

Review

# Olea europea and By-Products: Extraction Methods and Cosmetic Applications

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**Abstract:** Currently, in addition to the use of olive oil in cosmetics, the use of olive-derived bioactives and their incorporation into cosmetics is a growing trend. The olive oil industry produces vast quantities of by-products, such as olive mill wastewater, olive pomace and leaves from which new ingredients may be obtained for cosmetic use. In this way, by-products are revalorized, which contributes to the implementation of a sustainable economy or upcycling. This review intends to provide a detailed overview of the different extraction techniques reported in order to obtain the bioactive compounds of cosmetic value that can be found in olive by-products: fatty acids, tocopherols, polyphenols, phytosterols and squalene. Different extraction techniques are presented, including some traditional techniques (solid–liquid extraction) and more novel or “greener” ones: ultrasound, microwave, supercritical extraction, pressurized fluids and deep eutectic solvents. Additionally, different applications of olive by-products in skin care products are explored: emollient, antioxidant, anti-age, anti-inflammatory, antiviral, antifungal and antibacterial, and the perspective of consumers is also considered since they increasingly demand products formulated with natural ingredients.

**Keywords:** by-products; upcycling; extraction techniques; skin care; consumers



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## 1. Introduction

Virgin olive oils are obtained from the fruit of the olive tree (*Olea europaea* L.) solely by mechanical or other physical means under conditions that do not cause alterations in the oil, especially thermal conditions, and that have not been subjected to any other treatment other than washing, decantation, centrifugation or filtration [1]. Olive oil is a natural emollient that has been used for millennia for the treatment of the skin. This oil has a high percentage of pure oleic acid, which, together with two other essential fatty acids, linoleic and linolenic acid, also present in olive oil, play a fundamental role in maintaining the barrier function of the skin, facilitating the penetration of active substances into the deeper layers, reducing transepidermal water loss (TEWL) and improving the protective function [2]. Other components of olive oil of importance in skin care are antioxidants, especially phenols and polyphenols (tyrosol, hydroxytyrosol (HT), caffeic acid, and oleuropein, among others), in addition to tocopherols. Antioxidants are anti-aging actives used in skincare [3,4]. These compounds can be found both in the oil and in the by-products obtained during its production. The olive oil industry produces large amounts of waste, such as olive pomace, olive mill wastewater and leaves. Wet olive pomace, or “alpeorujo”, is the main by-product obtained by the two-phase centrifugation system during the extraction of virgin olive oil. It contains the solid residue of the olive (consisting of the stone, the skin and the mesocarp) together with the vegetation waters (natural water in the olive plus the water added during the extraction process). Alpeorujo is a semi-solid residue with a high moisture content (55–65%), and it includes a large amount of organic matter in its composition, among which many phenolic compounds can be

found [5]. Therefore, it is a very attractive by-product for obtaining compounds with high added value for cosmetics [6–8]. Olive leaves are waste from the pruning of the olive tree and represent 10% of its total weight [9]. They are usually used as animal feed [10,11]; however, they may be used in other applications, such as cosmetics, pharmaceuticals and food. The concentration of phenolic compounds in olive leaves changes with the quality, origin and variety of the olive tree [12]. Furthermore, many of the compounds found in olive leaves show a wide range of biological activity in addition to their antioxidant capacity, such as antifungal, antibacterial [13], antiviral [14] and therapeutic [15,16].

Therefore, this review compiles the cosmetic applications of the olive industry by-products as well as the main methods for obtaining products with cosmetic relevance from them. A final section on the perception of consumers about cosmetic products with active ingredients derived from olives is included.

## 2. Olive By-Products for Skin Care

The compounds derived from the olive tree, which are used in cosmetics because of their properties, can be categorized as hydrophilic and lipophilic agents. Hydrophilic compounds with important cosmetic uses are mainly polyphenols. The group of lipophilic compounds includes fatty acids, vitamins and squalene. All of them are extensively used in cosmetics and can be found in the olive, in olive leaves and branches, in the olive stone and in by-products that include olive mill wastewater (OMWW), alpeorujo, olive pomace and cake. Table 1 lists some of the bioactive compounds obtained from the olive and by-products and some of the more common cosmetic uses.

**Table 1.** Some of the bioactive compounds obtained from the olive and by-products and some of the more common cosmetic uses.

Compound	Present in	Use	Reference
Phenols and Polyphenols *	Bark, root, leaves, wood, stones, pomace, OMWW	Antioxidant, anti-aging, antiplatelet aggregation activity, anti-cancer activity, antimicrobial activity, cardioprotective activity, free radical scavenging activity, protect and reduce skin thickening and wrinkles, fibroblast proliferation	[6–8,17–21]
Triterpenes	Dry leaves (as herbal infusions)	antimicrobial, anti-tumor, anti-inflammatory, and anti-HIV	[20]
Pectins and oligosaccharides	Pomace, OMWW	Improve physical and structural properties of emulsions and oxidative stability, viscosity, texture, sensory characteristics and shelf-life of products	[6,17]
Other sugars, mannitol, cellulose, hemicellulose	Root, wood (no flavonoids), pruning material, stones, pomace	Physical and structural properties of hydration, oil holding capacity	[6,8,17]
Fatty acids *	Stones, olive oil	Decreases the permeability barrier (blocks complex lipids in sebum produced by sebaceous glands); signals keratinocytes to regulate epidermal homeostasis; promotes acidification of stratum corneum; increases hydration, softness, elasticity and the protective barrier of the skin	[6,22,23]
Essential amino acids	Stones		[6,24]
Squalene *	Pomace, olive oil	Emollient, moisturizing, biological filter of singlet oxygen; sink for lipophilic xenobiotics	[6,25,26]
Maslinic acid	Olive oil, olive pomace	Antioxidant, antiproliferative effect of murine melanoma cells, anti-inflammatory	[18,27,28]
Carotenoids * ( $\beta$ -carotene)	OMWW	Antioxidant	[19]
Tocopherols	OMWW	Antioxidant	[19]
K, Ca, Na	OMWW, pomace	Hydration, stiffness, controlling pH	[6,17]

\* Indicates the compounds most widely used in the cosmetic industry.

It is possible to review the information regarding commercial olive by-products and the derivatives (Table 2) available in the European Cosmetic Ingredient database (CosIng) [29]. CosIng is the EU database that includes cosmetic ingredients and their uses; it is a relevant source of information in the cosmetic field.

**Table 2.** Olive derivatives as raw materials for cosmetics in CosIng.

INCI Name	Functions
Olea europaea leaf	Skin conditioner
Olea europaea leaf cell extract	Flavoring, skin protecting
Olea europaea leaf oil	Perfuming
Olea europaea leaf powder	Skin conditioner, abrasive
Olea europaea leaf water	Skin conditioner
Hydroxytyrosol	Bleaching, skin conditioner
Caffeic acid	Antioxidant, fragrance
Ferulic acid	Antimicrobial
Oleuropein	Antioxidant, hair conditioner, fixing, waving and straightening
Squalene	Hair and skin conditioning, emollient, solvent
Olea europea fruit unsaponifiables	Antioxidant, hair and skin conditioner
Olive acid	Surfactant, cleansing
Olive glycerides	Humectant, surfactant, emulsifying
Oleic acid	Skin conditioning, emollient, surfactant, emulsifying

### 2.1. Hydrophilic Compounds: Polyphenols

Polyphenols stand out among the hydrophilic components due to their antioxidant capacity. Antioxidants are commonly used in cosmetics and dermatology to delay skin aging. Aging is a complex process that involves intrinsic and extrinsic factors. The most relevant extrinsic factors are ultraviolet radiation (UV), infrared (IR), visible light, blue light, alcohol consumption and cigarette smoke; they all have a cumulative effect on the skin. It becomes leathery, drier, showing signs of hyperpigmentation and deep wrinkles [30–37]. These factors are involved in the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which cause lipid peroxidation, damage to desoxyribonucleic acid (DNA), degradation of the extracellular matrix, protein damage and glycation, also generating an inflammatory response of the skin, which triggers the immune response and causes additional oxidative stress that increases the damage [38–40]. The cosmetic and pharmaceutical industries are investigating the mechanisms involved in these processes and are looking for new anti-aging active ingredients [30,41]. Polyphenols are powerful antioxidants with anti-free radical activity; they can prevent or reduce the damage caused by ROS and RNS [6,42,43] and produce significant improvements in the elasticity, thickness and moisture of the skin when used topically [32,39,44–46], thus resulting in good anti-aging agents [4,47–50].

Espeso et al. [51] provided a detailed assessment of the environmental impact of olive industry waste, taking into account data from Spain. Waste from the olive industry (pomace, alpeorujo, vegetation waters, leaves) is a greater source of polyphenols than olive oil. Rodríguez et al. [6] reported leaves, stones, olive mill waste-water (OMWW) and olive pomace and highlighted the contents of the bioactive products found, among which were polyphenols, mainly hydroxytyrosol (HT), tyrosol, oleuropein and verbacoside [7]. Fruit and olive mill wastewater are rich in HT and tyrosol, while the leaves are particularly rich in oleuropein and its derivatives [51,52]. HT has been studied extensively. It has the capacity to scavenge free radicals and stabilize ROS [53], thus reducing lipid peroxidation and enhancing anti-inflammatory actions and promoting cell proliferation [54]. HT and oleuropein showed greater antioxidant capacity in vitro and in vivo than vitamins C and E [55], being greater for HT than for oleuropein.

HT has several other properties, such as being an antiplatelet agent, an inducer of apoptosis, and providing in vitro activity against Gram-negative and Gram-positive

bacteria [55], as well as promoting tissue healing, preventing oxidative stress [52] and blocking UV radiation—it is a promising component of cosmetic products [18,56–60].

These components can be incorporated into emulsions and cleansing products, such as liquids, lotions, oleum and serums. Additionally, their antioxidant capacity makes them useful in formulations as preservative agents, protecting them from rancidity.

### 2.1.1. Polyphenols as Anti-Aging Agents

Rodríguez et al. [6] highlighted the antioxidant capacity associated with possible anti-aging action and protection from UVA and UVB radiation. Rodríguez et al. [43] mentioned the bioactives recovered from OMWW, which are particularly rich in polyphenols, HT, oleuropein, caffeic and ferulic acid, which could be used as complements to sunscreens, anti-aging and antibacterial agents. Tamasi et al. [61] analyzed extra virgin olive oil (EVOO) and the by-products from the olive industry, olive fruit and pomace, extracted the antioxidant components and identified and quantified tyrosol, HT and oleuropein. They performed cytotoxicity studies on the extracts and found that pomace is a promising source of bioactives and antioxidants with no cytotoxic effects.

### 2.1.2. Polyphenols in UV Protection

Additionally, it is important to highlight that olive derivatives rich in polyphenols have interesting properties in relation to photoprotection and antimicrobial activity. Olive polyphenols may contribute to photoprotection, and they increase the sun protection factor (SPF) when combined with UV filters, extending the UV protection to areas of the spectrum in which the UV filters do not have good absorption. Rodríguez et al. [43] also reviewed several studies in which natural antioxidants, among which olive antioxidants are found, contribute to improving the stability of UV filters and act as boosters of others. Table 3 provides information about the UV absorption of olive derivatives.

**Table 3.** Olive derivatives' contribution to UV absorption.

By-Product	Material	Results	References
OMWW *	Extracts combined with UV filters: TiO <sub>2</sub> , Benzophenone-3, Uvinul <sup>®</sup> A, Tinosorb <sup>®</sup> M, Octocrylene, Octylmetoxy cinnamate, Octyl dimethyl PABA.	Spectrum between 220–400 nm. SPF and UVA absorption increase in vitro. Phenols as UV filter boosters.	[62]
OMWW *	Extracts combined with UV filters: TiO <sub>2</sub> , Benzophenone-3, Uvinul <sup>®</sup> A, Octocrylene, Octylmetoxy cinnamate, Octyl dimethyl PABA	Spectrum between 220–400 nm from a combination of UV filters and combinations of different concentrations of phenols. Absorption increases in the UV range. Equations that relate SPF, polyphenol and UV filter content were obtained.	[63]
OIBPE **	OIBPE extracts	Spectrum between 290–320 nm (UVB), in vitro calculation of SPF. OIBPE may act as an UV protection booster, increasing the absorption of UV filters.	[59]

\* Olive mill wastewater (OMWW), obtained by concentration and vacuum filtration (26–30 °C). \*\* Oil industry by-products extracts (OIBPE), extracts from olive leaves; brand name: Sovena.

It is important to consider that the European Union (EU) defines UV filters as “substances which are exclusively or mainly intended to protect the skin against certain UV radiation by absorbing, reflecting or scattering UV radiation” [64]. Moreover, different legislations propose positive lists for UV filters. Some authors classify them as “synthetic” filters, and their safety is continually being revised. The use of the compounds that are not included in these lists and allow obtaining the desired levels of UVA and UVB protection using fewer amounts of filter is of interest [64–66]. Olive by-products belong to those types of compounds, and they offer the double advantage of being natural and being recovered

from waste. All these studies restate the idea that olive-derived polyphenols can be used as boosters in sunscreens.

### 2.1.3. Polyphenols as Antimicrobial Agents

Regarding antimicrobial activity, Ribeiro et al. [48] underlined a growing interest in finding natural components with antimicrobial activity that can replace synthetic antimicrobial preservatives. The phenolic compounds found in olive wastes may fit this purpose [48,49]. EU and Mercosur legislation, among others, establish requirements regarding the microbiological quality of cosmetic products and present positive lists of raw materials that can be considered preservatives, understanding by such “substances which are exclusively or mainly intended to inhibit the development of micro-organisms in the cosmetic product” [64,67]. However, it is possible to incorporate into cosmetic products compounds with antimicrobial activity not included in the positive lists. Olive by-products with antimicrobial activity, as well as other products of natural origin, could act as preservative boosters, partially replacing synthetic preservatives.

In relation to the legislation regarding microbiological quality, MERCOSUR established, as a requirement, the absence of pathogens, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and total and fecal Coliforms [68]. On the other hand, the US Pharmacopeia indicates the evaluation of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger* in order to perform the preservative efficacy test of products for topical use. In Table 4, information about the antimicrobial activity of olive derivatives is gathered, enhancing the fact that olive derivatives could be used as natural antimicrobial agents in cosmetics alone or as preservative boosters.

**Table 4.** Antimicrobial activity of olive by-products.

By-Product	Effective against	References
Olive mill waste	<i>Escherichia coli</i> , <i>Klebsiella pneumoniae</i>	[43]
Olive leaf phenolic extract	<i>Escherichia coli</i> , <i>Salmonella entérica</i> , <i>Pseudomonas aeruginosa</i> , <i>Bacillus cereus</i> , <i>Staphylococcus aureus</i> .	[69]
OIBPE *	<i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> , <i>Bacillus subtilis</i> , <i>Escherichia coli</i> , <i>Candida albicans</i> .	[59]
Olive pomace, hydrophilic and lipophilic components	<i>Staphylococcus aureus</i> , <i>Escherichia coli</i> .	[70]
Olive pomace SFE extract	<i>Staphylococcus aureus</i> , <i>Bacillus subtilis</i> , <i>Bacillus cereus</i> , <i>Klebsiella pneumoniae</i> , <i>Salmonella typhimurium</i> .	[71]

\* Oil industry by-products extracts (OIBPE), olive leaf extract, brand name: Sovena.

### 2.2. Lipophilic Compounds

Olive oil and its derivatives are included in many skin and hair care cosmetic products, where they play a variety of roles. They contribute to the replacement of natural lipids, to retaining water in the stratum corneum of the skin and to improving cell renewal, adding elasticity and suppleness to the skin. They facilitate combing and provide shine to the hair. They form the oily phase of emulsions and can act as over-greasing agents in detergents for the skin and hair. Additionally, olive oil can contribute to the improvement of skin conditions, such as atopic dermatitis, psoriasis and eczema, moisturizing the dry, flaky skin, which becomes supple and flexible [6,72]. Several lipophilic compounds in the olive tree are also present in the skin. Their properties range from being emollient, moisturizing, and protective to being an antioxidant; they show healing activity and repair the function of the lipid barrier [72,73], and also act as lubricants, resulting in soft, elastic and lubricated skin, providing a feeling of well-being. Their activity results from their capacity to remain on the surface of the skin for long periods of time [74–76]. They can also be found in other cosmetic formulations, such as emulsions, oils, suspensions and gels [77].

### 2.2.1. Fatty Acids

Oleic acid (C18:1), the most abundant fatty acid from the olive tree, holds a particular interest in topical formulations and may be used as a facilitator for the entry of certain active ingredients through the skin barrier [72,78,79].

Essential polyunsaturated fatty acids (EFAs) linoleic (C18:2) and linolenic (C18:3) are important components of the cell membrane that cannot be synthesized by our body and whose topical application may contribute to the improvement of skin conditions, such as psoriasis, topical dermatitis and eczema. They also have anti-irritant and anti-inflammatory effects, protecting the skin against UV-induced damage [22,23,72].

### 2.2.2. Vitamin E

Vitamin E occurs in eight isomeric forms,  $\alpha$ -tocopherol being the one with the highest concentration in olive oil [47,80]. Vitamin E is a very well-known non-enzymatic antioxidant [81]. It is widely used in topical formulations due to its free radical scavenging power, prevention of lipid oxidation of fatty acids and protection of cell membranes [69], participating, as well, in signaling pathways regarding inflammation, apoptosis and cell differentiation [80].

Vitamin E may be isolated from the oil or lipid waste, such as cake, and also from olive leaves [82] and from OMWW, associated with the remaining fat [19]. It is a lipophilic antioxidant, which could be used to complement hydrophilic antioxidants, the polyphenols, in formulations with both hydrophilic and lipophilic components, such as emulsions.

### 2.2.3. Carotenoids

Carotenoids are natural pigments with multiple conjugated double bonds, high antioxidant capacity and low water solubility, thus hindering their incorporation into cosmetic products. They may be extracted from pomace and the fatty remains of OMWW [19].  $\beta$ -carotene is found in the leaves of *Olea europea* and can be extracted with conventional techniques. When applied topically, it prevents UVA damage on the dermis, reduces oxidative stress, prevents the loss of antioxidant enzymes and the apoptosis of fibroblasts and shows anti-inflammatory effects [81]. Galanakis emphasized that more research is needed with regard to its extraction and cosmetic applications [83].

### 2.2.4. Squalene

Squalene is a hydrocarbon with several double bonds from the family of triterpenes [25,84]. It is one of the most abundant lipids on the skin's surface [73,85]. The European Cosmetic Ingredient database [29] describes it as an animal (fish) or plant-derived raw material used in cosmetics, such as hair and skin conditioner, emollient and solvent. It was first discovered as a component of liver oil from some varieties of sharks and was used in cosmetics for a long time. Vegetable sources are now mostly used to obtain squalene in order to protect biodiversity. Lozano-Grande et al. [86] highlighted *Olea europea* as the main source of squalene. It may be used topically as an antioxidant, antibacterial, or antifungal. It has also been pointed out that other sources of squalene related to the olive industry are olives, leaves, pomace, olive oil and alpeorujo [21,87].

Filipović et al. [26] assessed, in vivo, the efficacy of commercial emulsions containing squalene (Olifeel® SQ, from the olive oil) and Alp Rose stem cells as active components. A clinical study with 52 volunteers was carried out. Potential irritation was determined by measuring the erythema index (EI), and the efficacy was assessed by measuring the skin elasticity, the transepidermal water loss (TEWL) and moisture content. The authors found that the concentration of squalene affected the level of skin hydration and that the combination with stem cells in the said emulsions improved the barrier function and the recovery of skin cells. All formulations were found to be useful as moisturizers, and the ones with squalene could also be used on irritated and sensitive skin.

### 3. Extraction Technologies of Bioactive Compounds from Olive By-Products

In this section, a detailed perspective of the different extraction techniques reported to obtain bioactive compounds from olive by-products of cosmetic interest is provided. As previously mentioned, these compounds include mainly fatty acids, tocopherols, polyphenols, phytosterols and squalene [88]. The success of the extraction depends greatly on the selected extraction technology, among which are conventional technologies (solid–liquid extraction) and novel or “green” technologies (ultrasound, microwave, supercritical fluids, pressurized liquids and deep eutectic solvents). A brief description and the fundamentals of each one are provided, together with their advantages and disadvantages, including the cosmetic applications reported in the last few years. It is worth mentioning that, in some cases, no specific applications in the cosmetic field were found in the bibliography.

#### 3.1. Solid–Liquid Extraction

Solid–liquid extraction involves the removal of soluble compounds present in a vegetable matrix using organic solvents or mixtures of them. It results in a very convenient type of extraction since the solvent provides a physical medium to which the target molecules are transferred [89]. It is important to select the extraction solvent properly, as well as the experimental conditions (time, temperature, solid:liquid ratio, stirring, particle size), in order to achieve a quantitative extraction of the target compounds [90]. Hydroalcoholic mixtures are the most employed to recover phenolic compounds from olive by-products, especially when the extracts are intended to be used for cosmetic or food products since ethanol is considered a GRAS (generally recognized as safe) solvent; therefore, it does not implicate any harm to consumers.

Many authors have employed different solvents to extract polyphenols from olive leaves, such as water, ethanol, acetone, ethyl acetate and its aqueous solutions [91–93]. Recently, the potential use of olive leaf extracts from the “Negrinha do Freixo” and “Cornicabra” varieties, prepared by maceration in ethanol/water, was evaluated. The extracts, obtained in optimized conditions (6 h, 50% ethanol, 1:20 *w/v*), showed great potential as an anti-aging agent, exhibiting antioxidant activity as well as the ability to inhibit the enzymes elastase, collagenase and tyrosinase. They also exhibited antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus* [69]. Solid–liquid extraction with water has also been proposed as a green approach to recover phenolic compounds from olive pomace [94]. According to the authors, the experimental conditions that maximize the extraction of total phenols and some specific phenolic compounds (3-hydroxytyrosol, rutin and oleuropein) at the same time turned out to consist of one extraction step, 10 min, 25 °C, 1:30 (kg/L) and a pH = 5. In these conditions, samples of alperujo from different cultivars (Arbequina, Hojiblanca, Picual, among others) and geographic zones of Spain showed a wide range of total phenol yield (between 69 and 2817 mgGAE/kg), determined by Folin–Ciocalteu. Also, great variations were observed in the antioxidant activity, determined by the ABTS, DPPH and FRAP assays. This is proposed as a simple, feasible to be escalated method, with the additional advantage that the aqueous extracts obtained are fully compatible with additional purification steps, e.g., by using membranes. This point is of great importance for the cosmetic industry since there is a need to achieve high purity levels for using these extracts in cosmetic products [95]. The use of water as an extraction solvent in this type of extraction, which traditionally employs organic solvents, is oriented to develop extraction processes aligned with circular economy aspects and the Sustainable Development Goals (SDGs) proposed by the United Nations (UN), with several recent publications adopting this approach [96,97].

#### 3.2. Ultrasound-Assisted Extraction (UAE)

UAE is an extraction technology that has proved effective for extracting a wide range of compounds from different matrixes. It uses mechanical waves that propagate longitudinally through a fluid with a frequency between 20 and 2000 kHz [98]. The waves induced in the solvent alternate high- and low-pressure cycles, called compressions and

rarefactions, respectively, which promote the displacement and eviction of molecules from their original location [98,99]. The negative pressure generated during rarefaction exceeds the attraction forces between the liquid molecules, separating them by creating cavitation bubbles. This cavitation effect breaks down the vegetable matrix, leading to higher penetration of the solvent in the cell structure, higher mass transfer rates and shorter extraction times [88,100]. Extractions can be performed in an ultrasonic bath or using an ultrasonic probe immersed in the solvent [101]. In terms of operational costs, UAE offers advantages against other “green” technologies without sacrificing extraction efficiency. This was the conclusion after comparing UAE, MAE and PLE for recovering polyphenols from olive pomace [102]. UAE has also been used to extract polyphenols from olive pomace with water as a solvent [103]. The effect of three factors (power, time and the sample/solvent ratio) on the total phenol content and antioxidant activity of the extracts was studied by the application of a Box–Behnken design, proposing the optimal conditions as 2 g/100 mL of water, 250 W and 75 min at 30 °C. UAE improved extraction efficiency, obtaining extracts with high total phenol content (TPC) levels and strong antioxidant activity. As for olive leaves, Contreras et al. [104] studied the application of UAE to maximize the extraction yield, antioxidant activity, oleuropein content and TPC of olive leaf extracts from the Picual cultivar. The ethanol percentage in the ethanol/water mixture significantly affected the results, and the conditions for a multiple optimization were 50 min, 47% ethanol and a 5.9% solid/liquid ratio. A total of 29 phenolic compounds were identified in the extract (HPLC-MS/MS), with oleuropein being the one with the highest relative abundance (28%). Other authors have studied UAE using different solvents. In this sense, Irakli et al. [105] reported high antioxidant activity in olive leaf extracts using acetone as a solvent. In the suggested experimental conditions (50% acetone, 10 min, 60 °C) the TPC value was 37.4 mg GAE/g. In this particular case, however, the recovery of hydroxytyrosol and phenolic acids was higher when using water as a solvent. HT, the phenolic alcohol product of oleuropein hydrolysis, has been widely studied regarding its antioxidant properties; however, it also exhibits cosmetic potential as a photoprotector, antibacterial agent and anti-inflammatory. The use of UAE has also been reported under reduced pressure conditions, improving extraction efficiency regarding conventional extractions [106].

### 3.3. Microwave-Assisted Extraction (MAE)

Microwaves are radiations from the electromagnetic spectrum whose frequency oscillates between 300 MHz (radio radiation) and 300 GHz (infrared radiation) [107,108]. This technology implies the use of microwave energy to heat the extraction solvent in contact with the solid matrix. The heating produces an increase in the intracellular pressure that facilitates the rupture of the cell wall and the release of the compounds of interest into the solution [109]. The effect of microwaves on exposed materials is strictly related to the conversion of electromagnetic energy into heat [107]. Thus, the MAE refers to the use of the heat generated by microwaves to accelerate the processes of extraction with solvent. These extractions are characterized by a low energy cost and short process times, which are some of the main advantages [110]. Prolonged extraction times could negatively affect thermolabile compounds, such as polyphenols, due to localized heating, which is why this is an important parameter that must be considered for preserving the integrity of the compounds being extracted [111]. Other factors that may affect the efficiency of the extraction are the power used, type of solvent, sample:solvent ratio, particle size and the number of cycles. Therefore, it is important to find the optimal conditions when trying to scale up to the industrial level [112]. The use of microwaves in combination with other techniques has been reported in order to improve efficiency. For example, Silveira da Rosa et al. [113] studied the extraction of phenolic compounds from olive leaves from the variety Arbequina through maceration, UAE and MAE, obtaining the best results for the latter (104.2 mg GAE/g at 86 °C, 3 min). However, they observed that the use of MAE as a pretreatment in the ultrasound extraction increased the content of recovered phenols from the extract by 8%, reaching a TPC of 113.3 mg GAE/g. On the other

hand, Macedo et al. [114] proposed a type of extraction that simultaneously integrated the use of enzymes and microwaves to obtain phenolic compounds from alpeorujo. The use of enzymes in the extraction process has the advantage of using mild conditions, thus preserving the target compound [115]. The authors reported similar results in the MAE and extraction by maceration with water; however, the extraction times were shorter for the MAE (17 vs. 20 min) and had a better sample:solvent ratio (1:15 vs. 1:50 g/mL). By combining the use of MAE with enzymes (pectinase, cellulase and tannase), an increase in the total phenols extracted was achieved (341 vs. 272 mg GAE/g alpeorujo), obtaining a higher content of phenolic acids and phenolic alcohols in particular.

### 3.4. Supercritical Fluid Extraction (SFE)

SFE involves the use of fluids at pressure and temperature levels above their critical points [116]. Some of the characteristics of fluids in a supercritical state are the relatively low viscosity and high diffusion coefficient, compared with conventional organic solvents, which enhances their performance as extraction solvents, as they present mass transfer properties similar to those of a gas and solvation characteristics similar to those of a liquid [117]. Moreover, the density and diffusivity of supercritical fluids can be adjusted by modifying the temperature and pressure conditions, adapting their selectivity against different molecules. Since supercritical solvents operate at high pressures, the separation carried out between the solute and solvent is very simple and efficient by simply decompressing the system [118]. This also allows the obtention of solvent-free extracts [117]. The most widely used solvent in the food and pharmaceutical industries is supercritical carbon dioxide (sc-CO<sub>2</sub>). Due to its relatively low critical temperature and pressure values (31.1 °C, 7.39 MPa), moderate extraction conditions can be applied, protecting target molecules from thermal degradation. CO<sub>2</sub> is considered a nontoxic, non-flammable and GRAS substance, which allows safe working conditions. CO<sub>2</sub>'s low polarity makes it suitable for extracting nonpolar compounds, such as lipids and carotenoids. Polar modifiers or co-solvents can be added in order to efficiently extract other types of molecules, such as flavonoids or simple phenols.

The main reported use of supercritical technology in olive by-products is, by far, to recover phenolic compounds either from leaves, pomace or vegetation waters. In this sense, most of the published work points out the importance of using a co-solvent to maximize the extraction of this type of molecule and the antioxidant activity of the extracts, as well as the influence of operating parameters, mainly pressure and temperature [71,119–121]. Another factor to take into account is the drying and grinding process of the sample prior to extraction, given it can significantly influence the results [122,123].

Regarding cosmetic applications, the use of squalene, obtained from olive by-products by supercritical CO<sub>2</sub> extraction, has been suggested [87]. Squalene is used as a moisturizing/emollient agent, and it has also been studied as an antioxidant, anti-inflammatory, and even with properties to neutralize xenobiotics and treat skin conditions [25,84]. Being a low-polarity compound, its extraction with supercritical CO<sub>2</sub> results are convenient. Squalene is present in variable amounts in virgin olive oil; however, different authors have proposed extracting it from the deodorizer distillate of olive pomace oil, where it is present in higher concentrations and where a high-purity product can be obtained [124]. Other compounds with cosmetic interest that may be efficiently recovered by SFE are tocopherols. Due to their lipophilic nature, this group of compounds are highly compatible with supercritical CO<sub>2</sub>. Tocopherols, in general, but specially  $\alpha$ -tocopherol, exhibit antioxidant and vitamin E activities and may be used in cosmetic formulations, such as topical preparations or emulsions to prevent rancidity [125]. Dauber et al. [71] reported tocopherol values of 345–454 mg/kg (Coratina) and 232–274 mg/kg (Arbequina) in olive pomace extracts obtained by SFE, being in all cases that the  $\alpha$  isomer was the most abundant. High concentrations of tocopherols have also been reported for olive leaf SFE extracts [126].

### 3.5. Pressurized Liquid Extraction (PLE)

The basic principle underlying PLE is that the solvent is used at temperatures higher than its boiling point and at a high enough pressure to keep it in the liquid state during the extraction process. These conditions result in faster extractions, during which high yields are obtained with small volumes of solvent. The high temperatures increase the solubility of the analytes in the solvent while the viscosity and the superficial tension of the solvent decrease, allowing greater penetration of the solvent in the matrix [117]. The extractions can be performed in two ways: static or dynamic; the static one is the most widely used [127]. A particular case of extraction with pressurized liquids happens when the solvent used is water, which is known as extraction with subcritical water. At the temperatures used (between 100 and 374 °C, the subcritical temperature for water), the hydrogen bonds weaken, which changes the dielectric constant of water and, therefore, its polarity. Depending on the working temperature, we can selectively extract different compounds: the more polar ones at a lower temperature and the less polar ones at a higher temperature. In this way, the selectivity of subcritical water allows the manipulation of the composition of the extracts by changing the working conditions. Other factors that affect the extraction are time, pressure, the addition of an organic solvent or a surfactant and the water flow rate [117].

To the best of our knowledge, there are currently no publications reporting the specific uses of this technology in the cosmetic area, even though in recent years, research regarding the optimization of PLE for both olive leaves and alpeorujo has increased. For example, Cea et al. [128] showed great compositional variability in PLE for the alpeorujo extracts obtained under different extraction conditions, highlighting the selectivity of the technique. The extract obtained in optimized conditions contained 1659 mg/kg of the total phenols compared to 281.7 mg/kg of a conventional extract. Regarding the polyphenol profile, the amount of flavonoids and secoiridoids was three to four times greater in the PLE extract, which also showed a high concentration of hydroxytyrosol, which had not been detected in the conventional extract. Katsinas et al. [129] studied the use of static PLE in defatted alpeorujo, identifying hydroxymethyl oleuropein aglycone dialdehyde (3,4-DHPEA-DEA) as the most abundant polyphenol. Likewise, in the work of Dauber et al. [130], the effect of two independent variables (the temperature and percentage of ethanol used as a solvent) on the yield of extraction, TPC and antioxidant activity was assessed. Regarding the total phenol content and antioxidant activity of the extracts, the most favorable conditions were around 120 °C and low percentages of ethanol, with a maximum of around 60–75%. At higher temperatures, greater yields could be achieved; however, the thermal degradation of the thermolabile compounds became more important. Similar studies were reported for olive leaves, like the one performed by Martín-García et al. [131], where the extraction conditions were optimized by means of a Box–Behnken design depending on the temperature, ethanol/water ratio and the time to simultaneously maximize the yield, total phenol content, flavonoids, elenolic acids, secoiridoids and simple phenols. The optimal conditions applying the desirability function were 138 °C, 100% ethanol and 5 min. The authors concluded that this type of extraction could be used at the industrial level, claiming a low operation cost in relation to the short process time.

### 3.6. Deep Eutectic Solvents (DES)

One of the newest and least studied technologies in relation to the recovery of bioactive compounds from olive waste is the use of deep eutectic solvents (DES). DES is defined as a multimolecular solvent that contains a hydrogen bond donor (HBD) and a hydrogen bond acceptor (HBA). The HBD and the HBA interact through hydrogen bonds in order to generate a stable liquid that is quite viscous at room temperature, with a lower fusion point than those of the individual components [132]. One of the advantages of DES is the ease of its preparation and the fact that it can be generated from a variety of substances, typically, choline chloride ([Ch]Cl) as HBA, and sugars, organic acids and urea as HBD. This allows for the creation of “tailor-made” solvents, depending on each particular application.

They also present other benefits, such as low toxicity, low volatility and low cost [133]. One practical disadvantage that may arise when working with DES is the high viscosity; however, this can be solved by the addition of a small amount of water [134].

When compared to conventional extractions, the extraction with DES may be more efficient in extracting phenolic compounds from the olive by-products. According to de Almeida et al. [135], this technology allowed the extraction of 15% more phenols from olive leaves than a conventional ethanolic extraction, both under optimized conditions. In turn, according to the substance used as HBD (different carboxylic acids) in combination with choline chloride, different profiles of extracted phenols were verified. With the mixture of [Ch]Cl:acetic acid, the greatest amount of phenols was extracted (470.0 mg/kg), followed by malic acid, malonic and citric. On the other hand, Chanioti and Tzia [136] studied the use of DES in combination with other emergent extraction technologies, such as homogenization, UAE, MAE and high hydrostatic pressures (HHP), in order to extract phenolic compounds from alpeorujo. Almost all of the combinations of NADESs studied increased the extraction of phenols when compared to conventional aqueous ethanol extraction (70%). The highest efficiency was found with homogenization. From that standpoint, the use of DES in combination with the referred technologies is proposed for large-scale recoveries of phenolic compounds from pomace, proposing a sustainable option that offers compositional flexibility with greater efficacy and shorter times than conventional extractions. Other authors have also addressed the extraction of polyphenols through natural deep eutectic solvents (NADES), especially from alpeorujo [133,137], highlighting its potential use in the food and pharmaceutical industries in all cases without demanding the subsequent high-cost purification stages and thus contributing to a circular economy.

#### 4. Applications of Olive Extracts in the Cosmetic Industry

There is abundant information regarding studies about extracts from olive by-products and the cosmetic benefits of their components. Most of the extracts reported in the bibliographic references were obtained by solid–liquid extraction; for example, Oliveira et al. [69] obtained phenolic extracts by solid–liquid extraction from a mixture of pulverized olive leaves from two Portuguese cultivars (Negrinha do Freixo and Cornicabra), using different ethanol:water ratios at room temperature. The phenolic extracts exhibited high antioxidant capacity, and they inhibited enzymes in the skin associated with aging, such as elastase, collagenase and tyrosinase. The authors concluded that the extracts possessed anti-aging potential. Kishikawa et al. (2015) [138] reported that ethanolic extracts from olive leaves inhibit the growth of *Staphylococcus aureus* and reduce melanin synthesis in melanoma B16 cells. Galanakis et al. (2018) [62] obtained powdered extracts by concentrating OMWW for 15 h (26–30 °C) in an electric vacuum filter. The residual water was mixed with ethanol 96% (1:1 *w/w*), and the insoluble solute was removed. The supernatant liquid was mixed with maltodextrin and then spray-dried. These authors studied the potential of the extract obtained as UV filter boosters. They found that the UV absorption of the synthetic filters increased as a function of the concentration of olive phenols, while the relationship between the increase in SPF and the concentration of olive phenols was linear.

Lecci et al. [42] reported the results of their analysis of extracts obtained by ultrafiltration of the OMWW that came from six different Italian cultivars. All the extracts showed high contents of HT and tyrosol, and three of them showed high amounts of verbascoside. The antioxidant capacity was dependent on the cultivar and related to the polyphenol content. The cytotoxic effect was evaluated, and a determination of the concentration of polyphenols in the extracts that could be used safely was conducted by means of tests in cultures of human epidermal keratinocytes, adult (HEKa). In addition, the effect of the extracts on cultures of HEKa irradiated with UVA was evaluated. Polyphenols had an antioxidant and protective effect against UV damage and oxidative stress when applied before or during irradiation. When applied after irradiation, when the DNA of the keratinocytes had already been damaged, the extracts caused apoptosis of the cell by increasing the oxidative action, thus preventing the propagation of damaged cells.

Otero et al. [139] gathered information on research papers detailing green extraction techniques (high hydrostatic pressure, ultrasound-assisted extraction, microwave-assisted extraction, pulsed electric field, radio frequency drying, high-voltage electrical discharge, supercritical fluid extraction and pressurized liquid extraction) on the main bioactive compounds from different types of waste from the olive industry, such as leaves, pruning biomass, OMWW, olive cake, olive pomace and olive stone. The authors detailed the cosmetic effects of the different olive-derived components. Along the same lines, Carrara et al. [140] surveyed the potential uses of OMWW, rich in phenolic compounds (HT, tyrosol, oleuropein, verbascoside, ferulic and caffeic acid). The authors highlighted the anti-inflammatory and anti-aging effects, protection against UV radiation related to photoaging, wound healing, cancer prevention capacity and antimicrobial activity. Other researchers have investigated the commercial cosmetic applications of olive extracts and by-products. In Table 5, the olive by-products, actives and commercial cosmetic products are listed.

**Table 5.** Olive oil by-products and commercial cosmetic applications.

Commercial Products	Tests	References
O20, O30 *	Content and profile of polyphenols, antioxidant activity. Inhibition of hyaluronidase, elastase. Keratinocytes culture cytotoxicity, skin irritation using Episkin®. Potential efficacy for use in cosmetics.	[58]
OIBPE **	<b>Extract</b> In vitro: cytotoxicity, ocular and skin irritation. In vivo HRIPT **** 51 volunteers. Inhibition of hyaluronidase, elastase, collagenase, tyrosinase. Antioxidant capacity, reduction of ROS. Promising extract for cosmetic use.	[59]
Commercial cream with leaf extract ***	<b>O/W emulsion with OIBPE extract</b> In vivo clinical evaluation, 10 volunteers. Security (patch test). UVA protection (colorimeter). The application of the cream did not produce adverse effects, and it showed excellent compatibility with the skin.  In vivo evaluation of efficacy measured with non-invasive techniques; skin moisture, sebum, pH, melanin, erythema index, TEWL, skin texture and wrinkles. Thirty-six volunteers, twice a day, for two months. Results: reduction of wrinkles, improvement in texture, moisture and skin barrier function. Volunteers point out unpleasant color and smell.	[56]

\* Natac Company (Alcorcón, Madrid, Spain). \*\* Oil industry by-product extracts (OIBPE), olive leaf extract; brand name: Sovena. \*\*\* SuperHeal® O-Live Cream. \*\*\*\* Human Repeat Insult Patch Test.

The different extracts derived from the olive industry may be considered suitable natural raw materials for cosmetic products. However, little is reported in the literature on the cosmetic benefits of the extracts incorporated in a cosmetic formulation. Tarbiat et al. [60] used *Olea europea* leaf extract with an 80% oleuropein content to develop cosmetic emulsions with *Helichrysum Italicum* y Kumquat essential oils, providing no information on how the extracts were obtained. The following bioactive properties of the emulsions were evaluated: antioxidant activity, enzyme inhibition activity (hyaluronidase, tyrosinase, soybean 5-lypooxygenase) and antibacterial activity. The results from that study allowed them to conclude that the oleuropein-containing emulsion and the ones that contained essential oils in their formulations exhibited antioxidant capacities and inhibited all three enzymes.

The use of some olive derivatives or olive-derived extracts in cosmetic formulations could present several inconveniences or challenges regarding the technical or sensory aspects. The main problem is related to their stability, given that polyphenols are highly

susceptible to degradation. Another concern could be the fact that lipophilic compounds could result in undesirable absorption when applied to the skin. On the other hand, compounds that act as UV filters are hydrophilic, which is inadequate for their use in water-resistant formulations. The smell of some active compounds may be unpleasant for some consumers. This is the case with vitamin E, squalene and some fatty acids [141,142], which can also show stability problems. To overcome all these issues, encapsulation could be an alternative, thus preserving the bioactive products against oxidation, changes in environmental conditions and possible interactions with other active products in the formulation, as well as masking their smell. Microencapsulated olive oil may result in an attractive raw material for the cosmetic industry [143]. A few examples of encapsulated olive-derived extracts with cosmetic purposes are reported in the literature. Panagiotopoulou et al. [57] used microencapsulation as a means to favor a product's stability and to protect labile bioactives. These authors worked with an aqueous extract from olive leaves with high oleuropein and HT contents, obtained enzymatically and encapsulated by spray-drying. The microparticles were incorporated into a cosmetic cream, which was evaluated in terms of its rheology, thermal stability, microbiological and sensory characteristics. Meanwhile, Galanakis et al. [63] developed cosmetic formulations containing polyphenols encapsulated by spray-drying with maltodextrin in order to incorporate them into sunscreen formulations.

## 5. Consumer Perception of Cosmetic Products with Olive Oil and By-Products

Understanding consumer perception of a product is extremely important for the cosmetic industry. Due to the fact that the selection of a cosmetic product is a highly complex phenomenon, for commercial success, it is essential to understand consumers' expectations about their sensory attributes, efficacy and other aspects, such as packaging, brand, price, ingredients, etc., and even the socio-economical and ethical aspects [144,145].

Even though the demand for natural cosmetics has increased in the last years, the market has become increasingly competitive; therefore, it is important to explore and analyze the potential market opportunities for new products by researching the opinions, perceptions and purchase motives of potential consumers [146]. In the current case, it is essential to understand how consumers perceive cosmetic products that contain olive derivatives (olive oil and by-products, such as alpeorujo and leaves). Even though some studies have analyzed the factors that affect the purchasing intentions of consumers of natural/organic cosmetics [147], studies that focus on the opinions or perceptions of consumers of those products are scarce.

For the consumer, a product is more than the sum of its attributes; it also implies an emotional component and a symbolic meaning. One way to access the opinions and thoughts of consumers is through the application of sensory qualitative techniques, such as projective techniques. Projective techniques allow for the free interpretation and response to a stimulus, trying to explain motivations, feelings, beliefs, attitudes and reasons when participants select different answers from within a specific topic [148]. In the literature, projective techniques are classified or subdivided into five categories: construction, completion, choice ordering, expressive and association [149].

Within the association task, the word association (WA) technique has been the most used, where the stimulus presented is a word or a phrase. The participants are asked to provide the first four words (images, associations, thoughts or feelings) that come to their mind in relation to a product or a group of products. It is expected that the associations revealed through this process are closely related to the possible purchase decisions [150]. The word association (WA) technique is one of the most used projective techniques due to its easy application and effectiveness in exploring consumers' perceptions [151–154].

Yano et al. [155] explored the opinion of 901 Japanese women about cosmetic products that contained plant-derived ingredients by using the word association technique. The results showed that even though the respondents considered plant-derived cosmetics to be gentle on the skin and safe to use on sensitive skin, they also considered those products to be expensive and slow to show an effect. Some of the respondents were also skeptical

about the effectiveness of these products. Some respondents, especially older women, were worried about the possible negative effects, while younger women tended not to worry about the potential problems or the negative effects of the use of plant-derived products. Gambaro et al. [156] examined the perceptions of consumers of cosmetics creams containing olive oil and enriched with olive leaf extract using a word association technique. The authors found that potential consumers regarded cosmetic creams containing olive oil and enriched with olive leaf extract as effective and natural, but they also associated them with undesirable sensory characteristics (for example, oily and greasy texture and strong, unpleasant smells).

During the completion task, the participants were given an incomplete sentence, story, plot or conversation and were asked to complete it. This technique can be divided into three types: (a) sentence completion, (b) story completion and (c) dialogue completion. Visual stimuli, in the form of cartoons with bubbles, are generally used in the dialogue completion type, in which respondents are asked to fill in the thoughts of the characters. To date, most of the studies that reported the application of this technique belong to the food field [157–160].

Parente et al. [141] explored the perception of 334 Uruguayan women upon using an after-shower oil with olive oil, applying three variants of the completion projective technique: story completion, sentence completion and dialogue completion. This study allowed the defining of important aspects to be considered while designing a new cosmetic containing olive oil. The ignorance of consumers about the effect of olive oil on the skin was evident. While more than one-third of the respondents expressed an intention to buy and try the product, others were discouraged by some aspects, like the belief that the cream would feel greasy and sticky on the skin and the intense, disagreeable smell they imagined the product would have. These findings suggest that in order to enter the cosmetic market with a product containing olive oil and by-products, a company must offer products that are safe and effective, with a warranted quality and a good quality–price relationship. It would be important to provide a detailed description of the products and information related to their safety. Developing cosmetic products that could be supplied at reasonable prices and that have an immediate effect on the skin could be an effective marketing strategy.

## 6. Conclusions

The olive oil industry gives rise to plenty of by-products, which may be recycled using environmentally friendly methods, thus obtaining raw materials of interest for the cosmetic industry with high added value. It is important to consider sustainable extraction methods that promote adequate industrialization and residue management.

Olive derivatives have shown numerous cosmetic and health effects. The present review has placed emphasis on the extracts with a high polyphenol content, particularly their antioxidant potential associated with their anti-aging, UV filter booster and antimicrobial effects. In addition to the hydrophilic components derived from the olive, the lipophilic components mentioned in this review are also of interest in cosmetic formulations. The first ones may be incorporated in cosmetics formulations with an aqueous base, lotions and serums, while the second ones may be incorporated into oily formulations. Both of them may form part of the emulsion or biphasic systems. Even though there are several commercial raw materials derived from olive oil and its derivatives, as seen during the review of the CosIng database, there are cosmetic products available in the market containing them, with interest for new olive by-product derivatives currently growing. These offer an additional advantage that involves providing natural cosmetic activities while solving some environmental problems related to the disposal of waste. Nevertheless, when considering a new active or new extraction technique, safety and efficacy tests should be available. Although there is abundant information on the extraction methods and the potential of different compounds as cosmetic active ingredients, little has actually been put into practice. Therefore, there is room for a deeper awareness in this field.

For the success of a cosmetic product, it is essential, although not sufficient, to have consumers' acceptance, and one of the factors that influence acceptance is the sensory characteristics of the product. It is fundamental to consider the sensory profile of the raw materials derived from the olive that may become apparent within the cosmetic into which they are incorporated. The correct balance communication of these characteristics in cosmetics that include these actives is essential so that they are well-received by potential consumers.

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## Abbreviations

ABTS, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); DES, deep eutectic solvent; DNA, deoxyribonucleic acid; DPPH 1,1-diphenyl-2-picrylhydrazyl radical; EFAs, essential fatty acids; EU, European Union; EVOO, extra virgin olive oil; FRAP, ferric reducing antioxidant power; GAE, gallic acid equivalents; GHz, Gigahertz; GRAS, generally recognized as safe; INCI, international nomenclature of cosmetic ingredients; HBA, hydrogen bond acceptor; HBD, hydrogen bond donor; HEKa, human epidermal keratinocytes adult; HHP, high hydrostatic pressures; HPLC, high-performance liquid chromatography; HT, hydroxytyrosol; IR, infrared; MAE, microwave-assisted extraction; MHz, Megahertz; MS, mass spectrometry; NADES, natural deep eutectic solvents; OMWW, olive mill wastewater; PLE, pressurized liquid extraction; RNS, reactive nitrogen species; ROS, reactive oxygen species; SDGs, Sustainable Development Goals; SFE, supercritical fluid extraction; SPF, sun protection factor; TEWL, transepidermal water loss; TPC, total phenol content; UN, United Nations; UV, ultra violet; UVA, ultra violet A; UVB, ultra violet B; UAE, ultrasound-assisted extraction; WA, word association.

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