimization of the extraction of oleuropein, such as Artificial Neural Networks (Yasemi, 2020), Central Composite Design (CCD) (Lamprou *et al*., 2020), and Box Wilson-Central Composite Rotatable Design (Vural *et al*., 2020).

Microwave-assisted extraction

Similar to ultrasound, microwave-assisted techniques are also widely applied for the extraction of bioactive compounds from vegetal matrices.

Microwave extraction can be assumed to be an eco-friendly technique due to the low solvent requirement. This extraction can provide a good yield, since the heating caused by the waves leads to distension of the plant cells, causing the rupture of the structures where the compounds of interest are concentrated (Alupului *et al*., 2012). Moreover, this method requires a reduced extraction time when compared to other techniques (Ghassempour *et al*., 2008). According to Pérez-Serradilla and Luque de Castro (2011), the microwave extraction improves the extraction efficiency of phenolic compounds in a shorter time concerning conventional techniques that demand about 24 h of extraction. Rafiee *et al*. (2011) tested the extraction of phenolic compounds from olive leaves using a microwave device and different solvents, in which ethanol provided the best results with an extraction yield of 88.3 mg/g after 15 min of microwave exposure. Sahin *et al*. (2017) used response surface methodology (RSM) and artificial neural networks to optimize the yield extraction of oleuropein from olive leaves by

Table 3. Experimental conditions and yields of emerging technologies for the extraction of oleuropein from olive leaves.

Type of technology	Experimental conditions			Yield (mg/g)	Reference
	Temperature (°C)	Solvent (proportion)	Time (minutes)		
MAE (NR)	NR	Ethanol (100%)	15	88.3	Rafiee *et al*. (2011)
MAE (250–300 W)	NR	Water (100%)	2	60	Sahin *et al*. (2017)
SFE (300 bars)	NR	CO_2 (with 20% ethanol)	10	51	Xynos *et al*. (2012)
SFE (150 bars)	35	CO_2 (100%)	NR	360	Baldino *et al*. (2018)
PLE (300 bars)	115	Ethanol (100%)	5	43	Xynos *et al*. (2012)
	150	Water (100%)	5	34	
PLE (103 bars)	120	Ethanol: water (50:50)	20	NR	Lozano-Sánchez *et al*. (2014)
PLE (103 bars)	60	Ethanol (100%)	NR	73.6	Rosa *et al*. (2019)
MF (5000 Da)	NR	None	NR	2.6[1]	Khemakhem *et al*. (2017)
MF (300 Da)	NR	None	NR	26.2[1]	Khemakhem *et al*. (2017)
IAE	90	Ethanol: water (55.35:44.65)	221	14.0	Abi-Khattar *et al*. (2019)
Microchannels (inner diameter: 800 µm; length: 8.5 mm)	RT	Deionized water (100%)	<1	68.7[2]	Naleini *et al*. (2015)
Microchannels (inner diameter: 600 µm; length: 70 mm)	35	Ethyl acetate (100%)	0.5	96.3[2]	Yasemi *et al*. (2017)
Microchannels (inner diameter: 800 µm; length: 8.5 mm)	40	Deionized water (100%)	<1	70.9[2]	Heydarid *et al*. (2018)
Acid hydrolysis	40	2% H_2SO_4	NR	43.1	Lamprou *et al*. (2020)

[1]Values are relative to the oleuropein content in the retentate.
[2]Values expressed as percentages.
MAE: microwave-assisted extraction; SFE: supercritical fluid extraction; PLE: pressurized liquid extraction; MF: membrane filtration (values in brackets are pore sizes); IAE: infrared-assisted extraction; NR: not reported.

microwave-assisted extraction. The RSM design and the artificial neural networks model were found to be suitable for predicting the oleuropein amounts and the increment/decrement tendency of the extraction responses, with a maximum yield value of 60 mg/g of oleuropein. In particular, artificial neural networks models have been frequently used to optimize and control the oleuropein extraction process, aiming to save energy and time, also providing a more favorable product (Yasemi, 2020).

Supercritical fluid extraction

The extraction using supercritical fluid offers a great advantage related to the use of organic solvents, since it is possible to reduce their quantity, besides occurring low degradation of the target compounds (Reverchon and De Marco, 2006). In this method, the supercritical fluid is used as the solvent, since its solubility is similar to the liquid diffusivity and the gas (mainly carbon dioxide [CO_2]), thus allowing the dilution of a great variety of natural compounds (Zhang *et al*., 2018). In addition, it has solvency properties that change radically close to its critical points due to changes in temperature and pressure (Zhang *et al*., 2018). Supercritical fluid extraction is also considered a green technique due to the use of nontoxic solvents (El and Karakaya, 2009). Furthermore, if nonpolar compounds are used, it is possible to reduce energy expenditure on extraction (Khosravi-Darani and Mozafari, 2009). The most commonly used solvent for the extraction of oleuropein in supercritical media is CO_2, which is inert, nonflammable, and environmentally friendly (Ignat *et al*., 2011). Xynos *et al*. (2012) evaluated the extraction of oleuropein from olive leaves by supercritical fluid, testing nontoxic solvents such as CO_2, water, and ethanol (Table 3). The authors were able to extract 30% of oleuropein in the dry extract (equivalent to the recovery of 51 mg of oleuropein per gram of olive leaves), with 20% ethanol as co-solvent and supercritical CO_2 at 300 bars. However, Baldino *et al*. (2018) obtained higher yields using supercritical CO_2 at 150 bars and 35°C, with a maximum yield of 360 mg/g of oleuropein. A possible disadvantage of the extraction methods based on supercritical CO_2 is the requirement of high pressures, which increases the cost of the equipment and, consequently, the final product (Herrero *et al*., 2006).

Pressurized liquid extraction

Extraction with pressurized liquid is also known as accelerated solvent extraction, enhanced solvent extraction, fluid extraction, or high-pressure solvent extraction (Zhang *et al*., 2018). This technique applies high pressures during the extraction process, which preserves the solvents in the liquid state above their boiling point, resulting in high solubility and diffusion rates, and the introduction of the solvent into the matrix. In this method, the extraction time is reduced, and the solvent exhibits better behavior compared to the other techniques (Zhang *et al*., 2018). Pressurized liquid can be heated to elevated temperatures and pressures, allowing the solvent to remain in its liquid state even at higher temperatures (above boiling temperature). When water and ethanol are subjected to 200 bar pressure and up to 200°C, the liquids remain liquid, even under conditions above the atmospheric and boiling limits (Coppa *et al*., 2017). When this technique is used to extract compounds from plant sources, it is possible to facilitate the solubility of the components of interest with the solid matrix, by accelerating the release kinetics of the bioactive compounds from the solid plant material. The extraction of oleuropein through pressurized liquid technique has been used with "green" solvents, such as ethanol and water under pressures not exceeding 200 bar and temperatures varying from 25°C to 200°C, as presented in Table 3.

Xynos *et al*. (2012) compared the results obtained by the extraction of oleuropein from olive leaves through pressurized liquid using ethanol or water as solvents. Maximum yields of 43 and 34 mg/g were obtained using ethanol at 115°C and water at 150°C, respectively. It can be observed from these results that, using the pressurized liquid technique, a higher oleuropein yield was obtained due to the high pressures applied in this type of extraction. In spite of the great yields obtained using this method, the high temperatures applied can originate several undesirable compounds due to reactions in the matrix, which may also be extracted along with oleuropein (Herrero *et al*., 2013). Lozano-Sánchez *et al*. (2014) carried out a study to recover bioactive compounds from olive leaves by pressurized liquid extraction at 103 bars using different combinations of ethanol and water at temperatures ranging from 40 to 175°C. The best solvent proportion and temperature were ethanol: water (50:50) and 120°C, respectively. Although the authors reported that the main compounds found in the extracts were oleuropein and hydroxytyrosol, no quantification or extraction yields for oleuropein were provided. Rosa *et al*. (2019) also used pressurized liquid extraction with ethanol (100%) at 103 bars and 60°C and observed a yield of 73.6 mg/g of oleuropein.

Extraction with other emerging techniques

Among new extraction technologies, methods based on separation by membranes have been recently proposed for oleuropein and other bioactive compounds available in aqueous solutions (Otero *et al*., 2020). In membrane separation, the selective permeation of dissolved molecules is achieved through a semipermeable polymer or inorganic support, thus providing high concentration and purity of the compound (Yasemi *et al*., 2017). In addition, the process requires low energy consumption, since it is operated at low temperatures (Otero *et al*., 2020). Khemakhem *et al*. (2017) extracted oleuropein

by ultrafiltration (pore size of membrane: 5000 Da) and nanofiltration (pore size of membrane: 300 Da) processes, obtaining 10 times higher levels of oleuropein in the retentate from nanofiltration than from the nanofiltration process (yields of 2.6 and 26.2 mg/g, respectively).

An infrared-assisted extraction was proposed by Abi-Khattar *et al*. (2019) using ethanol: water (55.35:44.65) at 90°C for 221 min. The process was previously optimized by RSM in order to increase the recoveries of oleuropein and other polyphenols from olive leaves, yielding 14.0 mg/g of oleuropein. Microchannel devices have also been used for the extraction of oleuropein, since these systems are based on the principle that lower molecular distance leads to greater diffusion, increasing the possibility of mass transfer (Otero *et al*., 2020). Naleini *et al*. (2015) developed a microfluid device to extract oleuropein from ethyl acetate into aqueous phase, obtaining a yield of 68.7%. The authors stated that microchannel methods are efficient and more economical and environmentally friendly than conventional techniques applied for oleuropein extraction. In another study conducted by Yasemi *et al*. (2017), microchannels provided a higher yield of oleuropein (96.3%) after optimization of the operating parameters by RSM, when compared with Soxhlet, maceration, and ultrasound-assisted techniques. RSM optimization of microchannel extraction of oleuropein was also performed by Heydarid *et al*. (2018), who obtained a maximum extraction yield of 70.9% under the following conditions: deionized water as extractant phase, temperature of 40 °C, 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%.

A new method for extracting phenolic compounds from olive leaves based on acid hydrolysis by sulfuric acid was proposed by Lamprou *et al*. (2020). The authors optimized the experimental conditions according to a CCD comprising the most important factors that affect the extraction process, and the resulting yield of oleuropein was 43.1 mg/g.

Application of Oleuropein in Foods

The beneficial effects of oleuropein have attracted considerable research interest in recent years, aiming at its application as a natural product in many industrial areas, especially in foods (Erbay and Icier, 2010). The majority of studies on the potential food applications evaluated the incorporation of olive leaves' extracts containing known levels of oleuropein and its derivatives, as presented in Table 4. However, there is limited information on the use of purified oleuropein in food formulations. An important application of oleuropein-containing extracts is the improvement of the nutritional value of foods by increasing their polyphenol content. In this context, Chiou *et al*. (2007) enriched the palm tree, olive tree, and sunflower oils with olive leaf extracts containing 120 and 240 mg of total polyphenols/kg of oil, and observed high transference rates from the supplemented oils to potato chips during the frying process. This result was in agreement with the findings from Erbay and Icier (2010), who observed that polyphenols did not degrade significantly after absorption by fried foods. In fact, the frying process of potatoes in olive, sunflower, and soya oils enriched with 100 mg of total phenolic compounds/kg of oil retained 3–12% of oleuropein (Nunes *et al*., 2016). Furthermore, the consumption of approximately 200 g of these chips provided nearly 6 mg of oleuropein. However, it has been demonstrated that, throughout the frying process, a reduction in the oleuropein contents may occur, both in the oil and in the potatoes (Chiou *et al*., 2013; Nunes *et al*., 2016; Rahmanian *et al*., 2015). Ahmad-Qasem *et al*. (2014) also used olive leaves' extract rich in oleuropein at 19,280 mg/kg to improve the nutritional value of dehydrated apple cubes, concluding that the final products presented higher oleuropein contents (about 1.928 mg/100 g dry weight), when compared with controls.

Oleuropein as a natural antioxidant can be a potential replacement for synthetic food antioxidants, such as butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT), considering that their promotion effects on carcinogenesis have been speculated (Farag *et al*., 2007). According to Erbay and Icier (2010), the combined antioxidant activities of oleuropein and its derivative hydroxytyrosol are higher than BHA or BHT. Jimenez *et al*. (2011) reported the oxidative stability of sunflower, canola, and soybean oils supplemented with three types of olive leaf extracts: hydroalcoholic extract, pressed juice, and extracts obtained by supercritical CO_2. Each extract contained polyphenols at concentrations of 250 and 630 mg/kg of oil. For the extracts containing 250 mg/kg of oil, the induction times of the sunflower oil containing hydroalcoholic extracts, extract obtained from CO_2 supercritical, and pressurized juice were 21.1, 26.3, and 2.7 h, respectively. For canola oil containing the same order of extracts, the results were 9.5, 12.6, and 1.8 h, respectively. For soybean oil, induction times of 6.7, 6.9, and 1.7 h were found in the same order as the extracts described above. For the polyphenol concentration of 630 mg/kg oil, sunflower oil with hydroalcoholic extract, supercritical CO_2, and pressurized juice resulted in induction times of 30.7, 35.1, and 3.0 h, respectively. For the canola oil, the induction times were 11.0, 13.4, and 2.0 h, while for the soybean oil, the times were 7.6, 8.4, and 1.4 h for the same extract order as mentioned, respectively. These data are consistent with those reported previously by Salta *et al*. (2007), who evaluated the stability of vegetable oils enriched with olive leaves'

Table 4. The main applications of olive leaves' extracts containing known levels of oleuropein as a food additive.

Type of food	Oleuropein concentration (mg/L or kg)	Main outcomes	Reference
Palm oil, olive oil, and sunflower oil	120–240	High transference rates of oleuropein from the supplemented oils to the enriched potato chips during the frying process	Chiou *et al*. (2007)
Olive oil, palm oil, sunflower oil, and vegetable shortening	200	Antioxidant and oxidative capacities increased in types of oils, especially in the olive oil	Salta *et al*. (2007)
Sunflower, canola, and soybean oil	250 and 630	Oxidative stability (expressed as induction times) increased in all types of oils, with maximum values (6.7–35.1 h) at 630 mg/L	Jimenez *et al*. (2011)
Dried apple	19,280	Incorporation of oleuropein improved the nutritional value of dried apples, reaching 1928 mg/100 g dry weight	Ahmad-Qasem *et al*. (2014)
Cow's milk and yogurt	100 and 200	Oleuropein improved the functionality of milk and yogurt, without any effect on their sensory attributes	Zoidou *et al*. (2014)
Tabaq-Maz (fried mutton ribs)	3000, 6000 and 9000	Increase of lipid oxidative stability and storage quality, and reduction of mesophilic and psychrophilic counts, as well as mold and yeast counts by all oleuropein levels tested	Dua *et al*. (2015)
Extra-virgin and refined olive oil	30 and 60	Oxidative stability (expressed as induction times) increased in extra-virgin and refined oils at 30 and 60 mg/L	Coppa *et al*. (2017)
Frozen hamburger	2500, 5000, and 7500	Effective antioxidant activity at oleuropein levels ≥ 5000 mg/kg	Al-Rimawi *et al*. (2017a)
Fresh hamburger stored at 4°C	500, 1000, and 1500	Effective antioxidant activity and prolonged shelf life of hamburgersat 1500 mg/kg	Al-Rimawi *et al*. (2017b)
Pasteurized milk	3600	Oleuropein reduced mesophilic bacteria to an undetectable level after 6 days	Palmeri *et al*. (2019)
Halal minced beef	854 and 4272	Effective antioxidant activity at 4272 mg/kg, along with reduction of nearly 1.5 and 3 log cycles of pathogens (*Salmonella enterica* ser. Enteritidis, and *Escherichia coli* O157:H7) and psychrotrophic counts, respectively	Djenane *et al*. (2019)
Vegetable pâté	500 and 1000	Reduction of 0.5–1.0 log cycles of mesophilic bacteria, lactic acid bacteria, Streptococci, Enterococci, molds and yeasts, especially at 1000 mg/kg	Difonzo *et al*. (2019)

extracts, which contained about 185 mg oleuropein/kg of oil. Before the addition of the extracts in olive and palm oil, the samples had induction times of 13.3 and 24 h, respectively. After the enrichment of the oils, their stability increased to 20.8 and 43.1 h, respectively, confirming that the addition of extracts of olive leaves in vegetable oils contribute to the increase of their oxidative stability. Coppa *et al*. (2017) added lyophilized extracts rich in oleuropein in extra-virgin and refined olive oils, to verify the performance of the compound as a natural antioxidant. The results showed that the supplemented extra virgin and refined olive oils had 2-h and 1-h increases in their induction times, respectively, thereby confirming that the addition of oleuropein-containing extract increases the oxidative stability of olive oil.

The inclusion of 500 and 1000 mg/kg of oleuropein in vegetable pâtés reduced 0.5–1.0 log cycles of mesophilic bacteria, lactic acid bacteria, Streptococci, Enterococci, molds, and yeasts in the product, in a dose–response relationship (Difonzo *et al*., 2019). Oleuropein is also applicable as a natural preservative for pasteurized milk, since its fortification at 3600 mg/L reduced total mesophilic bacteria to an undetectable level after 6 days and increased its shelf life by 60% (Palmeri *et al*., 2019). Regarding meat products, Al-Rimawi *et al*. (2017a) evaluated the replacement of sodium erythorbate, a chemical antioxidant used in the meat industry, with oleuropein-rich olive leaves' extracts as a natural antioxidant in frozen hamburgers stored at –12°C. The samples were treated with different concentrations of oleuropein (2500, 5000, and 7500 mg/kg), and the storage period was from 1 to 6 months. The concentration of 5000 mg/kg oleuropein presented the best antioxidant effect in the experimental hamburgers, which could be used as an alternative natural additive in this food. A similar delay in the oxidation process was observed in fresh hamburgers added with olive leaf extract containing oleuropein at 5000 mg/kg and stored at 4°C (Al-Rimawi *et al*., 2017b). Dua *et al*. (2015) found that oleuropein increased the lipid oxidative stability and the storage quality of fried mutton ribs (Tabaq-Maz), and significantly reduced the mesophilic and psychrophilic counts, as well as the mold and yeast counts. In a recent study, Djenane *et al*. (2019)

observed that oleuropein at 4272 mg/kg in Halal minced beef reduced nearly 1.5 and 3 log cycles of pathogens (*Salmonella enterica* ser. Enteritidis and *Escherichia coli* O157:H7) and psychrotrophic counts, respectively.

Nunes *et al*. (2016) studied the effect of olive leaf extracts containing about 21% oleuropein and α-tocopherol on biodegradable films. A small interaction was observed between the polymer matrix and the antioxidants evaluated, besides the migration of the antioxidants to the food. This fact suggests that the material is only suitable for foods with a shorter shelf life (Marcos *et al*., 2014). Encapsulating bioactive compounds can help improve their properties, such as controlling the release of chemical constituents (Chatzidaki *et al*., 2016). As an example, Mohammadi *et al*. (2016) stated that the encapsulation of bioactive compounds could reduce the unpleasant flavors of oils enriched with olive leaf extracts. In addition, they may help to preserve bioactive compounds from high temperatures employed in the frying process. Several studies have demonstrated that encapsulation increases the stability of oleuropein and hydroxytyrosol in water/oil emulsions, thereby indicating that the technique may help to improve the organoleptic properties of foods added with oleuropein, also protecting bioactive compounds from degradation in the small intestine (Chatzidaki *et al*., 2016; Markopoulos *et al*., 2009; Souilem *et al*., 2014).

In addition to the bitter taste of oleuropein, which may potentially limit its inclusion in food preparations (Ozturk, 2014), the stability of this compound in food processing also deserves attention for deeper studies (Erbay and Icier, 2010). Zoidou *et al*. (2014) conducted the first study on the application of oleuropein in milk and yoghurt, aiming to improve their functional attributes. The authors found that oleuropein remained stable during milk heating and coagulation, and was not hydrolyzed by acid production neither metabolized by lactic acid bacteria nor inhibit its growth. Oleuropein was soluble at concentrations of 0.1 mg/mL or 0.2 mg/mL without adding any particular taste to the products. According to the authors, from a technological point of view, the presence of oleuropein did not interfere with the milk and yoghurt process, and considering the biological value of the compound, it can be added as an active ingredient in these products for the production of new functional foods with health benefits.

Concluding Remarks

Oleuropein and the related phenolic compounds present in olive leaves' extracts have antioxidant, antimicrobial, and anti-inflammatory activities, among other health benefit effects. Therefore, its potential application in many types of industries has been explored. The most applied procedures for the extraction of oleuropein from olive leaves include solid–liquid, ultrasound-assisted, microwave, supercritical fluid, and pressurized liquid techniques. These procedures have several practical advantages and satisfactory oleuropein yields, although ultrasound-assisted and microwave methods are considered as low cost and environment-friendly techniques due to the use of nontoxic solvents. In recent years, statistical optimization approaches and new emerging technologies have been proposed for the extraction of oleuropein, with microchannels gaining increased research interest because of the higher yields of oleuropein provided, low operational costs, and low environmental impact of the procedure.

The main application of oleuropein extracts in food products is intended to replace synthetic antioxidants and also act as a natural preservative due its antimicrobial activity. As indicated by the outcomes of several studies available in the literature, the use of small quantities of oleuropein can improve the shelf life of products with high fat contents, including margarine, butter, meat, and edible oils, thereby avoiding lipidic oxidation and providing health benefits to the consumers. However, studies addressing food applications using purified oleuropein are strongly needed. The bitterness and the low stability of oleuropein are important limitations for the general use of olive leaves' extracts or pure oleuropein in food preparations. Encapsulation techniques have demonstrated promising results to improve the sensorial properties of supplemented foods and to protect the bioactive compounds in the food matrix. Further studies are needed to evaluate the stability of encapsulated oleuropein in complex food matrices under different storage conditions.

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Conflict of Interest Disclosure

The authors declare that there are no conflicts of interest relevant to this study.

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