JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

pubs.acs.org/JAFC Review

Polyphenol-Rich Extracts from Agroindustrial Waste and Byproducts: Results and Perspectives According to the Green Chemistry and Circular Economy

Published as part of Journal of Agricultural and Food Chemistry virtual special issue "International Conference on Polyphenols (ICP2023)".

Roberta Bernini,* Margherita Campo, Chiara Cassiani, Andrea Fochetti, Francesca Ieri, Andrea Lombardi, Silvia Urciuoli, Pamela Vignolini, Noemi Villanova, and Chiara Vita

Cite This: *J. Agric. Food Chem.* 2024, 72, 12871−12895

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: Polyphenols are natural secondary metabolites found in plants endowed with multiple biological activities (antioxidant, anti-inflammatory, antimicrobial, cardioprotective, and anticancer). In view of these properties, they find many applications and are used as active ingredients in nutraceutical, food, pharmaceutical, and cosmetic formulations. In accordance with green chemistry and circular economy strategies, they can also be recovered from agroindustrial waste and reused in various sectors, promoting sustainable processes. This review described structural characteristics, methods for extraction, biological properties, and applications of polyphenolic extracts obtained from two selected plant materials of the Mediterranean area as olive (*Olea europaea* L.) and pomegranate (*Punica granatum* L.) based on recent literature, highlighting future research perspectives.

KEYWORDS: *polyphenols, Olea europaea L., Punica granatum L., hydroxytyrosol, oleuropein, punicalagin, green chemistry, circular economy, agroindustrial byproducts, chemical functionalization, biological activities*

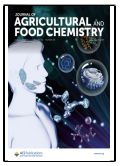
1. INTRODUCTION

Plants are an important source of biologically active natural compounds that play crucial roles not only in plant biology but also in maintaining ecosystem health, supporting animal life, and promoting human well-being. These molecules include secondary metabolites, synthesized through specialized species-specific pathways that determine chemical diversity in the plant world and enable plants to defend themselves and survive in a complex ecosystem such as a natural one. In fact, they perform important physiological and defense functions for plants, control the biological properties of other species in the environment, and exert a crucial role in their coexistence and coevolution. Through photosynthesis, they contribute to climate regulation, provide energy, and serve as renewable resources. In addition, some medicinal plants are used to improve human well-being and to prevent the onset of various diseases, including cardiovascular, neurodegenerative, and cancer.

Polyphenols make up a class of secondary metabolites characterized by at least one aromatic ring bearing one or more hydroxyl groups, either free or conjugated to form ethers, esters, or glycosides. Simple and complex structures originate from this basic unit. They include phenyl acetic acids and alcohols, benzoic acids, cinnamic acids, coumarins, chromones, xanthones, quinones, flavonoids, stilbenes, pyrones, lignans, lignin, tannins deriving from the shikimate pathway, the acetate/malonate pathway, or the combination of the two biogenetic pathways. These compounds are known for their broad spectrum of biological properties such as antioxidant, antimicrobial, anti-inflammatory, and anticancer activity and are the active ingredients in foods, pharmaceuticals, nutraceuticals, antimicrobials, and innovative materials.[1]

Due to their importance and the high commercial demand for these compounds, they can also be extracted from agricultural waste and byproducts in line with the principles of green chemistry and the circular economy. The green chemistry found its origins in 1998, with Paul Anastas and John C. Warner providing the 12 principles on which it is based, laying the foundation for the design and implementation of sustainable chemistry based on atomic and energy efficiency, pollution prevention, and biomass reuse.[2] The circular economy concept is very recent. In fact, it was first introduced in 2016 by Walter Stahel, who devised an economic model for industrial processes focused on job creation, improved economic performance, resource conservation, and waste prevention.[3] Green chemistry and circular economy are strongly connected to each other: in fact, green chemistry

Received: January 30, 2024
Revised: May 17, 2024
Accepted: May 17, 2024
Published: June 3, 2024



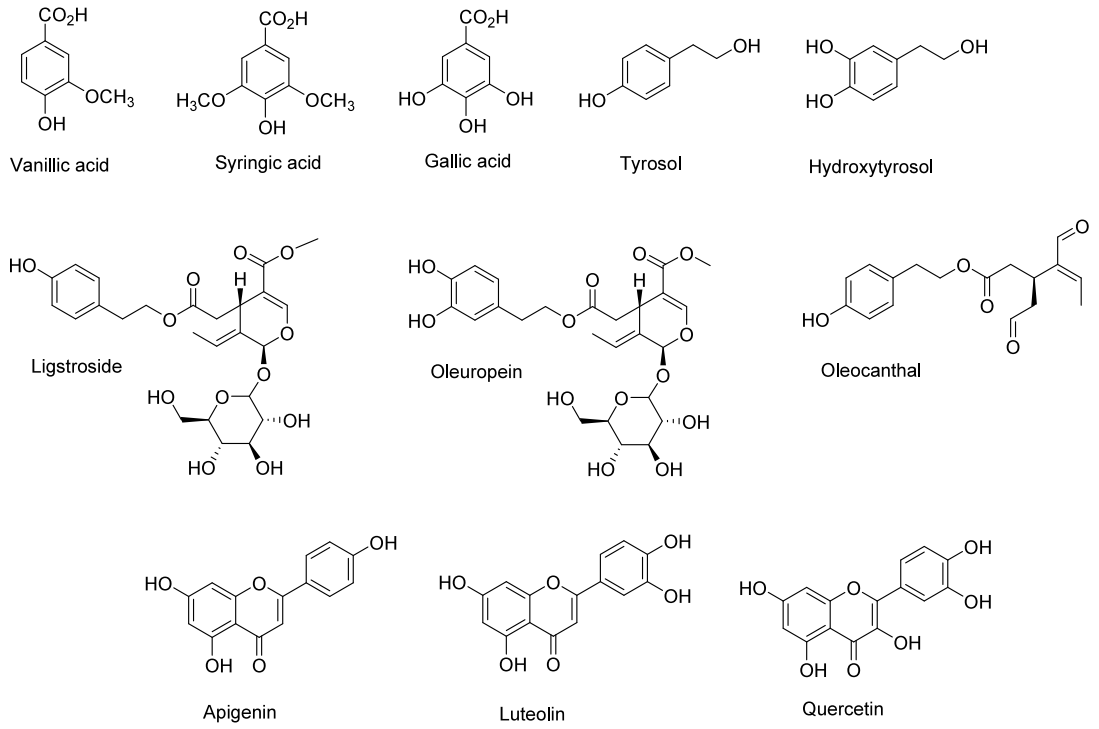


Figure 1. Main phenolic compounds found in *Olea europaea* L.

represents a tool for realizing the goals of the circular economy with environmental benefits.[4]

Given the breadth of these topics in the literature, this review focuses on polyphenols found in two selected plant materials from the Mediterranean region, olive (*Olea europaea* L.) and pomegranate trees (*Punica granatum* L.). Based on the recent literature, our experience and knowledge, structural characteristics, extractive methods, chemical functionalization, stabilization and delivery system, biological properties, applications of polyphenols, and polyphenols-rich extracts have been described.

2. POLYPHENOLS FOUND IN *OLEA EUROPAEA* L

2.1. Structural Features. The olive tree (*Olea europaea* L.) is one of the world's most important and widespread fruiting tree species, whose origin is lost in history. Various sources trace it to the Mediterranean's eastern coast, including present-day southern Turkey, Syria, Lebanon, Palestine, and Israel. From its original area, olive cultivation was introduced to Greece and Egypt; it later spread to all countries in the Mediterranean basin, in parallel with the progress of trade.

The fruit of the olive tree is an oval-shaped drupe. It consists of epicarp (skin), mesocarp (pulp), and endocarp (stone). The epicarp, covered with wax, turns green, purple, or almost black during growth and fully ripens in the late autumn. The mesocarp, with soft pulp, accounts for 84−90%, while the endocarp, containing the seed, can range from 13 up to 30% (both percentages are referred to as the total fruit mass). The distribution and structure of the chemical components of the drupe are complex and depend on several parameters such as variety, cultivation practices, geographical origin, and ripening level. However, generally, it contains protein (1.6%), oil (22%), carbohydrates (19.1%), cellulose (5.8%), and polyphenols (1−3%).[5]

Lipophilic and hydrophilic polyphenols are responsible for the browning of the fruit and, in addition to contributing to the sensory and aromatic characteristics of the olive, provide antimicrobial properties and human health benefits. The main compounds include phenolic acids, e.g., vanillic acid, syringic acid, and gallic acid; phenolic alcohols, e.g., tyrosol (Tyr) and hydroxytyrosol (HTyr); secoiridoids, e.g., ligstroside, oleuropein (Ole), and oleocanthal; and flavonoids, e.g., apigenin, luteolin, and quercetin (Figure 1).

Extra virgin olive oil (EVOO) is obtained from the pressing of drupes through purely mechanical techniques, preserving the composition of the lipid fraction and limiting autoxidation reactions and consequently chemical alterations of bioactive compounds. Byproducts of processing are pomace and olive mill wastewater (OMWW). Additional wastes are leaves collected during both EVOO production and olive tree pruning.

EVOO is renowned for its wide spectrum of benefits exerted on humans and animals. The high content of nutrients allows them to act as key components of a balanced diet and healthy lifestyle, providing nutraceutical, antimicrobial, antioxidant, anti-inflammatory, and anticancer activities.[6,7] The nutraceutical properties of olive dietary polyphenols have led the European policymaker, through the Reg. CE 432/2012, to authorize the health claim "*olive oil polyphenols contribute to the protection of blood lipids from oxidative stress*" for products referring to a daily intake of 20 g of olive oil containing at least 5 mg of HTyr.[8]

HTyr is the main phenol in olives,[9] but pomace and OMWW represent alternative and renewable sources of this compound.[10,11] Structurally, it is a small molecule bearing the catechol moiety responsible for many biological properties, ranging from antioxidant[12,13] to anti-inflammatory to health benefits related to cardiovascular diseases.[14,15] However, HTyr is poorly soluble in fats and its bioavailability depends on the

https://doi.org/10.1021/acs.jafc.4c00945
J. Agric. Food Chem. 2024, 72, 12871−12895

Scheme 1. Hydrolysis of Ole

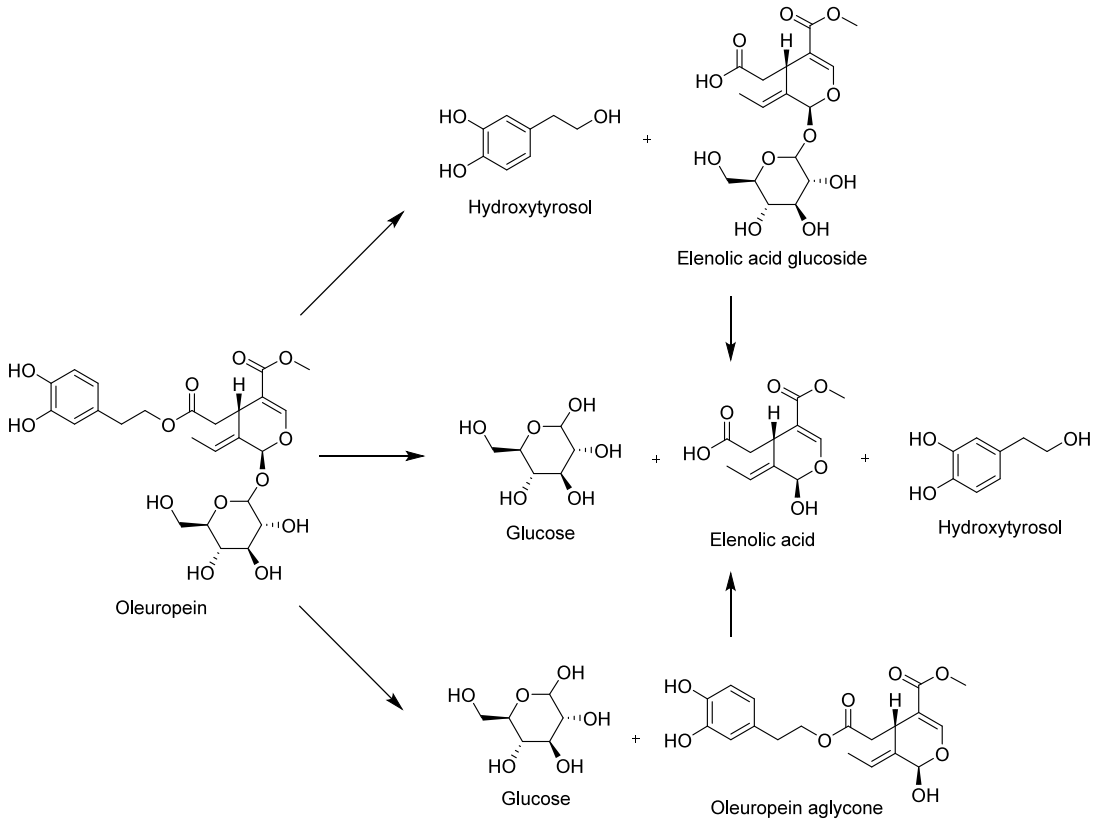


Table 1. Some Polyphenols Extraction Methods from Olive Oil Waste and Byproducts at Lab Scale

extraction methods	solvent(s)	olive plant material	main phenolic compound	ref
solvent	methanol/water = 90/10 (v/v)	leaves	Ole	27
solvent	ethanol/water = 70/30, acetic acid 1% (v/v)	leaves	Ole	28
NADES	citric acid/fructose = 1/1, water 19%	OMWW		30
Soxhlet	ethanol/water= 80/20 (v/v)	leaves	Ole	31
UAE	methanol/water = 80/20 (v/v)	leaves	Ole	32
MAE	ethanol/water = 70/30 (v/v)	leaves	Ole	35
SFE	sCO_2, ethanol 60%	leaves	Ole	36
SFE, PSE	none	leaves	HTyr, chlorogenic acid, caffeic acid, ferulic acid	37
membrane technologies	none	leaves	Ole	38
membrane technologies	none	pomace	HTyr	39,40

food matrix with which is administered.[16] Ole is a secoiridoid only found in plants of the Oleaceae family, mainly present in olive leaves.[17] High amounts of Ole (up to 14% of dry weight in unripe olives) are in the unprocessed olive fruit and are responsible for the bitter taste of these matrices.[18] During olives maturation and EVOO production, Ole concentration progressively decreases being hydrolyzed by enzymes into HTyr, elenolic acid, and glucose (Scheme 1).[19]

Ole shows anti-inflammatory,[20] antioxidant,[21] and antiproliferative[22] properties with protective behavior at neurological,[23] cardiovascular, and metabolic levels.[24] Recently, it has indeed been demonstrated that Ole can lower the amount of glucose in the blood, evidencing potential employment in diabetes treatment.[25]

2.2. Extractive Methods. The extraction process from a plant source is a crucial step in the search for natural substances. Generally, this stage is complex and depends strictly on various factors related to plant materials (e.g., kind of matrix, location of the plant material, methods of collection and preservation, chemical properties of compounds to be extracted). At the same time, it must be reliable and reproducible to give standardized extracts of high-added value for applications.[26] Therefore, the choice of the extraction method should be made by considering the application purpose of the extract, which may also require scalability of the production.

Olive oil polyphenol extraction from waste and byproducts (pomace, OMWW, and leaves) can be carried out by conventional and innovative methods. Conventional methods include maceration, solvent extraction, and Soxhlet extraction; innovative methods include ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), supercritical fluid extraction (SFE), and pressurized fluid extraction (PFE).

In the literature, many references are reporting the application of these methods to olive oil wastes and byproducts. This review aims to report a few examples at the lab scale without claiming to be exhaustive (Table 1) and some

https://doi.org/10.1021/acs.jafc.4c00945
J. Agric. Food Chem. 2024, 72, 12871−12895

industrial procedures, at the end of the section, to demonstrate the scalability of some processes.

Maceration and solvent extraction consist of treating the plant material with a solvent or a mixture of solvents at a fixed temperature. Generally, water or ethanol/water are the solvents of choice, being safe, but also methanol, ethyl acetate, hexane, diethyl ether, and acetone can be used depending on the chemical properties of the molecules to be recovered. In these cases, even with satisfactory extraction efficiency, there is the possibility of toxic residues being found in the final extracts. Cho et al. (2020)[27] treated dried olive leaves powder with different solvents at different percentages: water (100%), ethanol/water (50, 70, and 90%, v/v), methanol/water (50, 70, and 90% v/v), and acetone/water (50, 70, and 90% v/v) for 1 h at room temperature. The highest extraction efficiency of polyphenols (20.4%), with a total polyphenol content of 231.98 mg GAE/100 g, was obtained using aqueous methanol 90% v/v.[27] HPLC analysis detected that Ole was the main component, with 26.10 ± 0.20 g/L; HTyr and Tyr were found in small amounts (0.74 and 0.07 ± 0.02 g/L, respectively). Coppa et al. (2017) obtained 18 g of Ole by macerating 100 g of dried olive leaves with aqueous ethanol 70% v/v acidified with acetic acid (1%) as solvent of extraction (olive leaves/solvent mass ratio = 1/3).[28]

In recent times, natural deep eutectic solvents (NADES) have gained great popularity as green solvents for their biodegradability and biocompatibility[29] and found application in the efficient and sustainable extraction of polyphenols from plant materials. In a recent study, six NADES composed of citric acid and fructose at different mass ratios were formulated and tested to extract polyphenols from OMWW. The NADE containing citric acid/fructose = 1/1 and water (19%) was the most effective extractive solvent, allowing recovery of 4 g of polyphenols per kg of fresh vegetation waters.[30]

The Soxhlet extraction is a commonly employed laboratory method with several drawbacks, such as high solvent and energy consumption and possible degradation of thermolabile compounds. However, generally, it is efficient because the sample is always in contact with the solvent. A range of extraction solvents, from water to organic solvents, can be used with this technique. A study performed by Yateem et al. (2014) evidenced an Ole recovery up 19.0 ± 0.66 mg per gram of dry plant material when olive leaves dry powder was extracted with ethanol/water = 80/20 at 60 °C for 4 h in a Soxhlet apparatus.[31]

Ultrasound-assisted extraction (UAE) is a nonconventional technique widely used in applied chemistry, being a green solution for obtaining extracts. It shows the advantage of shortening the extraction time in comparison to conventional extraction methods, mainly due to the physical and chemical effects promoted by cavitation, a phenomenon responsible for the acceleration of chemical reactions. In addition, it significantly reduces the amount of solvent employed in the process. On the other hand, it presents some disadvantages, such as the formation of free radicals responsible for oxidation processes and chemical modifications of bioactive compounds. Wu et al. (2015) treated 1 g of powdered olive leaves of 29 different varieties with methanol/water = 80/20 (v/v) under UAE for 40 min (40 kHz, 180 W). Analytical data evidenced a high efficiency of extraction and an Ole content varying in a range of 1.56−19.58% depending on the olive variety.[32]

Microwave-assisted extraction (MAE) is a green chemistry technique that exploits microwave energy to heat solvents in contact with samples, allowing for reduced extraction time and costs as well as lower amounts of solvents. Despite these advantages, MAE presents some scale-up limitations from laboratory to a larger scale, the main of which concerns the low penetration depth and, consequently, the difficulty of homogeneously treating large quantities of raw material.[33,34] Different conditions were used to extract phenolic compounds from olive leaves and pomace using MAE. Some authors compared three different techniques (maceration, UAE, and MAE) to extract phenolic compounds from olive leaves. The results evidenced that aqueous ethanol 70% (v/v) at T = 86 °C MAE was highly efficient in extracting phenolic compounds in a very short extraction time (3 min).[35] More recently, some authors reported on olive pomace dried powder extraction by innovative technologies including UAE and MAE compared to conventional maceration.[35]

Supercritical fluid extraction (SFE), due to lower operating temperatures, is a technology particularly recommended to extract thermolabile compounds. For their chemical physical properties (low viscosity, absence of surface tension), supercritical fluids are similar to both gas and liquids, being able to penetrate a solid matrix allowing an efficient extraction of bioactive compounds. On the other hand, the expensive required equipment limits their uses, and only industrial applications can justify the initial substantial investment. The most used supercritical fluid is carbon dioxide (CO_2) due to its nonflammable and inert nature. Caballero et al. applied SFE at 300 bar on three different olive residues (pruning biomass, leaves, and exhaust pomace) using ethanol 60% as cosolvent. HTyr, chlorogenic acid, caffeic acid, and ferulic acid were the main phenolic compounds found in the extract.[36]

Another technique that enables the time-reduced extraction of bioactive compounds is pressurized solvent extraction (PSE), which operates up to 200 bar at 25−200 °C. Water and ethanol are solvents of choice to extract polyphenols. Combining PLE with PSE, Ole was recovered from olive leaves up to 46.6%.[37]

In recent years, membrane technologies have found wide applications for the separation, purification, and concentration of bioactive compounds from aqueous solutions. Low temperatures and energy consumption are the advantages of this technique, which uses only physical phenomena to obtain extracts based on the selective permeation of soluble molecules employing membranes. Microfiltration (MF), ultrafiltration (UF), and nanofiltration (NF) can be used independently or in combination to obtain polyphenolic extracts. A sequence of MF, UF and NF processes allowed to recover 1685 mg of Ole per 100 g of olive leaves extract.[38] With a similar process, HTyr-enriched extracts containing 60.53 ± 0.41 mg/g of HTyr were obtained from olive pomace.[39,40]

Despite most of the described, extractive procedures were achieved on a lab scale, both HTyr and Ole were successfully isolated and purified from wastes at preindustrial or industrial scale. As an example, Fernandez-Bolanos et al. (2002) patented a purification system to obtain up to 3.5 kg of 90−95% pure HTyr from 1000 kg of liquid−solid waste after acidic treatment and without the employment of organic solvents.[41] Similarly, Fava et al. (2017) designed an automated process for polyphenol extraction from the OMWW to obtain about 2 kg of polyphenolic extracts by treatment of 300 L of the OMWW, and the procedure proved to be reproducible for up to three cycles. Residues, consisting mainly in Tyr and HTyr, were characterized with HPLC and showed an average purity

https://doi.org/10.1021/acs.jafc.4c00945
J. Agric. Food Chem. 2024, 72, 12871−12895

of the latter of 70%.[42] HTyr fractions with different purities were obtained from byproducts generated during olive oil manufacturing process: columns filled first with a strong anion exchanger (Amberjet 4200-Cl, Rohm-Haas Co., Chauny, France) and then with a polymeric absorption resin XAD type allowed for the isolation to HTyr up to 96% pure.[43] On a smaller scale, Hamden et al. (2009) reported on the purification of HTyr from OMWW extracts using silica gel chromatography and preparative thin-layer chromatography.[44] Some authors reported on obtaining pure Ole by using different sorbent materials on MAE Ole-enriched extracts, handling up to 81.2 mg of Ole for each gram of extract.[45]

2.3. Biological Properties. Extensive investigations into the healthful effects of HTyr revealed promising potential in addressing metabolic syndrome and gastrointestinal tract disorders, including inflammatory bowel disease (IBD) and Crohn's disease.[46,47] These benefits can be highlighted by including molecules, extracts, or byproducts as innovative ingredients while developing new functional foods and dietary supplement formulations.[48]

Along with nutrition, olive oil has gained growing attention as a promising source of natural antimicrobial agents. Over the past few decades, HTyr has emerged as main responsible for the antimicrobial properties of EVOO and byproducts. The higher activity of HTyr compared to that of Ole could be attributed to its ability to permeate the bacterial or fungal membrane more readily due to the absence of glycosylation in its structure.[49]

The assessment of extracts rather than isolated compounds typically acquired by chemical suppliers could open a new strategy of antimicrobial evaluations due to the possible synergistic effects. Leaves extract revealed activity against *Listeria monocytogenes* via reducing biofilm production and motility, leading to flagella losses.[50] The antimicrobial properties of natural active ingredients from olives could represent a critical alternative to fight multidrug-resistant pathogens. The enrichment of a leaves extract with HTyr demonstrated an enhanced activity against *Campylobacter spp.* resistant strains compared to individual compounds.[51] In addition, the development of novel natural-based antimicrobial drugs could facilitate food safety management along chain and production processes. Olive leaves extracts showed promising performances in replacing chemicals during anchovy fillet pickling or nitrate and nitrite in sausage ripening.[52,53]

Using extracts from pruning and oil production wastes and byproducts as active ingredients in food safety paves the way for novel sustainable approaches following circular economy principles, resulting in a lower environmental impact by minimizing the reliance on synthetic preservatives. From an economic standpoint, this approach brings several benefits, beginning with a decrease in food waste production. Additionally, the enhanced quality of food could result in increased consumer demand and lower healthcare cost.

In this respect, the development of novel active materials using olive phenolic compounds or extracts revealed an interesting field of business and research, offering the opportunity to harness their dual antioxidant and antimicrobial properties.[54] As an example, promising antioxidant and technological performance were achieved by preparing active materials for food packaging by using poly(vinyl alcohol) (PVA) films combined with HTyr- and Ole-enriched extracts.[55] The potential of active packaging is further amplified when they are in direct contact with food products, especially those prone to rapid spoilage. For instance, the addition of olive leaves extract in a chitosan-based edible film helped to prevent lipid oxidation, microbial growth, and texture changes in pork burgers.[56] Within this framework, encapsulation of the active olive-based ingredients on the polymeric matrix enabled extended food shelf life through enhanced stabilization of phenolic compounds.[57] Additionally, biobased and biodegradable polymers represent an eco-friendly approach to mitigate the environmental impact of plastic consumption, particularly for disposable products. In this regard, olive pomace demonstrated its potential as an ingredient for innovative disposable thermopressed tableware.[58]

Beyond food safety and preservation concerns, the antioxidant and antimicrobial properties of olive phenolic compounds can be applied to prevent infections caused by harmful microorganisms in humans and animals. Extracts from olive oil mill wastewater using ethanol, methanol, or ethyl acetate as solvents exhibited antimicrobial activity against a wide range of pathogens as *Enterococcus faecalis*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa*, *Streptococcus uberis*, and *Staphylococcus aureus*.[59] In contrast to other *in vitro* studies focused on Ole and leaves extracts, no inhibition was found against *Candida albicans*.[60,61] In this case, as in many instances, including HTyr, discrepancies in findings between studies may arise. A leading cause to these inconsistencies could be attributed to the application of different analytical methodologies during the assessment of the antimicrobial activity of natural phenolic compounds.[62] This variable, likewise, the choice of bacterial and fungal strains, poses significant challenges in conducting comparative studies within the literature. Furthermore, the assessment of antimicrobial performance of extracts should be coupled with an analytical quali-quantitative chemical characterization, e.g., using chromatographic techniques. A sample obtained through NADES extraction from Coratina cultivar showed significant antibacterial performance against *Chlamydia trachomatis*, targeting its extracellular forms known as elementary bodies, which are responsible for infection transmission and movement within the host. This activity is achieved by damaging the external layers of chlamydial cells, compromising their structure and, consequently, their pathogenic functions. These results suggest potential avenues for developing novel drug strategies against this pathogen increasingly associated with antibiotic resistance, responsible for a sexually transmitted disease.[63] Deoiled pomace extracts, obtained by biorefinery and characterized through an HPLC-DAD/MS analysis, revealed promising *in vitro* activity against *Trichophyton interdigitale*, a dermatophyte responsible for human mycosis and athlete' foot disease. Efficacy of the extract could be attributed to the presence of Tyr and HTyr.[64]

The beneficial effects against biotic and abiotic stresses of human skin of olives and byproducts have gained widespread use in cosmetic and dermocosmetic formulations. This is attributed to the potent antioxidant and radical-scavenging activity of polyphenols, which could play a crucial role as antiaging agents and UV protectors while simultaneously serving as natural preservatives.[65]

Ole and HTyr revealed positive effects on aging management by regulating various signaling pathways, including AMP-activated protein kinase, SIRT1, autophagy, and inflammatory processes.[66,67]

https://doi.org/10.1021/acs.jafc.4c00945
J. Agric. Food Chem. 2024, 72, 12871−12895

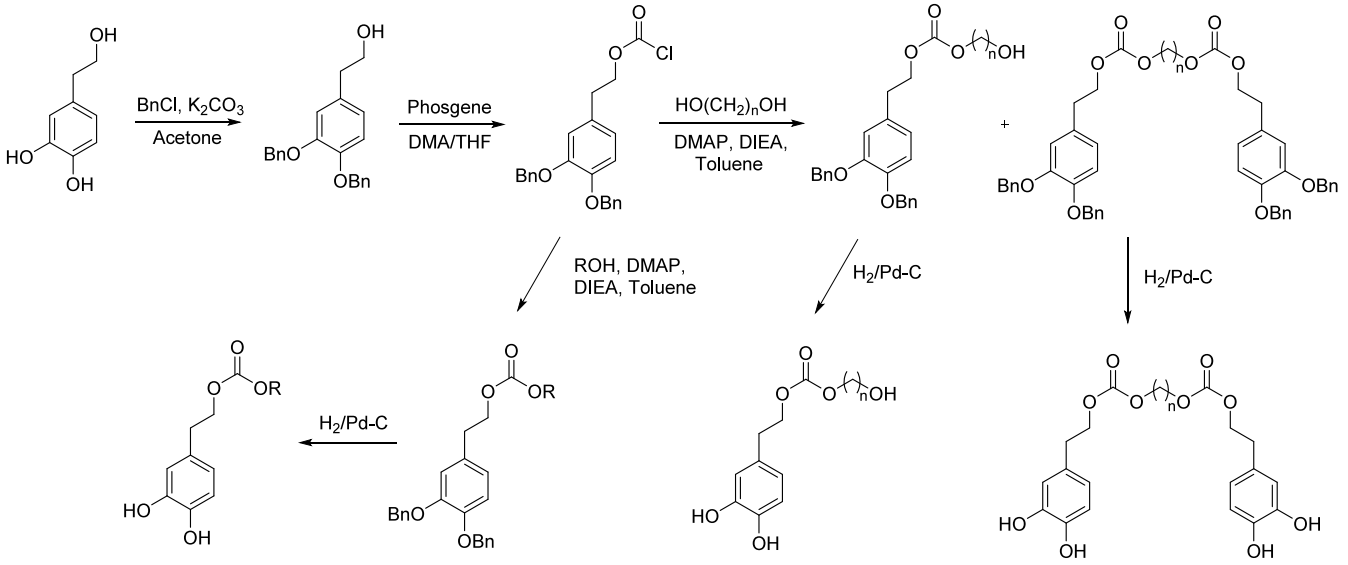
Table 2. Biological Activities of *Olea europaea* L. Polyphenols

application field	extract/pure compound	target	ref
Antimicrobial Activity			
drug discovery	Ole-rich extract: Ole hydrolysate, frozen olive extract	*Lactobacillus plantarum*	49
		Lactobacillus brevis	
		Pediococcus cerevisiae	
		Leuconostoc mesenteroides	
		Staphylococcus aureus	
		Bacillus subtilis	
		Enterobacter aerogenes	
		Enterobacter cloacae	
		Escherichia coli	
		Salmonella typhitmurium	
		Pseudomonas fluorescens	
		Pseudomonas solanacearum	
		Pseudomonas lachrymans	
		Erwinia carotovora	
		Phoma tracheiphila	
		Xanthomonas vescicatoria	
		Corynebacterium michiganese	
		Saccharomyces rosei	
		Saccharomyces cerevisiae var. ellipsoideus	
		Hansenula subpelliculosa	
		Kloeckera apiculata	
		Debaromyces membranaefaciens	
		Pichia membranaefaciens	
		Candida krusei	
food	olive leaves extract	*Listeria monocytogenes*	50
		Escherichia coli	
		Salmonella enteritidis	
food	olive leaves extract	*Campylobacter jejuni*	51
		Campylobacter coli	
food	olive leaves extract	*Pseudomonas fluorescens*	52
		Pseudomonas fragi	
		Pseudomonas putida	
		Brochotrix thermosphacta	
		Clostridium sporogenes	
		Listeria innocua.	
drug discovery	olive mill wastewater extract	*Bacillus spizizenii*	59
		Bacillus cereus	
		Staphylococcus aureus	
		Escherichia coli	
		Klebsiella aerogenes	
		Pseudomonas aeruginosa	
		Streptococcus uberis	
		Enterococcus faecalis	
		Candida albicans	
medicine	olive leaves extract	*Candida albicans*	60
		Candida dubliniensis	
medicine	Ole	*Candida albicans*	61
drug discovery	HTyr	*Erwinia carotovora*	62
		Klebsiella peneumoniae	
		Pseudomonas aeruginosa	
		Escherichia coli	
		Yersinia enterocolitica	
		Salmonella typhimurium	
		Aeromonas hydrophila	
		Shigella sonnei	
		Pediococcus acidilactici	
		Kocuria rhizophila	
		Listeria monocytogenes	
		Staphylococcus aureus	
drug discovery	EVOO extract	*Chlamydia trachomatis*	63
food; medicine	olive leaves extract	*Trichophyton interdigitale*	64
Antioxidant Activity			
aging	HTyr and Ole	inflammatory cell lines	66,67
biomedicine	EVOO	J774A.1 cell lines	70
Anti-inflammatory Activity			
medicine	EVOO	metabolic syndrome patients	71
medicine	HTyr	THP-1, PBMC, MRC-5, RAW 264.7 cell lines	74
medicine	HTyr	inflammatory diseases animal models	77−81
medicine	EVOO	nephropathic patients	84,85
medicine	EVOO	microglia cells	87
medicine	olive leaves extract	McA-RH7777 cells	88
medicine	aqueous olive leaves extract	paw edema	89
Antitumoral Activity			
drug discovery	HTyr	SH-SY5Y cell line	97
drug discovery	Ole-rich olive leaves extracts	colon carcinoma, breast cancer, chronic myeloid leukemia and melanoma	98
drug discovery	oleocanthal	MDA-MB-231 cells	101
drug discovery	Ole, Tyr, HTyr, EVOO extracts	HUVEC	105

EVOO polyphenols are potent inhibitors of LDL oxidation, a significant risk factor for atherosclerosis and cardiovascular diseases.[68] Several studies associate this behavior with the ability to bind human LDL particles.[69] Furthermore, EVOO biophenols inhibit cell-mediated oxidation of LDL by enhancing mRNA transcription of the antioxidant enzyme glutathione peroxidase (GSH-Px).[70] EVOO intake can improve glycemic and insulin sensitivity. It also modulates transcription of genes involved in metabolism, inflammation, and carcinogenesis, leading to shifts of the inflammatory phenotype of circulating inflammatory peripheral blood mononuclear cells (PBMCs) into a less harmful inflammatory

https://doi.org/10.1021/acs.jafc.4c00945
J. Agric. Food Chem. 2024, 72, 12871−12895

Scheme 2. Synthesis of ω-Hydroxyalkylcarbonates from HTyr



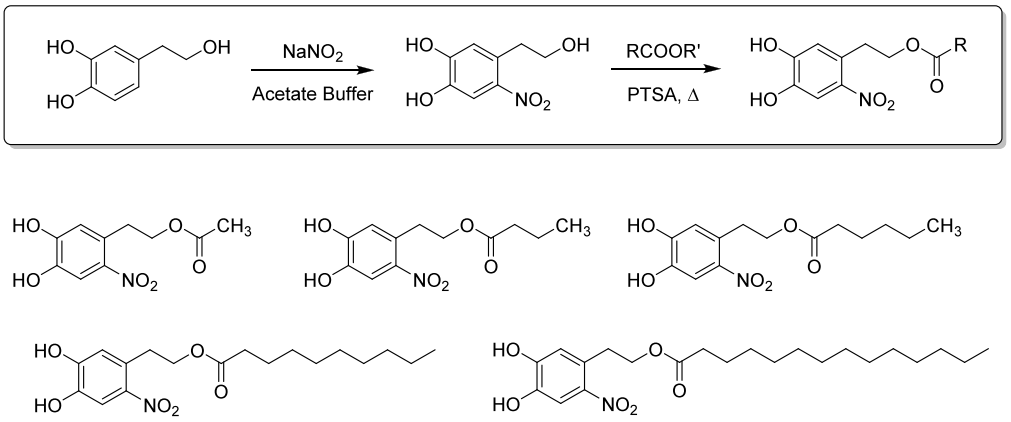
cell phenotype. This effect has been observed in both healthy individuals and patients with metabolic syndrome.[71] Nevertheless, the bioavailability of major olive polyphenols, standing on their hydrophilic nature, could be improved through structural modification (see section 2.4) and/or encapsulation. Liposomes or biobased nanovesicles represent a practical solution to facilitate the absorption and delivery of phenols and polyphenolic extracts, enabling them to exert their antioxidant and anti-inflammatory properties more efficiently.[72,73] *In vivo* studies have demonstrated that polyphenols-enriched formulations mitigate inflammatory response by inhibiting the production of pro-inflammatory cytokines (IL-1β, TNF-α, IL-6) and enzymes (p38MAPK).[74] Additionally, *in vitro* and *in vivo* studies revealed that purified HTyr is able to counteract the increase in multiple inflammatory mediators, thereby modulating inflammation and autophagy.[75,76] Administration of pure HTyr to human and murine cell lines effectively modulates inflammatory biomarkers (THP-1, PBMC, MRC-5, and RAW 264.7). HTyr downregulates the expression of the innate immune receptor TLR-4 and pro-inflammatory cytokines, chemokines, and acute phase proteins.[74] Furthermore, the anti-inflammatory effects of HTyr have been validated *in vivo* by using animal models of inflammatory diseases.[77−79] HTyr can also exert anti-inflammatory activity by modulating the quali-quantitative composition of guttle microbiota.[80,81] Among olive phenolic compounds, oleocanthal has been found to exhibit anti-inflammatory activity, inhibiting key enzymes involved in the inflammatory process through mechanisms similar to nonsteroidal anti-inflammatory drugs.[82,83]

Noce et al. carried out an *in vivo* study on nephropathy patients and demonstrated the anti-inflammatory effect with a significant reduction of IL-6 and C-reactive protein (CRP) after the intake of EVOO rich in oleocanthal. The daily intake of EVOO rich in phenolic compounds has also shown a cardioprotective action in nephropathic patients.[84,85] Inflammation is a complex biological process that involves several mechanisms, including cytokine cascade. Dysregulation is associated with several chronic diseases. The anti-inflammatory properties of EVOO contribute to cytokines downregulation and help to balance and control the inflammatory response.[86] In addition, recent research has unveiled the impact of EVOO's polyphenols on microglia cells in the central nervous system (CNS), enhancing the expression and activity of triggering receptor expressed on myeloid cells 2 (TREM2), a receptor associated with anti-inflammatory and neuroprotective effects.[87] Leaves extracts from an Italian cultivar rich in Ole and luteolin-7-*O*-glucoside revealed promising anti-inflammatory activity in McA-RH7777 cells.[88] Using the carrageenan-induced inflammation assay, the potential anti-inflammatory effect of the aqueous extract of olive leaves was assessed, showing significant inhibition of paw edema in rats at doses of 400, 200, and 100 mg/kg. The extract demonstrated the ability to fight both the early and the late stages of inflammation. The results revealed a significant reduction in all of the measured pro-inflammatory markers (TNF, IL-1, COX2, and NO), compared to the effect of the control drug (diclofenac), confirming the anti-inflammatory potential of the extract and reducing the concentration of proinflammatory cytokines at the site of inflammation.[89] As a result, incorporating EVOO into diet can be a valuable strategy for mitigating inflammation and promoting overall well-being.[90]

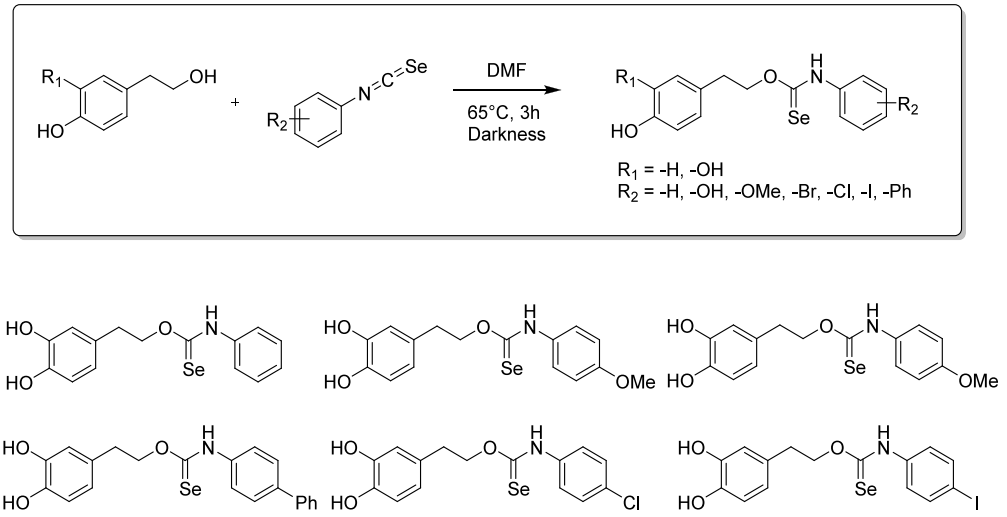
The antitumoral effects of EVOO polyphenols, as extracts or pure compounds, have garnered considerable attention, opening new paths for understanding and potentially treating various types of cancer.[91−94] The antitumoral effects of EVOO polyphenols operate through various mechanisms, influencing multiple stages of cancer progression; a crucial aspect is their ability to induce programmed cell death by apoptosis at low concentrations.[95−97] The anticancer effects of Ole-rich olive leaves extracts have also been investigated. In a 2020 study, the inhibition of glycolysis by Ole on cancer cells under-regulating GLUT-1, PKM2, and MCT4 was demonstrated, this has been highlighted in colon carcinoma, breast cancer, chronic myeloid leukemia, and melanoma.[98] Oleocanthal has demonstrated potent *in vitro* and *in vivo* neuroprotective and antiproliferative activities against various human cancer cells.[99,100] Additionally, oleocanthal revealed capability to trigger a caspase-dependent apoptosis pathway at the concentration of 25 μM, showing caspase-8, caspase-3 activation, and death domain kinase cleavage in breast adenocarcinoma MDA-MB-231 cells.[101] Peri et al. (2022) studied the effect of an oleocanthal-rich extract of

https://doi.org/10.1021/acs.jafc.4c00945
J. Agric. Food Chem. 2024, 72, 12871−12895

Scheme 3. General Synthetic Procedure and Chemical Structures of Novel Nitrohydroxytyrosol Esters



Scheme 4. General Synthetic Procedure and Chemical Structures of Novel Selenium Organohydroxytyrosol Derivatives



EVOO on the gastric adenocarcinoma cell line (AGS wt) and drug-resistant AGS cells. The study demonstrated that treatment with the extract enhances the drug's efficacy and anticancer activity on gastric adenocarcinoma cells.[102]

Both single compounds such as Ole, Tyr, and HTyr as well as EVOO polyphenolic extracts demonstrated antiangiogenic effects, preventing the formation of new blood vessels.[103,104] A polyphenolic extract was able to significantly reduce stimulated angiogenesis in human umbilical vein endothelial cells (HUVEC).[105] The combined effects of olive oil's entire polyphenolic profile are more effective than the individual purified components. This suggests that polyphenols work synergistically, enhancing each other's action.[106]

The *Olea europaea* L. compounds' activities, field of applications, and target against which they are active are summarized in Table 2.

2.4. Chemical Functionalization of HTyr and Ole. The absorption and bioavailability of polyphenols as active ingredients in humans are important issues that are often underestimated, especially in the field of food supplements. Semisynthetic derivatives of natural phenols have been developed throughout the years to enhance the biological activities of naturally occurring phenols and increase the bioavailability. In this section, our attention will be focused on chemical modifications of HTyr and Ole.

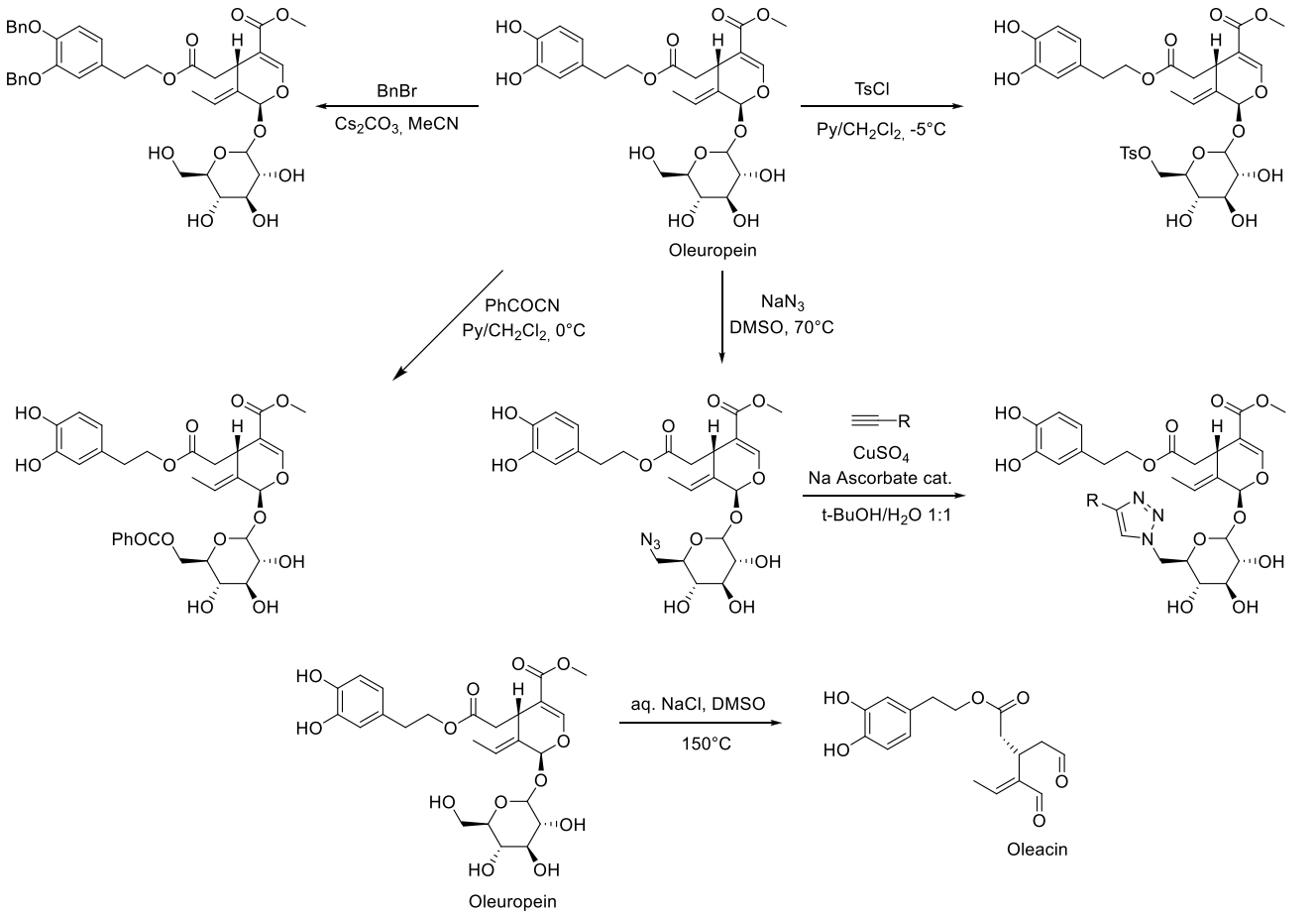
Among all possible HTyr functionalizations, esterification is by far the most employed.[107−111] The regioselective esterification of aliphatic hydroxyl groups of polyphenols via acyl nucleophilic substitution under nucleophilic catalysis requires the protection of phenolic hydroxyls. For example, esterification reactions, coupled with protection and deprotection of hydroxyl groups, have been performed to achieve the formation of ω-hydroxyalkylcarbonate derivatives starting from HTyr (Scheme 2).[112] These derivatives showed increased antimicrobial activity against *Tripanosoma brucei* compared to HTyr.

Appendino et al. (2002) reported on the esterification of HTyr carried out via acyl nucleophilic substitution.[113] Regioselective HTyr fatty acids esters synthesis has also been reported in high yields (81%) using immobilized lipase from *Candida antarctica* (Novozym 435), in a solventless reaction under vacuum.[114] Mitsunobu's reaction has been widely employed to obtain a series of HTyr esters with cinnamic acids that were subsequently tested as monoamine oxidase inhibitors and for the treatment of breast cancer.[115,116]

Nitrohydroxytyrosol and its esters have been successfully synthesized by Trujillo et al. (2014);[117] HTyr was recovered from OMWW and then treated with sodium nitrite in acetate buffer. The following acid-catalyzed esterification afforded the corresponding nitrohydroxytyrosyl alkyl esters (Scheme 3);

https://doi.org/10.1021/acs.jafc.4c00945
J. Agric. Food Chem. 2024, 72, 12871−12895

Scheme 5. Functionalization Reactions of Ole



some of them showed significantly increased antioxidant activity compared to HTyr.

HTyr phosphodiesters, obtained in a six-step procedure by Romanucci et al. (2021),[118] are suitable structures for both the prevention and therapy of Alzheimer's disease and novel potential antioxidants 2-arylhydroxytyrosol derivatives were synthesized via Suzuki−Miyaura cross-coupling.[119]

Etherification reaction has also been explored throughout the years because of the biological relevance of HTyr alkyl ethers. For example, HTyr ethyl ether showed intestinal anticarcinogenic activity,[120] while hexyl ether exhibited antiangiogenic[121] and antiplatelet effects.[122]

HTyr has also been employed to synthesize the isochroman moiety, a scaffold occurring in natural products as well as drugs and agrochemicals.[123] The regioselective oxa-Pictet−Spengler reaction has been employed to achieve the formation of 1-substituted-6,7-dihydroxyisochromans as the intramolecular cyclization takes place mainly in the less sterically hindered position.[124,125]

More recently, HTyr has been employed as a starting material for the synthesis of a panel of selenocarbamates via direct nucleophilic coupling between the phenolic derivative and the selenocumulene (Scheme 4). Due to the strong antioxidant properties of selenium-containing compounds, products obtained this way showed significant *in vitro* antioxidant activity alongside antiproliferative activity on tumor cell lines.[126]

Compared with HTyr, the derivatization of Ole has been scarcely investigated. The primary hydroxyl group in the structure is the most reactive position for selective functionalization. Benzoylation on the primary hydroxyl group was performed by Jerbi et al. upon treatment of 80% pure Ole with benzoyl cyanide at 0 °C under an inert atmosphere. The same authors also reported benzylation on the catechol moiety, tosylation, and conversion of the hydroxyl group into azide moiety, which was subsequently involved in the copper-catalyzed "click-reaction" to give the corresponding triazole.[127] All of the described reactions are reported in Scheme 5.

Vougogiannopoulou et al., treating natural Ole with aqueous sodium chloride in dimethyl sulfoxide (DMSO) at 150 °C under Krapcho decarbomethoxylation conditions, obtained semisynthetic oleacein that subsequently proved to target 5-lypoxygenase.[128]

As reported in the section 2.2, both HTyr and Ole were successfully isolated and purified from olive oil waste and byproducts; therefore, the above synthetic procedures described for commercial HTyr and Ole could be extended to these compounds obtained from plant materials, according to the circular economy concept.[29−45]

Moreover, HTyr-enriched extracts from *Olea europaea* L. waste and byproducts have been successfully employed for chemical derivatization under green chemistry conditions over the years. For example, an HTyr-enriched extract from olive pomace was functionalized with dimethyl carbonate in the presence of a catalyst to achieve the corresponding lipophilic carbonate in 92% yield, which preserved a high radical scavenging activity but resulted soluble in a nonaqueous medium.[39] HTyr-enriched extracts were esterified with acyl

https://doi.org/10.1021/acs.jafc.4c00945
J. Agric. Food Chem. 2024, 72, 12871−12895

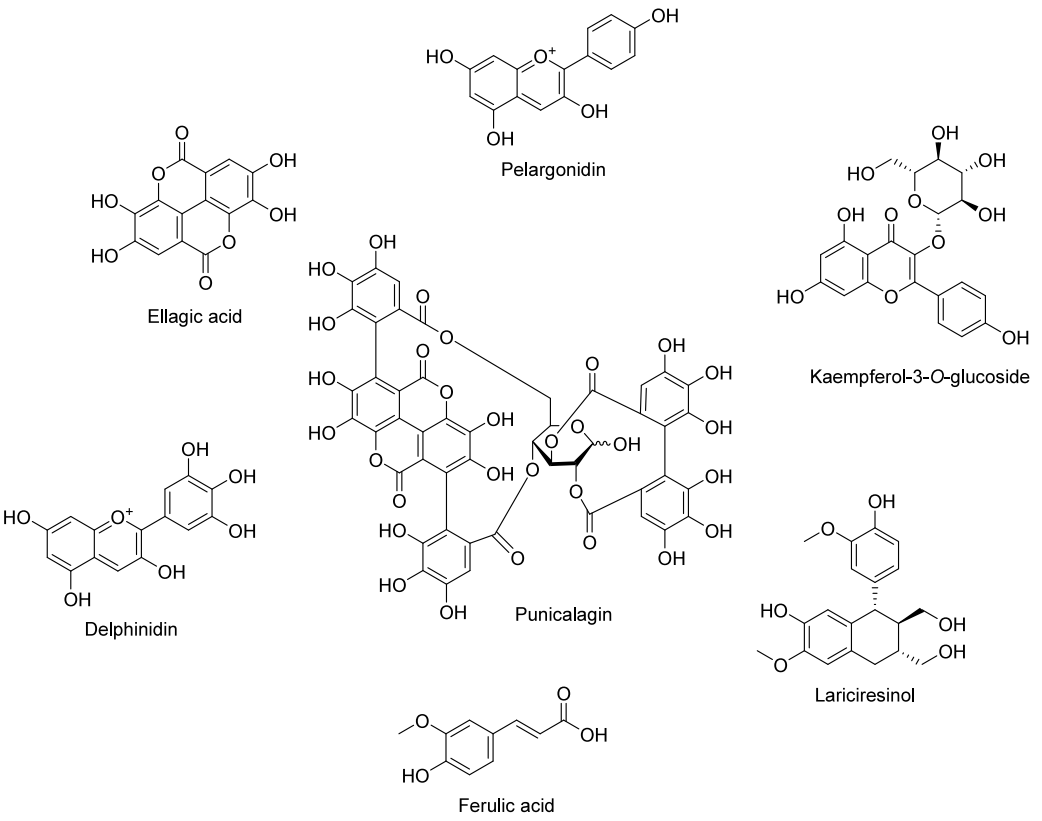


Figure 2. Main phenolic compounds found in *Punica granatum* L.

chlorides to obtain extracts containing both HTyr and the corresponding esters (HTyr butanoate, octanoate, and oleate). After the synthesis and characterization, they were tested *in vitro* on a model of colorectal cancer cells (HCT8-β8). The experimental results evidenced that all extracts showed an antiproliferative activity on cancer cells, and the most effective was the one containing HTyr oleate due to the presence of the unsaturated C-18 chain.[40]

2.5. Stabilization and Delivery System. Based on their biological activities, EVOO's and waste matrices' polyphenols represent high value-added resources with significant application in food, cosmetic, and medical fields. Due to their hydrophilic nature, the potential attributed to HTyr, Ole, and enriched extracts, manifests low bioavailability in humans, and their susceptibility to environmental factors, including light, temperature, and oxygen, represents a liability to their absorption and stability.[81,129]

To overcome these limitations, structural modification (as discussed in section 2.4)[97,130] and/or encapsulation techniques could be applied to identify innovative drug delivery systems.[131]

Encapsulation stands out as an intriguing strategy for the preservation of bioactive compounds, allowing them to exert their beneficial effects.[132] Within this context, liposomes or biobased delivery systems emerge as practical solutions to enhance polyphenols absorption and delivery, enabling them to exert their antioxidant and anti-inflammatory properties more effectively.[72,73] Concerning the encapsulation efficiency, Ole showed greater efficacy compared to that of HTyr and Tyr in zwitterionic liposomes. The encapsulation resulted in decreased cytotoxicity of all products toward fibroblasts compared to direct treatments with phenolic compounds.[131]

While liposomes boast low toxicity, high biocompatibility, and controlled release ability, they face challenges related to chemical stability at room temperature and liability to hydrolysis and oxidize.[133,134] Additionally, these vesicles are inclined to aggregation and fuse over time.[135] An eye drop formulation based on Ole liposomes has been proposed to alleviate dry eye symptoms. However, the stability of Ole in an aqueous solution is poor. Nevertheless, the drug-in cyclodextrin-in liposome system allowed overcoming of the sensitivity to light and hydrolysis, enabling Ole as an attractive option for ophthalmic use.[136]

A sophisticated technique for delivering hydrophilic bioactive compounds is water-in oil-in water microencapsulation, employing polymers as shell material.[137−139] With this method, Tyr and HTyr showed high encapsulation efficacy in ethyl cellulose microparticles, revealing an improved bioaccessibility and stability under simulated gastrointestinal conditions. These results could be referred to a protective effect conferred by the microparticles against the first-pass metabolism in the small intestine.[137,138]

Sustainable micro- and nanoparticles could be developed by employing olive extracts or byproducts in combination with natural-based polymeric matrices.

In food science field, chitosan nanoparticles loaded with an olive leaves extract, prepared by ionotropic gelation, exerted at high concentration a greater antifungal activity against *Fusarium proliferatum* compared with pure extract.[140] Additionally, methylcellulose microparticles loaded with an olive mill pomace extract have been formulated to counteract lipid oxidation in three types of olive oil (extra virgin, virgin olive oil, and a blend of virgin and refined olive oil). Fortifying these oils with encapsulated antioxidants led to improved quality

https://doi.org/10.1021/acs.jafc.4c00945
J. Agric. Food Chem. 2024, 72, 12871−12895

Table 3. Some Polyphenols Extraction Methods from Pomegranate Waste and Byproducts

extraction methods, experimental conditions, additional processes	pomegranate plant material	extracts/main extracted compounds	ref
squeezing, concentration by membrane technology	grains/pulp	juice, concentrated juice	186,187
squeezing, concentration by ultrafiltration, constant volume diafiltration	grains/pulp	high yields of polyphenols; low glucose and fructose	190,191
UAE (water, 50 °C, 7 min)	peel	polyphenols, higher yield than simple water bath extraction	198
UAE (extraction time, 20−40 min; ultrasonic power, 500−800 W; ethanol 40−60%, sample−solvent ratio 1:10−1:30 g/mL)	peel	high yields in punicalagin: 505.9 mg/g	179
UAE (pretreatment by freeze-drying, particle size from 125 μm, 45 °C)	peel	polyphenols	181
UAE (liquid−solid ratio 17, 43% ethanol, 10 min of ultrasounds treatment, 300 W power)	flowers	polyphenols	201
PEF (water, 50 °C, 7 min)	peel	polyphenols (partially selective for ellagic acid); higher yield than UAE at the same conditions, higher stability than HVED	198
HVED (water, 50 °C, 7 min)	peel	polyphenols (partially selective for gallic acid); higher yield than UAE and PEF at the same conditions	198
MAE (aqueous ethanol 50%, solvent/solid ratio 60/1 mL/g, power 600 W)	peel	polyphenols (high yield in punicalagin: 143.64 mg/g dry matter); higher yield than UAE	178
MAE (pretreatment with 0.6% Viscozyme L; acidified 30% ethanol, power 443.5 W, time 131.0 min, and solvent-to-solid ratio 23.6:1)	peel	total phenolics (amounts by *in vitro* assays)	179
pretreatment by acid hydrolysis: 4 M HCl in water at 90 °C, 24 h; solvent extraction after acid hydrolysis dimethyl sulfoxide/methanol = 50:50 (v/v)	husk, peels, mesocarp	polyphenols, extractable and nonextractable ellagitannins	180
pretreatment by digestion with 10 L of 6 M HCl, 40 °C, 2 h; solvent extraction after acid hydrolysis	peel	nonextractable polyphenols	181
PWE (40 °C, static time 5 min; particle sizes, as small possible but not smaller than 65 μm)	peel	polyphenols; as effective as conventional methanol extraction	182
PUAE	peel	polyphenols punicalagin: 146.5 mg/g	189
aqueous ball milling (pH = 7, 40 °C)	peel	phenolics, 11.7 g; punicalagin, 8.6−9.5 g/100 g dry peel	190
solvent extraction (EtOH 70%)	leaves	polyphenols, mainly ellagic acid	191

levels, with delayed oxidation and rancidity processes observed during storage via preserving total phenolic content and related antioxidant properties.[141]

The encapsulation of olive leaves extract in lipid nanovectors (OLE-NLC) and its incorporation into a pectin−sodium caseinate hydrogel have shown enhanced shelf life of functional foods while maintaining excellent antioxidant properties over time.[142]

An emerging strategy, oleogelation, could pave the way for innovative approaches to EVOO consumption, especially as a fat substitute.[143] In this context, the application of olive emulsion gels as fat replacers in frankfurters has emerged as an effective strategy for the development of functional foods that are well-received by consumers, in the meantime preserving food from oxidative spoilage, thanks to an enhanced polyphenols content.[144]

The interest and use of delivery systems as active components in matrices, given (i) the protection of the active compounds, (ii) the enhanced polyphenols content, and (iii) the unaltered or enhanced biological activity, are opening the doors to the identification of new biocompatible, biodegradable, and sustainable ways to delivery.

3. POLYPHENOLS FOUND IN *PUNICA GRANATUM* L.

3.1. Structural Features. Pomegranate (*Punica granatum* L.) is considered to be natively from northern India and Iran; nevertheless, it has been widely cultivated all over the world, and in particular in the Mediterranean area. The fruit, called balausta, has a rounded appearance, with a pericarp, formed by a hard-outer layer (exocarp or peel) and a soft inner husk (mesocarp or albedo), enclosing many grains, improperly and commonly called arils, formed by seeds surrounded by a juicy fleshy coat that constitute the edible part of the fruit.[145] The fruit is consumed directly as fresh grains as well as fresh juice.

The chemical characterization of pomegranate focused not only on the edible parts, but also on the inedible ones, which represent 40−50% by weight of the entire fresh fruit and constitute the byproducts of juice extraction.[146] The different parts of the pomegranate, such as the seeds, peel, and mesocarp, contain different phytochemicals such as phenolic acids, flavonoids, and hydrolyzable tannins (Figure 2).

The grains contain polysaccharides, pectins, vitamins, organic acids, fatty acids, and appreciable quantities of flavonoids, mainly anthocyanins, responsible for the red color of the juice. The juice contains water (85.4%), polyphenols (approximately 1%), sugars (10.6%), and pectins (1.4%), but also minerals and elements such as cobalt, sodium, calcium, magnesium, cesium, selenium, and zinc.[147,148] The pigments identified in the pomegranate fruit are cyanidin, delphinidin, and pelargonidin 3-glucoside and 3,5-diglucosides. The same compounds are found also in the peel at different percentages.[149−151] Among anthocyanidins, delphinidin-3,5-*O*-diglucoside was chosen as a marker for obtaining a variety fingerprint, not being detected in all of the 15 selected varieties.[152] On the other hand, Hamutal et al. (2011) suggested that relative proportions of delphinidins and cyanidins were accession and season-dependent, while pelargonidins were detected only in winter fruit and always in small concentrations.[149] Gómez-Caravaca and co-workers, identified five principal anthocyanin-flavanols, including afzelechin-delphinidin-3-*O*-hexoside and gallocatechin-cyanidin-3-*O*-hexoside, whose presence in pomegranate was described also by Sentandreu at very low concentrations.[153,154]

The juice contains organic acid as chlorogenic acid, citric acid, and gallic acid and flavonoids as quercetin, rutin, and kaempferol-3-*O*-glucoside[155] but also low quantities of hydrolyzable tannins including ellagitannins and gallotannins, such as punicalagin and punicalin, and numerous galloyl es-

https://doi.org/10.1021/acs.jafc.4c00945
J. Agric. Food Chem. 2024, 72, 12871−12895

ters.[149,150,153] The seeds and seed oil contain high levels of polyunsaturated fatty acids. The seeds exhibit antioxidant capacity due to the presence of polyphenols as 3,4-dihydroxybenzoic acid, ferulic acid, vanillic acid, and syringic acid.[156] The grains are separated by a white membrane called mesocarp, with which the exocarp or outer leathery skin constitutes the pericarp, the main waste component of pomegranate juice production. Pomegranate pericarp is a rich source of tannins, flavonoids, other polyphenols, and anthocyanins. Gallic and ellagic acids were found in pomegranate peel, whereas kaempferol 3-*O*-glucoside was the major flavonoid even if luteolin and quercetin were also found. Anthocyanidins, including mainly cyanidin, pelargonidin, and delphinidin, are present in the peel.[157] The peel is particularly rich in hydrolyzable tannins, specifically ellagitannins, ellagic acid derivatives,[158] and punicalagins, which are multiple esters of gallic acid and glucose, (hexahydroxydiphenoyl)-gallagyl-hexoside (HHDP).[159] Peel contains, specifically, punicalin, tellimagrandin, pedunculagin, granatin B, punicalagin, and gallagyldilactone. Ambigaipalan and co-workers identified proanthocyanidins in pomegranate outer skin, where the dominant proanthocyanidin was procyanidin dimers (Ambigaipalan, 2016).[160] Lignans (e.g., isolariciresinol) were found in the pericarp of pomegranate.[161] Pomegranate fresh pericarp contains high percentages of water (70−75%), simple sugars (30−35%), phenolic compounds (10−20%), and polysaccharides (10−15%). Polysaccharides and pectins, with high percentages of galacturonic acid, can be included among the bioactive components of the fruit along with phenolic compounds.[152]

3.2. Extractive Methods. Different suitable extraction techniques are available for pomegranate fruits and byproducts according to the process scale, characteristics of raw materials, and targeted subclasses of compounds. A summary of the methods is reported in Table 3. Pomegranate juice can be obtained by pressing the grains or the pulp after peeling the fruit; this process does not require the use of any solvents, and it is used for both lab-scale extraction and industrial purposes, even though juices for the food market, also as ingredients for other processed products, are generally concentrated to improve their shelf life and facilitate transport and storage.[162,163] In this case, the quality and characteristics of the final product are strongly influenced by the technology employed for concentration. Membrane technology, in particular nanofiltration and reverse osmosis, was reported as a very effective method to preserve the original properties of the juice, whereas thermal evaporation can compromise the stability of color and bioactive compounds.[164,165] Membrane technology also allows the recovery of purified bioactive polyphenols from clarified juice. Conidi et al. (2017 and 2020) obtained a retentate with low glucose and fructose and high yields of polyphenols by ultrafiltration concentration followed by constant volume diafiltration, with the perspective of using the high polyphenols retentate as an ingredient for nutraceutical products.[166,167] After peeling and squeezing pomegranate fruits to extract the juice, peel and pressed pulp are the resulting waste, where peels amount for 50% of weight of the fruit,[168] which contains an important portion of the bioactive polyphenols present in pomegranate fruit.

Conventional extraction methods of polyphenols from pomegranate as maceration or Soxhlet extraction can allow good yields of targeted compounds, but they often need medium-high temperatures, causing the degradation of thermolabile molecules above 40−45 °C;[169,170] in addition, long times of extraction, high costs, and the environmental impact of organic solvents led to the search for new alternative techniques.[171] Recent articles discuss new sustainable methodologies for extracting polyphenols from pomegranate by-products. However, many of these studies focus on laboratory-scale applications and lack thorough investigation of their industrial applicability. Among these techniques, the most widespread are UAE and MAE, enzymatic extraction, and PFE. Other authors also reported the use of NADES, extraction assisted by pulsed electric fields (PEF) or high voltage electrical discharge (HVED), and supercritical CO_2 combined with polar solvents preceded by enzymatic pretreatment.[172,173]

UAE feasibility has been studied on an industrial scale due to its affordability resulting from reduced installation costs, easy maintenance, and low energy and solvent consumption.[174] In a study carried out by Rajha et al. (2019),[175] ultrasounds were compared with PEF and HVED in enhancing water extraction of pomegranate peel, at 50 °C. After 7 min of extraction, the concentration of phenolic compounds in the extracts was higher for UAE than for simple water bath extraction. On the other hand, the efficiency of PEF and HVED was significantly higher. HVED allowed for higher yields in polyphenols, but the extracted compounds resulted in more stability at PEF extraction, which is also suitable for industrial implementation. These two methods also allow for partial selectivity. The PEF method enhances the recovery of ellagic acid, while HVED allows higher yields in gallic acid compared with the other tested methodologies. This represents a further advantage compared to UAE and MAE, which are less selective.[174,175] On the other hand, UAE has been applied to pomegranate peel to increase punicalagin yield. Liu et al.[176] studied the influence of time, ultrasonic power, amount of ethanol, and sample−solvent ratio by single-factor experimental design, evidencing that the maximum yield of punicalagin occurs with an extraction time of 20−40 min, an ultrasonic power in the range 500−800W, a quantity of ethanol between 40% and 60%, and a sample−solvent ratio between 1:10−1:30 g/mL. In these conditions, punicalagin was 505.9 mg/g from pomegranate peel powder, 26.7% higher than the value obtained by maceration (390.9 mg/g). Cano-Lamadrid et al. (2023)[177] focused their study on the optimization of UAE parameters with a polynomial regression model, evaluating pre-extraction variables including cultivar, drying method, particle size, and some variables directly implicated in the extraction process: time and temperature. This study evidenced that punicalagin content was lower for convective drying at 60 °C than for freeze-drying samples, and small particles and 45 °C were the best conditions for extraction. UAE was also used to obtain phenolic compounds from pomegranate flowers. The best process parameters obtained by using Box−Bohnken design were a liquid−solid ratio of 17 in 43% ethanol and 10 min of ultrasound treatment with 300 W power. The extracts prepared in these conditions showed antimicrobial activity against *Staphylococcus mutans* and its biofilm activity, moreover, they had good antioxidant and radical-scavenging ability.[178]

Concerning MAE, Kaderides et al. (2019)[179] compared this technique to UAE extraction. After optimizing the extraction parameters, they obtained yields 1.7 times higher than with UAE in shorter time (4 min MAE vs 10 min UAE). Scanning electron microscope (SEM) analysis highlighted an intense cell disruption by microwave treatment, which may be mainly

https://doi.org/10.1021/acs.jafc.4c00945
J. Agric. Food Chem. 2024, 72, 12871−12895

Table 4. Biological Activities of *Punica granatum* L.

application field	part of plant/ extract	results/target	ref
Antimicrobial Activity			
veterinary	flowers	*Staphylococcus* species associated with bovine mastitis	200
medicine	peels	*Candida albicans*, *Pseudomonas aeruginosa*, Escherichia coli, Salmonella spp., *Staphylococcus aureus*, Listeria monocytogenes, Enterococcus faecalis, *Bacillus cereus*, *Candida albicans*, and *Aspergillus niger*: cell lysis	199
medicine	juice	*Streptococcus mutans* and *Aeromonas hydrophila*: positive effects; *Klebsiella pneumoniae and Candida albicans*, no activity	219
Antiviral Activity			
medicine	leaves	prevent viruses from adhering to the cell membrane	199
medicine	whole fruit	maintains the integrity of the endothelium and limits the activation of inflammatory cells and tissue invasion in COVID-19 patients	217
Antioxidant Activity			
medicine	leaves	preserve antioxidant enzymes	199
medicine	flowers	inibition of nitrite generation	199
medicine	flowers	greater antioxidant activity of Garsi and Zaghwani varieties	201
medicine	peel	high content of phenolic compounds in comparison with seed and juice	202
medicine	peel	prevent toxic effects of ROS, ionizing and xenobiotic factors	203−205
medicine	whole fruit	the whole homogenized fruit has 20 times greater activity than the juice	206
medicine	whole fruit	effects against oxidative stress and cytotoxicity, protective action against hydrogen proxide	196
medicine	peel	reduction of proinflammatory cytokines in bovine mammary epithelial cells	213
medicine	peel	reduction in C-reactive protein and serum amyloid-A and total cholesterol in rats	213
Anticancer Activity			
medicine	flowers	inibition of nitrite generation and tumor necrosis (TNF-α)	200
medicine	leaves	action on the cell cycle and promotes aoportosis by interrupting mitochondrial activity in human multiple myeloma	221
medicine	leaves	in lung and prostate cancer effects in a dose- and time-dependent way	221
medicine	whole fruit	activity on cell proliferation and proapoptotic affects in ovarian carcinoma, pancreatic adenocarcinoma, cervical carcinoma, liver cancer, hepatocarcinoma, and colorectal cancer.	222
medicine	juice	proapoptotic effects on breast, liver and colon cancer cells	219

responsible for the yield enhancement. The MAE extract also showed a high content of punicalagin (143.64 mg/g dry matter). The extraction of polyphenols can be further enhanced by combining enzymatic pretreatments with the described extraction techniques. High yields of polyphenols from pomegranate peel were obtained by pretreating the biomass with Viscozyme L, a cellulolytic enzyme mixture, followed by MAE with acidified 30% ethanol.[180]

Pomegranate peel has also been reported for its content in nonextractable polyphenols: this fraction may represent a considerable part of the total polyphenols contained in the plant tissues, and it needs further procedures to be extracted. Performing acid hydrolysis with HCl at 90 °C for 24 h, the yield of peel polyphenols increased from 549.1 to 750.6 mg/g dry weight.[181] Sun et al. (2021) performed the extraction of the nonextractable polyphenols by digestion with HCl, followed by column fractionation for analysis and identification.[182] The highest polyphenols yield was obtained with 6 M HCl, for 2 h at 45 °C by using a solid/liquid ratio = 1:20.

Among hydrolyzable tannins present in *Punica granatum* L., punicalagin has been extensively studied in recent years.[183−187] Given its pharmacological properties, research has made efforts to optimize extraction techniques that could isolate this compound and maximize its extraction yield from pomegranate peel.[188] Cam et al. (2010)[189] compared the results obtained from the extraction of pomegranate peels with different solvents (methanol, ethanol, ethyl acetate, acetone, and water) with those obtained by pressurized water extraction (PWE), which proved to be a very effective and faster technique, optimizing temperatures and extraction time as well as the dimensions of the matrix. Kazemi et al.[190] optimized the pulsed ultrasound-assisted extraction (PUAE) conditions obtaining 146.5 mg/g punicalagin. Talekar et al. (2019)[191] described a fast and sustainable method to obtain high-punicalagin extracts by aqueous ball milling at pH = 7 and 40 °C from fresh pomegranate peel. The authors highlighted the effectiveness of this technique that allows for extracting up to 11.7 g of phenolics (8.6−9.5 g of punicalagin) for every 100 g of dry peel. Even if further studies are needed to assess the suitability of this methodology for industrial applications, it seems to be an interesting technique to be upgraded because of its simplicity and rapidity, low-energy consumption, and absence of chemicals.

Also, pomegranate leaves are reported as a raw material to obtain polyphenol-rich extracts for different applications on a lab-scale. One ethyl acetate fraction high in polyphenols was prepared from an extract of pomegranate leaves in 70% ethanol and then analyzed by UPLC-PDA-UV and LC-MS-MS analysis. The main compound was ellagic acid, but the other 23 polyphenolic compounds were identified according to their spectrophotometric and spectrometric data. The ethyl acetate fraction was used to test a green synthesis of silver nanoparticles with antimicrobial activity against different Gram-positive and Gram-negative bacteria.[192]

3.3. Biological Properties. Based on the kind and content of bioactive components (flavonoids, ellagitannins, punicalagins, ellagic acid, vitamins, minerals), pomegranate has been described as a "Nature's power fruit".[193,194] The biological properties have been described since ancient times, and over the years the scientific community has explored several applications.[195,196] The interest is due to the polyphenols present in the juice and whole fruit, including leaves, flowers, peel, and arils.[197]

This section and Table 4 provide an overview of some recent studies on the biological properties of *Punica granatum* L., focusing on the results of the antioxidant, anti-

https://doi.org/10.1021/acs.jafc.4c00945
J. Agric. Food Chem. 2024, 72, 12871−12895

inflammatory, antimicrobial, antiviral, and anticancer activities that the scientific community has recently published.

Studies of the antioxidant properties have been collected for all parts of the fruit and plant including leaves and flowers.[198] Machado et al. (2023) related the antioxidant and anti-inflammatory properties of *Punica granatum* L. leaves extracts to the presence of polyphenols and terpenes.[199] Both *in vivo* and *in vitro* studies are described showing nephroprotective action and reduction of markers of renal damage while preserving antioxidant enzymes. Antioxidant and anti-inflammatory effects are highlighted, resulting in a stabilization of the erythrocyte membrane, decreasing the quantity of hemoglobin derived from hemolysis. The flower extract inhibited nitrite generation and tumor necrosis (TNF-α) and showed activity against induced ischemia and brain damage in rats.[200] A recent study on *Punica granatum* L. flower extract demonstrated the antioxidant and antibacterial activity against mastitis pathogens by reducing postinfection oxidative stress, highlighting the use of this natural extract for the treatment of this pathology. Bekir et al. (2013) characterized seven varieties of pomegranate flowers and found that the varieties Garsi and Zaghwani are the ones with greater antioxidant activity.[201] The antioxidant capacity of pomegranate peel has been largely investigated by the scientific community in the last 20 years. The pomegranate peel is the plant material with the highest content of phenolic compounds, flavonoids, proanthocyanidins, and ascorbic acid. This evidence also demonstrated a high antioxidant capacity related to the content of active compounds even in comparison with seeds and juice which is the most consumed.[202] In this context, the antioxidant activity of pomegranate peel plays an important role in preventing the toxic effects of reactive oxygen species (ROS) and their ability to damage important and sensitive biological substrates such as RNA, DNA, lipids, and plasma membrane proteins which cause diseases such as cancer, cardiovascular diseases, and diseases induced by factors such as exposure to ionizing and xenobiotic factors.[203−205] Studies have also been carried out on arils and pomegranate juices to evaluate the polyphenol content and its correlation with antioxidant capacity. Tzulker et al. (2007) studied 29 types of pomegranate and related juices and analyzed antioxidant activity, total polyphenol content, total anthocyanins content, and the levels of four major hydrolyzable tannins. They demonstrated that the antioxidant activity in the juices was significantly correlated with the total content of polyphenols and anthocyanins, but the homogenates prepared from the whole fruit showed antioxidant activity approximately 20 times higher than the level found in the aril juice. In this case, the level of antioxidant compounds in the homogenates is significantly correlated to the content of four hydrolyzable tannins in which punicalagin is the most abundant, while they did not find correlation with the level of anthocyanins.[206] Liu et al. (2019) demonstrated that pomegranate extract enhanced the effects against oxidative stress and cytotoxicity thanks to the protective action against hydrogen peroxide by ellagic acid and punicalagin. These molecules reduced reactive oxygen species and cell apoptosis by up to 8.26%.[207] Several studies have shown that juice preparation technology affects the content of active molecules and antioxidant properties. Esposto et al. (2021) showed that in pomegranate juices, the most abundant molecules are punicalins among the main ellagitannins, while the predominant anthocyanin was cyanidin 3,5-diglucoside, followed by cyanidin 3-glucoside. During the production of the juices, the content of active molecules varies and consequently also the antioxidant capacity, affecting the functional quality of the final product.[208] Experiments carried out by Aloqbi et al. (2016) demonstrated that pomegranate juice has greater antioxidant power than punicalagin by scavenging free radicals. However, punicalagin showed significant iron chelating activity and potency-reducing ability in a dose-dependent manner compared to pomegranate juice.[209]

The anti-inflammatory activity is often related to the antioxidant properties of the pomegranate. A high *in vitro* anti-inflammatory activity of *Punica granatum* L. leaves extract has been observed on erythrocyte hydrolysis because it stabilizes the erythrocyte membrane and decreases the amount of hemoglobin deriving from hemolysis. *In vivo Punica granatum* leaves extract showed activity against ischemia-induced brain damage in rats that were treated with doses of 200−400 mg/kg for 7 days. This treatment reduced the brain changes that cause ischemia (edema, vascular congestion, and release of proinflammatory proteins and cytokines).[200] The study by Bekir et al. shows analyses of the anti-inflammatory properties of seven varieties of pomegranate flowers.[201] The results show that all varieties are active, but the variety with the greatest activity is Zaghwani (2.5 ± 0.1 mg/L). In this study, statistical analysis showed that the variety of pomegranate flowers is a significant factor ($P < 0.01$) influencing the chemical composition and biological activities. Pomegranate flower extract also showed analgesic and anti-inflammatory effects on reducing edema in rats, proposing this extract for uses and applications against inflammation and pain.[210] Xu et al. (2017) investigated the anti-inflammatory capacity of pomegranate flower ethanol extract in lipopolysaccharide (LPS)-induced RAW264.7 cells. The study shows that the extract inhibits the production of nitric oxide (NO), PGE2 (prostaglandin E2), and proinflammatory cytokines (TNF-α, IL-6, IL-1β) and also inhibits the protein expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX2) in RAW264.7 macrophages stimulated with LPS and blocks the nuclear translocation of nuclear factor kappa light chain enhancer of activated B cells (NF-KB).[211] Some studies about the anti-inflammatory properties of pomegranate peel extracts linked to the presence of punicalagin, punicalin, strictinin A, and granatin B, which significantly reduce the production of NO and PGE2 by inhibiting the expression of pro-inflammatory proteins. Studies carried out on human neutrophils show the inhibitory activity of extracts from *Punica granatum* L. peels of myeloperoxidase and the enzymatic production of HCl from hydrogen peroxide at a concentration of 50 ng/mL. Pomegranate peel extracts have been used for the reduction of inflammation, edema, and pain in rats.[212] The anti-inflammatory effect of the aqueous pomegranate peel extract was tested in an *in vitro* and *ex vivo* study, and the results showed a reduction of the proinflammatory cytokine IL8 in TNF-stimulated Caco-2 cells and suppressed the gene expression of proinflammatory cytokines (IL1A, IL6, and IL8) from colon tissues subjected to LPS. The tested extract was rich in punicalagin, and this evidence suggests the possible use of the aqueous pomegranate peel extract for the prevention of inflammation of the gastrointestinal system.[213] In 2020, the same extract was tested for the first time on bovine mammary epithelial cells (BME-UV1) to evaluate the anti-inflammatory and antioxidant activity; in fact, the pomegranate peel extract reduced production of reactive oxygen species and expressions of proinflammatory cytokines, showing an anti-inflammatory

https://doi.org/10.1021/acs.jafc.4c00945
J. Agric. Food Chem. 2024, 72, 12871−12895

effect on BME-UV1 treated with LPS. This could allow the use of peel pomegranate extracts for the nutritional supplementation of dairy cattle.[214] Salama et al. conducted an *in vivo* study on rats fed a high-fat diet to evaluate the anti-inflammatory and antiatherogenic effects of the administration of pomegranate peel extract powder. The rats treated with the pomegranate extract showed a reduction in C-reactive protein and serum amyloid-A and total cholesterol. The analyses of the portions of the thoracic aortas of the rats treated with the pomegranate peel extract also had less atherosclerotic damage.[215] In the review by Singh et al. (2023), the anti-inflammatory effects of pomegranate peel extract are summarized, including the reduction of the levels of COX-2, iNOS, TNF-α, IL-1β, and IL-6 and proinflammatory cytokines and inhibition of NF-KB. These effects are mainly due to the presence of ellagic acid and punicalagin in pomegranate peel extracts.[216] Several studies have been reported relating to the anti-inflammatory properties due to the presence of gallotannins and ellagitannins (punicalagin and granatins), flavonoids (quercetin, catechin), and ellagic acid found in pomegranate seed oil.[217]

Pomegranate has been known for its antimicrobial properties since ancient times. This plant is used against both Gram-positive and Gram-negative bacteria, even against more resistant strains. Pomegranate extracts are also active against fungi such as *Candida albicans*. Positive synergistic effects have also been highlighted in combining pomegranate extracts with antibiotics, which increases their effectiveness. Pomegranate leaves extracts, also rich in pigments, steroids, and terpenoids, have shown the ability to penetrate the microbial membrane and cause cell lysis. These effects have been demonstrated on strains of *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, *Listeria monocytogenes*, *Enterococcus faecalis*, *Bacillus cereus*, *Candida albicans*, and *Aspergillus niger*.[200] Regarding pomegranate peels, a review by Singh et al. collected *in vivo* and *in vitro* studies on the antibacterial and antifungal activity of pomegranate peel extracts rich in punicalagins, punicalins, gallic acid, ellagic acid, and gallic acid. The phytocomplex showed a broad-spectrum antimicrobial action against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Lactobacillus acidophilus*, *Actinomyces viscosus*, *Streptococcus mutans*, *Streptococcus sanguinis*, *Streptococcus salivarius*, *Listeria monocytogenes*, *Escherichia coli*, and *Yersinia enterocolitica*. Singh et al. showed how different cultivars have different antimicrobial targets (bacteria and fungi).[216] A recent study of concentrated pomegranate juice showed positive effects on the antimicrobial action on *Streptococcus mutans* and *Aeromonas hydrophila*. However, the same study showed no antibacterial activity against *Klebsiella pneumoniae* at any concentration and indicated no antifungal activity against *Candida albicans*.[218] A recent study published activities comparing the characteristics of the different parts of the pomegranate fruit. Regarding the antimicrobial properties, they have been classified in decreasing order starting from the pomegranate peel that has more phenolic compounds and greater antimicrobial activity, followed by the mesocarp, then the juice and last the seeds.[219]

In recent years, attention to antiviral properties has increased due to the Covid-19 pandemic, and studies on the antiviral properties of pomegranate have been investigated. Machado et al. collected studies on pomegranate leaves extracts rich in ellagic acid which would prevent viruses from adhering to the cell membrane. The authors described studies relating to the synergy between tannins, flavonoids, and terpenes, showing anti-HIV-1 action.[199] Alexova et al. published a paper on the potential antiviral effect of pomegranate polyphenols against Covid-19. The study showed results of *in silico* and *in vitro* studies on ellagic acid, hydrolyzable ellagitannins, and the phytocomplex extracted from the whole fruit on Covid-19, evidencing a greater effect of the phytocomplex. The authors explained this action with the maintaining of endothelium integrity, limiting the activation of inflammatory cells and tissue invasion, and integrating the antioxidant systems in the body. The use of these pomegranate extracts in patients suffering from Covid-19 and in the presence of other pathologies can integrate the body's antioxidant defense.[220] In this context, in-depth and *in vivo* studies are necessary.

In the literature, studies on different cell lines of the anticancer effects of pomegranate extracts are reported. The mechanisms of action seem to interfere in the process of cell proliferation, promoting apoptotic pathways and reducing the migratory capacity of cells. Regarding pomegranate leaves extracts, studies have been carried out on human multiple myeloma, and the extract interfered with the cell cycle by promoting apoptosis by interrupting mitochondrial activity. In lung and prostate cancer, the extract showed anticancer effects in a dose- and time-dependent way.[221] Rahimi et al. (2020) published a review in which they collected more than 40 *in vivo* and *in vitro* studies on the anticancer effect of pomegranate extracts.[222] Regarding studies on prostate cancer, pomegranate juice, pomegranate seed oil, and pomegranate extracts rich in ellagic acid, punicalagin, luteolin, and urolithin were tested. Regarding breast cancer, extracts of pomegranate peel, fermented pomegranate juice, ellagic acid, and punicalagin were tested. Among the active compounds that characterize *Punica granatum* L., the most tested is ellagic acid, which shows activity on cell proliferation and proapoptotic effects in ovarian carcinoma, pancreatic adenocarcinoma, cervical carcinoma, liver cancer, hepatocarcinoma, and colorectal cancer. The authors highlight a limitation in the use of ellagic acid due to its low bioavailability in both humans and animals. In a recent study, concentrated pomegranate juice was also tested on several cancer cell lines. The results obtained by Habib et al. (2023) show that concentrated pomegranate juice has proapoptotic effects on breast, liver, and colon cancer cells.[219]

3.4. Stabilization and Delivery Systems. As pomegranate extracts can be used in food, nutraceuticals, and biomedical sectors, considerations about their stability, metabolism, and bioavailability are crucial, mainly due to the high content of tannins with very complex structures and physicochemical properties. Oral administration of punicalagin exerted positive effects in *in vivo* studies,[223−225] but, for its low bioavailability, low stability at the acidic conditions of the stomach, and enzymatic degradation across the intestinal tract by the microbiota, it is not yet known whether or if the biological effects are due to this molecule or to its metabolites, such as ellagic acid.[226,227] Furthermore, assuming that a small percentage of punicalagin and other high molecular weight tannins are absorbed intact, it remains difficult to predict their distribution within the organism and evaluate the possibility that they reach the biological targets as such or in the form of active metabolites.

To the best of our knowledge, the literature does not report studies about chemical derivatizations of pomegranate polyphenols to improve their stability and activity, probably because of the structural complexity of the bioactive molecules.

https://doi.org/10.1021/acs.jafc.4c00945
J. Agric. Food Chem. 2024, 72, 12871−12895

However, in recent years, some studies described stabilization and delivery systems of punicalagin and pomegranate polyphenols. A spray drying technology was tested by Savikin et al. (2021) to stabilize pomegranate waste extracts and their bioactive compounds by obtaining encapsulated powders, comparing carbohydrate-based and protein-based carrier materials (maltodextrin and whey protein, respectively).[228] The effects of carrier type and concentration on process efficiency and product characteristics such as hygroscopicity, water solubility, and content of punicalin, punicalagin, gallic, and ellagic acid were evaluated. Maltodextrin, in 100% concentration with respect to the liquid extract amount, was the best carrier material, and it allowed a better preservation of the extracted polyphenolic compounds after spray drying, with an encapsulation efficiency of 88.63%, hygroscopicity of 15.17%, and a water solubility index of 87.04%. The main polyphenols in the encapsulated extract were gallic acid, punicalin, punicalagin anomers, ellagic acid, with a high predominance of punicalin, according to literature.[228−229230]

Structures known as herbosomes were prepared using standardized pomegranate extracts by a spray drying method to improve the bioavailability and activity of the extracts.[231] Herbosomes are complexes consisting of standardized plant extracts or their isolated compounds with phospholipids. In the reported study, phosphatidylcholine was used to envelop the extracted polyphenols from pomegranate, thanks to its ability to interact with phenolic groups by the choline head and to form the lipophilic cells with the fatty acid portion. Herbosomes and simple pomegranate extracts were compared by assessing their protective effects on carbon-tetrachloride-induced acute liver damage in rats. By administration of herbosomes, the serum concentration of punicalagins reached approximately 2.5 times that obtained with the conventional pomegranate extract. Furthermore, the levels of various enzymes of the hepatic glutathione system, superoxide dismutase and catalase, were preserved, avoiding the increase of thiobarbituric acid reactive substances, confirming the efficiency of herbosomes in improving the antioxidant and hepatoprotective activities of pomegranate extracts.[231]

In a recent work, montmorillonite was tested as a nanocarrier for oral administration of pomegranate extracts to improve the cellular uptake of the bioactive polyphenols. The results of the *in vitro* tests showed that montmorillonite can adsorb selectively pomegranate phenolics and release them in a controlled manner, also improving cellular uptake on the tested cells. Therefore, this could be an ideal kind of formulation for oral administration of pomegranate polyphenols-rich extracts.[230]

Recently, a lipidic nanocarrier system was optimized by encapsulating punicalagin and ketogenic amino acids (tryptophan, methionine, threonine, lysine, and leucine) with chia seed phospholipids through homogenization, emulsification, and cold ultrasonication. Quality and characteristics of punicalagin and ketogenic amino acid-loaded nano lipid carriers were assessed, and then they were tested for their ability to enhance mitochondrial lipolytic function and stimulate the ketogenesis pathway. The lipidic nanocarrier system was more effective than punicalagin in increasing mitochondrial efficiency and counteracting obesity-associated comorbidities. This suggests that the efficacy and lipolytic potential of punicalagin were enhanced by including it in the ketogenic amino acids−nano lipid carrier system.[232]

Mannose-decorated punicalagin nanocarriers were designed to increase their affinity toward bone marrow macrophages that show abundant mannose receptors on their surface and are involved in alleviating methotrexate-induced neutropenia. Computational studies predicted the interactions of punicalagin and mannose with their specific target regions on mannose receptors via hydrogen bonds, showing that this nanocarrier should be more extensively investigated as a possible lead molecule to regulate the incidence of drug-induced neutropenia.[233]

Many of the stabilization and delivery systems for punicalagin and pomegranate polyphenols are described in very recent studies, and some of them were only analyzed by computational methods or *in silico* experiments, but the reported outcomes suggest proceeding with experimental design and laboratory tests. This could lead to obtain effective delivery systems, allowing a gradual and targeted release of pomegranate active principles, overcoming stability and bioavailability issues.

4. PERSPECTIVES ACCORDING TO THE GREEN CHEMISTRY AND CIRCULAR ECONOMY

The valorization of agroindustrial wastes and byproducts is to convert these plant materials, mostly obtained downstream from agricultural production and the food industry, into high-value-added products (soil conditioners, compost, animal feed, cosmetic and food product ingredients, food packaging and building materials,...) or energy.[234]

With this review, we wanted to contribute to the scientific literature by highlighting the application potential of olive oil and pomegranate processing waste and byproducts typical of the Mediterranean area. These materials represent a valuable source of molecules, e.g., polyphenols, endowed with multiple biological activities (antimicrobial, antioxidant, anti-inflammatory) that can be recovered through conventional and unconventional extraction techniques and used as active ingredients in the formulation of cosmetics, functional foods, dietary supplements, animal feeds, and innovative packaging materials.

The process of waste and byproduct valorization, when properly conducted, contributes to reducing environmental pollution, conserving resources, and generating new revenue streams for industries in the sector. Its sustainable implementation can result from the synergy between green chemistry[2] and the circular economy,[3,4] which are based on the following common principles.

(1) Resource Efficiency. Both green chemistry and circular economy recommend the efficient use of resources. Green chemistry aims to minimize the consumption of raw materials and energy by maximizing the use of renewable resources and reducing waste generation from the design stage of a production process. Similarly, the circular economy focuses attention on keeping resources in use as long as possible through recycling and reuse, so as to use as few raw materials as possible and limit resource depletion.

(2) Waste Reduction and Valorization. Green chemistry and circular economy have in common not only the goal of reducing waste production but also of valorizing it. The methods and technologies that can be developed through green chemistry make it possible to convert waste materials into products or resources with high added value through selective processes such as chemical functionalization of phenolic molecules recovered or recoverable from olive oil

https://doi.org/10.1021/acs.jafc.4c00945
J. Agric. Food Chem. 2024, 72, 12871−12895

waste and byproducts, as reported in section 2.4. In this context, the circular economy provides a framework for closing the loop and reintroducing waste materials back into the production cycle, minimizing waste generation, and maximizing resource recovery.

(3) Innovation in Sustainable Process Design. Green chemistry prioritizes innovation and recommends the design of safe and sustainable chemicals, materials, and processes, taking into account human and environmental impact factors such as toxicity, energy efficiency, and environmental impact. Similarly, the circular economy promotes the design of durable, repairable, and recyclable products and systems, facilitating the transition to a closed-loop system in which resources circulate and are continuously reused.

(4) Interdisciplinarity. Both green chemistry and the circular economy place contamination of diverse knowledge, interdisciplinarity, and promoting collaborations between universities and industries at the basis of sustainability development and process innovation. Both approaches rely on supportive policies, regulations, and economic incentives to encourage companies to adopt sustainable practices, invest in research and development, and shift to circular business models.

(5) Global Perspective and Sustainable Development Goals. The green chemistry and the circular economy are closely aligned with global efforts to achieve the Sustainable Development Goals outlined in the United Nations 2030 Agenda. By promoting resource efficiency, waste reduction, and sustainable innovation, both green chemistry and the circular economy can make an active contribution to achieving these goals and creating a sustainable and resilient future.

In summary, the perspectives of green chemistry and circular economy complement each other and offer a comprehensive framework for promoting sustainability in various sectors. By integrating the principles of both approaches in the valorization of agroindustrial waste and byproducts, it is possible to create sustainable solutions to reducing environmental pollution and waste generation but also generate economic value by promoting resource efficiency and development of a circular economy.[235]

5. CONCLUSIONS

There has been steady growth in the polyphenol market in recent years, which certainly reflects an increase in consumer awareness of the health benefits of polyphenols and their use in various sectors. In 2022, the global market size was estimated at $1.68 billion and is expected to grow at a compound annual growth rate (CAGR) of 7.4% from 2023 to 2030.

The olive oil sector is one of the most studied in terms of polyphenol recovery from byproducts, particularly in the Mediterranean area, for several reasons. First, there is the strong spread of olive cultivation in these areas, which added the complex environmental issue related to the management of waste generated during the olive oil production process and the cost of disposal. Suffice it to say that in Italy alone, annual pomace production is about three million tons. Like the olive sector, pomegranate byproducts are produced in large quantities if we consider that processing 1 ton of pomegranate yields about 550 kg of waste, mainly represented by peel and mesocarp.

The recovery and valorization of polyphenols from *Olea europaea* L. and *Punica granatum* L. through green chemistry processes are examples of the application of the circular economy strategy, strongly recommended by the European Union to reduce the high economic and environmental impacts associated with the disposal of agroindustrial byproducts and wastes.

This review reports some of the most recent published data on extractive methods to obtain polyphenol-rich extracts from *Olea europaea* L. and *Punica granatum* L., biological properties and applications, chemical modifications, and stabilization of pure molecules and extracts.

Due to the wide number of biological activities, potential applications range across many sectors, from food to cosmetics and pharmaceuticals. However, some papers do not report the chemical composition of the tested extracts, and there are still few *in vivo* experiments demonstrating the biological activity of pure extracts and/or phenols. In addition, there is generally a lack of life cycle assessment (LCA) related to the extraction and valorization process, which is essential to evaluating the real economic and environmental impact of recovering and reusing these plant materials.

Therefore, the concrete use of these wastes depends on various factors, including investment in research and development of new technologies, industry regulations, and the availability of funding for innovative initiatives. In addition, addressing challenges such as the scalability of optimized processes will be critical to making them competitive in the marketplace.

AUTHOR INFORMATION

Corresponding Author

Roberta Bernini − *Department of Agriculture and Forest Sciences (DAFNE), University of Tuscia, 01100 Viterbo, Italy*; orcid.org/0000-0002-2548-3876; Email: roberta.bernini@unitus.it

Authors

Margherita Campo − *Department of Statistics, Informatics, Applications "G. Parenti" (DiSIA), PHYTOLAB Laboratory, University of Florence, 50019 Sesto Fiorentino, Florence, Italy*

Chiara Cassiani − *Department of Statistics, Informatics, Applications "G. Parenti" (DiSIA), PHYTOLAB Laboratory, University of Florence, 50019 Sesto Fiorentino, Florence, Italy*

Andrea Fochetti − *Department of Agriculture and Forest Sciences (DAFNE), University of Tuscia, 01100 Viterbo, Italy*

Francesca Ieri − *Institute of Bioscience and BioResources (IBBR), National Research Council of Italy (CNR), 50019 Sesto Fiorentino, Florence, Italy*

Andrea Lombardi − *Department of Agriculture and Forest Sciences (DAFNE), University of Tuscia, 01100 Viterbo, Italy*

Silvia Urciuoli − *Department of Statistics, Informatics, Applications "G. Parenti" (DiSIA), PHYTOLAB Laboratory, University of Florence, 50019 Sesto Fiorentino, Florence, Italy*

Pamela Vignolini − *Department of Statistics, Informatics, Applications "G. Parenti" (DiSIA), PHYTOLAB Laboratory, University of Florence, 50019 Sesto Fiorentino, Florence, Italy*

Noemi Villanova − *Department of Agriculture and Forest Sciences (DAFNE), University of Tuscia, 01100 Viterbo, Italy*

Chiara Vita − *QuMAP - PIN, University Center "Città di Prato" Educational and Scientific Services for the University of Florence, 59100 Prato, Italy*

Complete contact information is available at:

https://doi.org/10.1021/acs.jafc.4c00945
J. Agric. Food Chem. 2024, 72, 12871−12895

https://pubs.acs.org/10.1021/acs.jafc.4c00945

Author Contributions

Roberta Bernini: conceptualization, literature collection, writing, revision and editing manuscript. Margherita Campo, Andrea Fochetti, Andrea Lombardi, Silvia Urciuoli, Pamela Vignolini, Noemi Villanova: literatura collection, writing and revision manuscript. Chiara Cassiani, Francesca Ieri: literature collection, writing manuscript; Chiara Vita: literature collection. All authors have read and agreed to the submitted version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This article is intended as a tribute to Prof. Annalisa Romani, who passed away prematurely, and who devoted much of her research to the world of polyphenols. We acknowledge Lazio Region for the PSR 2014-2020 project entitiled "Valorizzazione della qualità e delle proprietà nutraceutico funzionali dell'olio extravergine d'oliva di Sonnino" (CUP F29H23000010009). The authors also thank Rome Technopole (Piano Nazionale di Ripresa e Risilienza, Missione 4, Investimento 1.5, Next Generation EU, Code project: ECS00000024) for a research contract; the University of Tuscia (Viterbo, Italy) and Bioricerche Srl (Castell'Azzara, Grosseto, Italy) for a PhD grant (DM 1061/2021, PON Ricerca e Innovazione 2014-2020); the University of Florence for a research contract (DM 1062/2021, PON Ricerca e Innovazione 2014-2020).

■ ABBREVIATIONS

AGS, gastric adenocarcinoma cell line; BME-UV1, bovine mammary epithelial cell line; CAGR, compound annual growth rate; COX2, cyclooxygenase; CNS, central nervous system; CRP, C-reactive protein; DMSO, dimethyl sulfoxide; EVOO, extra virgin olive oil; GSH-Px, glutathione peroxidase; HHDP, hexahydroxydiphenoyl)-gallagyl-hexoside; HPLC, high pressure liquid chromatography; HTyr, hydroxytyrosol; HUVEC, human umbilical vein endothelial cells; HVED, high voltage electrical discharge; IBD, inflammatory bowel disease; IL, interleukin; iNOS, inducible nitric oxide synthase; LCA, life cycle assessment; LPS, lipopolysaccharide; MAE, microwave-assisted extraction; MF, microfiltration; NADES, natural deep eutectic solvents; NF, nanofiltration; NF-kB, nuclear factor kappa light chain enhancer of activated B cells; NO, nitric oxide; Ole, oleuropein; OLE-NLC, olive leaves extract in lipid nanovectors; OMWW, olive mill wastewater; PVA, poly(vinyl alcohol); PBMCs, peripheral blood mononuclear cells; PEF, pulsed electric fields; PFE, pressurized fluids extraction; PGE2, prostaglandin E2; PUAE, pulsed ultrasound-assisted extraction; ROS, reactive oxygen species; SEM, scanning electron microscope; SFE, supercritical fluids extraction; TNF, tumor necrosis factor; TREM2, triggering receptor expressed on myeloid cells 2; Tyr, tyrosol; UF, ultrafiltration; UAE, ultrasound-assisted extraction

■ REFERENCES

(1) Quideau, S.; Deffieux, D.; Douat-Casassus, C.; Pouysegu, L. Plant polyphenols: chemical properties, biological activities, and synthesis. *Angew. Chem.* **2011**, *50* (3), 586−621.

(2) Anastas, P. T.; Warner, J. C. *Green Chemistry: Theory and Practice*; Oxford University Press: New York, 1998.

(3) Stahel, W. R. The circular economy. *Nature.* **2016**, *531*, 435−438.

(4) Ncube, A.; Mtetwa, S.; Bukhari, M.; Fiorentino, G.; Passaro, R. Circular economy and green chemistry: the need for radical innovative approaches in the design for new products. *Energies.* **2023**, *16*, 1752.

(5) Ghanbari, R.; Anwar, F.; Alkharfy, K. M.; Gilani, A. H.; Saari, N. Valuable nutrients and functional bioactives in different parts of olive (*Olea europaea* L.). A review. *Int. J. Mol. Sci.* **2012**, *13*, 3291.

(6) Servili, M.; Esposto, S.; Fabiani, R.; Urbani, S.; Taticchi, A.; Mariucci, F.; Selvaggini, R.; Montedoro, G. F. Phenolic compounds in olive oil: Antioxidant, health, and organoleptic activities according to their chemical structure. *Inflammopharmacology.* **2009**, *17*, 76−84.

(7) Romani, A.; Ieri, F.; Urciuoli, S.; Noce, A.; Marrone, G.; Nediani, C.; Bernini, R. Health effects of phenolic compounds found in extra-virgin olive oil, by-products, and leaf of *Olea europaea* L. *Nutrients.* **2019**, *11*, 1776.

(8) EFSA panel on dietetic products, nutrition and allergies (NDA). *EFSA J.*, **2011**, *9*, 2033. www.efsa.europa.eu/efsajournal.

(9) Blekas, G.; Vassilakis, C.; Harizanis, C.; Tsimidou, M.; Boskou, D. G. Biophenols in table olives. *J. Agric. Food Chem.* **2002**, *50*, 3688−3692.

(10) Fernandez-Bolanos, G. J.; Lopez, O.; Fernandez-Bolanos, J.; Rodriguez-Gutierrez, G. Hydroxytyrosol and derivatives: isolation, synthesis, and biological properties. *Curr. Org. Chem.* **2008**, *12* (6), 442−463.

(11) Obied, H. K.; Allen, M. S.; Bedgood, D. R., Jr; Prenzler, P. D.; Robards, K. Investigation of Australian olive mill waste for recovery of biophenols. *J. Agric. Food Chem.* **2005**, *53*, 9911.

(12) Visioli, F.; Galli, C. Olive oil phenols and their potential effects on human health. *J. Agric. Food Chem.* **1998**, *46*, 4292−4296.

(13) Bernini, R.; Fabrizi, G.; Pouysegu, L.; Deffieux, D.; Quideau, S. Synthesis of biologically active catecholic compounds via ortho-selective oxygenation of phenolic compounds using hypervalent iodine(V) reagents. *Curr. Org. Synth.* **2012**, *9* (5), 650−669.

(14) Hohmann, C. D.; Cramer, H.; Michalsen, A.; Kessler, C.; Steckhan, N.; Choi, K.; Dobos, G. Effects of high phenolic olive oil on cardiovascular risk factors: A systematic review and meta-analysis. *Phytomedicine.* **2015**, *22*, 631−640.

(15) Tejada, S.; Pinya, S.; Bibiloni, M. D. M.; Tur, J. A.; Pons, A.; Sureda, A. Cardioprotective effects of the polyphenol hydroxytyrosol from olive oil. *Curr. Drug Targets.* **2017**, *18*, 1477−1486.

(16) Bender, C.; Strassmann, S.; Golz, C. Oral bioavailability and metabolism of hydroxytyrosol from food supplements. *Nutrients.* **2023**, *15* (2), 325.

(17) Kuwajima, H.; Uemura, T.; Takaishi, K.; Inoue, K.; Inouyet, H. A secoiridoid glucoside from Olea europaea. *Phytochemistry.* **1988**, *27*, 1757.

(18) Tan, H. W.; Tuck, K. L.; Stupans, I.; Hayball, P. J. Simultaneous determination of oleuropein and hydroxytyrosol in rat plasma using liquid chromatography with fluorescence detection. *J.Chromat. B* **2003**, *785*, 187−191.

(19) Gambacorta, A.; Tofani, D.; Bernini, R.; Migliorini, A. High yielding preparation of a stable precursor of hydroxytyrosol by total synthesis and from the natural glucoside oleuropein. *J. Agric. Food Chem.* **2007**, *55*, 3386−3391.

(20) Castejon, M. L.; Rosillo, M. A.; Montoya, T.; Gonzalez-Benjumea, A.; Fernandez-Bolanos, J. G.; Alarcon-de-la-Lastra, C. Oleuropein down-regulated IL-1_-induced inflammation and oxidative stress in human synovial fibroblast cell line SW982. *Food. Funct.* **2017**, *8*, 1890−1898.

(21) Ahamad, J.; Toufeeq, I.; Khan, M. A.; Ameen, M. S. M.; Anwer, E. T.; Uthirapathy, S.; Mir, S. R.; Ahmad, J. Oleuropein: A natural antioxidant molecule in the treatment of metabolic syndrome. *Phytother. Res.* **2019**, *33*, 3112−3128.

(22) Imran, M.; Nadeem, M.; Gilani, S. A.; Khan, S.; Sajid, M. W.; Amir, R. M. Antitumor perspectives of oleuropeinand its metabolite hydroxytyrosol: Recent updates. *J. Food Sci.* **2018**, *83*, 1781−1791.

(23) Zhang, W.; Liu, X.; Li, Q. Protective effects of oleuropein against cerebral ischemia/reperfusion by inhibiting neuronal apoptosis. *Med. Sci. Monit.* **2018**, *24*, 6587−6598.
(24) Bulotta, S.; Celano, M.; Lepore, S. M.; Montalcini, T.; Pujia, A.; Russo, D. Beneficial effects of the olive oil phenlic components oleuropein and hydroxytyrosol: Focus on protection against cardiovascular and metabolic diseases. *J. Tsansl. Med.* **2014**, *12*, 219.
(25) Zheng, S.; Huang, K.; Tong, T. Efficacy and mechanisms of oleuropein in mitigating diabetes and diabetes complications. *J. Agric. Food Chem.* **2021**, *69*, 6145−6155.
(26) Romani, A.; Campo, M.; Urciuoli, S.; Marrone, G.; Noce, A.; Bernini, R. An industrial and sustainable platform for the production of bioactive micronized powders and extracts enriched in polyphenols from *Olea europaea* L. and *Vitis vinifera* L. wastes. *Front. Nutr.* **2020**, *7*, 120.
(27) Cho, W. Y.; Kim, D. H.; Lee, H. J.; Yeon, S. J.; Lee, C. H. Evaluation of effect of extraction solvent on selected properties of olive leaf extract. *J. Food Quality.* **2020**, *2020*, 3013649.
(28) Sengling Cebin Coppa, C. F.; Rosim, R. E.; Fernandes de Oliveira, C. A.; da Costa Rodrigues, C. E.; Bernado Gonçalves, C. Extraction of oleuropein from olive leaves using a hydroalcoholic solvent. *Braz. J. Food Technol.* **2017**, *20*, e2016169.
(29) Liu, Y.; Friesen, J. B.; McAlpine, J. B.; Lankin, D. C.; Chen, S. N.; Pauli, G. F. Natural deep eutectic solvents: properties, applications, and perspectives. *J. Nat. Prod.* **2018**, *81*, 679−690.
(30) Carmona, I.; Aguirre, I.; Griffith, D. M.; García-Borrego, A. Towards a circular economy in virgin olive oil production: valorization of the olive mill waste (OMW) "alpeorujo" through polyphenol recovery with natural deep eutectic solvents (NADESs) and vermicomposting. *Science of The Total Environment.* **2023**, *872*, 162198.
(31) Yateem, H.; Afaneh, I. A.; Al-Rimawi, F. Optimum conditions for oleuropein extraction from olive leaves. *Int. J. Appl. Sci. Technol.* **2014**, *4*, 153−157.
(32) Wu, Z.-Q.; Yue, G.-Z.; Zhu, Q.-P.; Jiang, Y.-J.; Tang, K.-Y.; Chen, H.-P.; Yang, Z.-S.; Huang, Q.-M. Purification, dynamic changes and antioxidant activities of oleuropein in olive (Olea europaea l.) leaves. *Journal of Food Biochemistry.* **2015**, *39*, 566−574.
(33) Lucarini, M.; Durazzo, A.; Bernini, R.; Campo, M.; Vita, C.; Souto, E. B.; Lombardi-Boccia, G.; Ramadan, M. F.; Santini, A.; Romani, A. Fruit wastes as a valuable source of value-added compounds: A collaborative perspective. *Molecules.* **2021**, *26* (21), 6338.
(34) Belghith, Y.; Kallel, I.; Rosa, M.; Stathopoulos, P.; Skaltsounis, L. A.; Allouche, N.; Chemat, F.; Tomao, V. Intensification of biophenols extraction yield from olive pomace using innovative green technologies. *Biomolecules.* **2023**, *13*, 65.
(35) da Rosa, G. S.; Vanga, S.; Gariepy, Y.; Raghavan, V. Comparison of microwave, ultrasonic and conventional techniques for extraction of bioactive compounds from olive leaves (*Olea europaea L.*). *Innovative Food Science & Emerging Technologies.* **2019**, *58*, 102234.
(36) Caballero, A. S.; Romero-García, J. M.; Castro, E.; Cardona, C. A. Supercritical fluid extraction for enhancing polyphenolic compounds production from olive waste extracts. *J. Chem. Technol. Biotechnol.* **2020**, *95*, 356−362.
(37) Xynos, N.; Papaefstathiou, G.; Psychis, M.; Argyropoulou, A.; Aligiannis, N.; Skaltsounis, L. Development of a green extraction procedure with super subcritical fluids to procedure extracts enriched in oleuropein from olive leaves. *Journal of Supercritical Fluids.* **2012**, *67*, 89−93.
(38) Khemakhem, I.; Gargouri, D.; Dhouib, A.; Ayadi, A.; Bouaziz, M. Oleuropein rich extract from olive leaves by combining microfiltration, ultrafiltration and nanofiltration. *Separation and Purification Technology.* **2017**, *172*, 310−317.
(39) Romani, A.; Pinelli, P.; Ieri, F.; Bernini, R. Sustainability, innovation and green chemistry in the production and valorization of phenolic extracts from *Olea europaea* L. *Sustainability.* **2016**, *8*, 1002.
(40) Bernini, R.; Carastro, I.; Palmini, G.; Tanini, A.; Zonefrati, R.; Pinelli, P.; Brandi, M. L.; Romani, A. Lipophilization of hydroxytyrosol-enriched fractions from Olea europaea L. by-products and evaluation of the in vitro effects on a model of colorectal cancer cells. *J. Agric. Food Chem.* **2017**, *65*, 6506−6512.
(41) Fernandez Bolanos, J.; Rodriguez, J.; Rodriguez, R.; Heredia, A.; Guillen, R.; Jiménez, A. Production in large quantities of highly purified hydroxytyrosol from liquid-solid waste of two-phase olive oil processing or "alperujo. *J. Agric. Food Chem.* **2002**, *50*, 6804−6811.
(42) Fava, G.; Di Mauro, M. D.; Spampinato, M.; Biondi, D.; Gambera, G.; Centonze, G.; Maggiore, R.; D'Antona, N. Hydroxytyrosol recovery from olive mill wastewater: process optimization and development of a pilot plant. *CLEAN-soil Air Water* **2017**, *45*, 1600042.
(43) Rodriguez, G.; Rodriguez, R.; Fernandez-Bolanos, J.; Guillen, R.; Jimenez, A. Antioxidant activity of effluents during the purification of hydroxytyrosol and 3,4-dihydroxyphenyl glycol from olive oil waste. *Eur. Food Res. Technol.* **2007**, *224*, 733−741.
(44) Hamden, K.; Allouche, N.; Damak, M.; Elfeki, A. Hypoglycemic and antioxidant effects of phenolic extracts and purified hydroxytyrosol from olive mill waste in vitro and in rats. *Chem. Bio. Interact.* **2009**, *180*, 421−432.
(45) Fiorito, S.; Collevecchio, C.; Spogli, R.; Epifano, F.; Genovese, S. Novel procedures for olive leaves extracts processing: selective isolation of oleuropein and elenolic acid. *Food Chem.* **2024**, *447*, 139038.
(46) Peyrol, J.; Riva, C.; Amiot, M. J. Hydroxytyrosol in the prevention of the metabolic syndrome and related disorders. *Nutrients.* **2017**, *9*, 306.
(47) Arangia, A.; Marino, Y.; Impellizzeri, D.; D'Amico, R.; Cuzzocrea, S.; Di Paola, R. Hydroxytyrosol and its potential uses on intestinal and gastrointestinal disease. *Int. J. Mol. Sci.* **2023**, *24*, 3111.
(48) Foti, P.; Occhipinti, P. S.; Romeo, F. V.; Timpanaro, N.; Musumeci, T.; Randazzo, C. L.; Caggia, C. Phenols recovered from olive mill wastewater as natural booster to fortify blood orange juice. *Food Chem.* **2022**, *393*, 133428.
(49) Fleming, H. P.; Walter, W. M.; Etchells, J. L. Antimicrobial properties of oleuropein and products of its hydrolysis from green olives. *Appl. Microbiol.* **1973**, *26*, 777−782.
(50) Liu, Y.; McKeever, L. C.; Malik, N. S. A. Assessment of the antimicrobial activity of olive leaf extract against foodborne bacterial pathogens. *Front. Microbiol.* **2017**, *8*, 113.
(51) Silvan, J. M.; Guerrero-Hurtado, E.; Gutierrez-Docio, A.; Prodanov, M.; Martinez-Rodriguez, A. J. Olive leaf as a source of antibacterial compounds active against antibiotic-resistant strains of *Campylobacter jejuni* and *Campylobacter coli*. *Antibiotics.* **2023**, *12*, 26.
(52) Testa, B.; Lombardi, S. J.; Macciola, E.; Succi, M.; Tremonte, P.; Iorizzo, M. Efficacy of olive leaf extract (*Olea europaea* L. cv Gentile Di Larino) in marinated anchovies (*Engraulis encrasicolus*, L.) process. *Heliyon* **2019**, *5* (5), No. e01727.
(53) Difonzo, G.; Totaro, M. P.; Caponio, F.; Pasqualone, A.; Summo, C. Olive leaf extract (OLE) addition as tool to reduce nitrate and nitrite in ripened sausages. *Foods.* **2022**, *11*, 451.
(54) Lombardi, A.; Fochetti, A.; Vignolini, P.; Campo, M.; Durazzo, A.; Lucarini, M.; Puglia, D.; Luzi, F.; Papalini, M.; Renzi, M.; Cavallo, A.; Bernini, R. Natural active ingredients for poly (lactic acid)-based materials: state of the art and perspectives. *Antioxidants.* **2022**, *11*, 2074.
(55) Luzi, F.; Pannucci, E.; Clemente, M.; Grande, E.; Urciuoli, S.; Romani, A.; Torre, L.; Puglia, D.; Bernini, R.; Santi, L. Hydroxytyrosol and oleuropein-enriched extracts obtained from olive oil wastes and by-products as active antioxidant ingredients for poly (vinyl alcohol)-based films. *Molecules.* **2021**, *26*, 2104.
(56) Carrapiso, A. I.; Pimienta, M.; Martín, L.; Cardenia, V.; Andrés, A. I. Effect of a chitosan coating enriched with an olive leaf extract on the characteristics of pork burgers. *Foods.* **2023**, *12*, 3757.
(57) Medfai, W.; Oueslati, I.; Dumas, E.; Harzalli, Z.; Viton, C.; Mhamdi, R.; Gharsallaoui, A. Physicochemical and biological

characterization of encapsulated olive leaf extracts for food preservation. *Antibiotics.* **2023**, *12*, 987.
(58) Grzelczyk, J.; Oracz, J.; Gałązka-Czarnecka, I. Quality assessment of waste from olive oil production and design of biodegradable packaging. *Foods.* **2022**, *11*, 3776.
(59) Sar, T.; Akbas, M. Y. Antimicrobial activities of olive oil mill wastewater extracts against selected microorganisms. *Sustainability.* **2023**, *15*, 8179.
(60) Zorić, N.; Kopjar, N.; Kraljić, K.; Oršolić, N.; Tomić, S.; Kosalec, I. Olive leaf extract activity against *Candida albicans* and *C. dubliniensis* - the in vitro viability study. *Acta Pharm.* **2016**, *66* (3), 411−421.
(61) Zorić, N.; Kopjar, N.; Bobnjarić, I.; Horvat, I.; Tomić, S.; Kosalec, I. Antifungal activity of oleuropein against *Candida albicans*. The in vitro study. *Molecules.* **2016**, *21*, 1631.
(62) Medina-Martinez, M. S.; Truchado, P.; Castro-Ibanez, I.; Allende, A. Antimicrobial activity of hydroxytyrosol: a current controversy. *Biosci. Biotechnol. Biochem.* **2016**, *80*, 801−810.
(63) Di Pietro, M.; Filardo, S.; Mattioli, R.; Bozzuto, G.; Molinari, A.; Mosca, L.; Sessa, R. Extra virgin olive oil-based formulations: a "green" strategy against *Chlamydia trachomatis*. *Int. J. Mol. Sci.* **2023**, *24*, 12701.
(64) Lombardi, A.; Campo, M.; Vignolini, P.; Papalini, M.; Pizzetti, M.; Bernini, R. Phenolic-rich extracts from circular economy: chemical profile and activity against filamentous fungi and dermatophytes. *Molecules.* **2023**, *28*, 4374.
(65) Dauber, C.; Parente, E.; Zucca, M. P.; Gámbaro, A.; Vieitez, I. *Olea europea* and by-products: extraction methods and cosmetic applications. *Cosmetics.* **2023**, *10*, 112.
(66) Menicacci, B.; Cipriani, C.; Margheri, F.; Mocali, A.; Giovannelli, L. Modulation of the senescence-associated inflammatory phenotype in human fibroblasts by olive phenols. *Int. J. Mol. Sci.* **2017**, *18*, 2275.
(67) De Pablos, R. M.; Espinosa-Oliva, A. M.; Hornedo-Ortega, R.; Cano, M.; Arguelles, S. Hydroxytyrosol protects from aging process via AMPK and autophagy; a review of its effects on cancer, metabolic syndrome, osteoporosis, immune-mediated and neurodegenerative diseases. *Pharmacol. Res.* **2019**, *143*, 58−72.
(68) Leenen, R.; Roodenburg, A. J. C.; Vissers, M. N.; Schuurbiers, J. A. E.; Van Putte, K. P. A. M.; Wiseman, S. A.; van de Put, F. H. M. M. Supplementation of plasma with olive oil phenols and extracts: influence on LDL oxidation. *J. Agric. Food Chem.* **2002**, *50*, 1290−1297.
(69) Lombardo, L.; Grasso, F.; Lanciano, F.; Loria, S.; Monetti, E. Broad-Spectrum health protection of extra virgin olive oil compounds. *Stud. Nat. Prod. Chem.* **2018**, *57*, 41−77.
(70) Masella, R.; Varì, R.; D'Archivio, M.; Di Benedetto, R.; Matarrese, P.; Malorni, W.; Scazzocchio, B.; Giovannini, C. Extra virgin olive oil biophenols inhibit cell-mediated oxidation of LDL by increasing the mRNA transcription of glutathione-related enzymes. *J. Nutr.* **2004**, *134* (4), 785−791.
(71) D'Amore, S.; Vacca, M.; Cariello, M.; Graziano, G.; D'Orazio, A.; Salvia, R.; Sasso, R. C.; Sabbà, C.; Palasciano, G.; Moschetta, A. Genes and miRNA expression signatures in peripheral blood mononuclear cells in healthy subjects and patients with metabolic syndrome after acute intake of extra virgin olive oil. *Biochim. Biophys. Acta* **2016**, *1861* (11), 1671−1680.
(72) Sklenarova, R.; Allaw, M.; Perra, M.; Castangia, I.; Frankova, J.; Luis Pedraz, J.; Letizia Manca, M.; Manconi, M. Co-delivering of oleuropein and lentisk oil in phospholipid vesicles as an effective approach to modulate oxidative stress, cytokine secretion and promote skin regeneration. *Eur. J. Pharm. Biopharm.* **2023**, *185*, 126−136.
(73) Romero-Márquez, J. M.; Navarro-Hortal, M. D.; Forbes-Hernández, T. Y.; Varela-López, A.; Puentes, J. G.; Pino-García, R. D.; Sánchez-González, C.; Elio, I.; Battino, M.; García, R.; Sanchez, S.; Quiles, J. L. Exploring the antioxidant, neuroprotective, and anti-inflammatory potential of olive leaf extracts from Spain, Portugal, Greece, and Italy. *Antioxidants.* **2023**, *12*, 1538.
(74) Velotti, F.; Bernini, R. Hydroxytyrosol interference with inflammaging via modulation of inflammation and autophagy. *Nutrients.* **2023**, *15*, 1774.
(75) Serreli, G.; Deiana, M. Extra virgin olive oil polyphenols: Modulation of cellular pathways related to oxidant species and inflammation in aging. *Cells.* **2020**, *9*, 478.
(76) Rosillo, M. A.; Alarcon-de-la-Lastra, C.; Castejon, M. L.; Montoya, T.; Cejudo-Guillen, M.; Sanchez-Hidalgo, M. Polyphenolic extract from extra virgin olive oil inhibits the inflammatory response in IL-1-activated synovial fibroblasts. *Br. J. Nutr.* **2019**, *121*, 55−62.
(77) Pirozzi, C.; Lama, A.; Simeoli, R.; Paciello, O.; Pagano, T. B.; Mollica, M. P.; Di Guida, F.; Russo, R.; Magliocca, S.; Canani, R. B.; Raso, G. M.; Galignano, A.; Meli, R. Hydroxytyrosol prevents metabolic impairment reducing hepatic inflammation and restoring duodenal integrity in a rat model of NAFLD. *J. Nutr. Biochem.* **2016**, *30*, 108−115.
(78) Aparicio-Soto, M.; Sanchez-Hidalgo, M.; Cardeno, A.; Gonzalez-Benjumea, A.; Fernandez-Bolanos, J. G.; Alarcon-De-La-Lastra, C. Dietary hydroxytyrosol and hydroxytyrosyl acetate supplementation prevent pristane-induced systemic lupus erythematous in mice. *J. Funct. Foods.* **2017**, *29*, 84−92.
(79) Fuccelli, R.; Fabiani, R.; Rosignoli, P. Hydroxytyrosol exerts anti-inflammatory and antioxidant activities in a mouse model of systemic inflammation. *Molecules.* **2018**, *23*, 3212.
(80) Miao, F. Hydroxytyrosol alleviates dextran sodium sulfate-induced colitis by inhibiting NLRP3 inflammasome activation and modulating gut microbiota in vivo. *Nutrition.* **2022**, *97*, 111579.
(81) Micheli, L.; Bertini, L.; Bonato, A.; Villanova, N.; Caruso, C.; Caruso, M.; Bernini, R.; Tirone, F. Role of hydroxytyrosol and oleuropein in the prevention of aging and related disorders: focus on neurodegeneration, skeletal muscle dysfunction and gut microbiota. *Nutrients.* **2023**, *15*, 1767.
(82) Parkinson, L.; Keast, R. Oleocanthal, a phenolic derived from virgin olive oil: a review of the beneficial effects on inflammatory disease. *Int. J. Mol. Sci.* **2014**, *15* (7), 12323−12334.
(83) Pang, K. L.; Chin, K. Y. The biological activities of oleocanthal from a molecular perspective. *Nutrients.* **2018**, *10* (5), 570.
(84) Noce, A.; Marrone, G.; Urciuoli, S.; Di Daniele, F.; Di Lauro, M.; Pietroboni Zaitseva, A.; Di Daniele, N.; Romani, A. Usefulness of extra virgin olive oil minor polar compounds in the management of chronic kidney disease patients. *Nutrients* **2021**, *13*, 581.
(85) Marrone, G.; Urciuoli, S.; Di Lauro, M.; Ruzzolini, J.; Ieri, F.; Vignolini, P.; Di Daniele, F.; Guerriero, C.; Nediani, C.; Di Daniele, N.; Noce, A. Extra virgin olive oil and cardiovascular protection in chronic kidney disease. *Nutrients* **2022**, *14*, 4265.
(86) Hornedo-Ortega, R.; Cerezo, A. B.; de Pablos, R. M.; Krisa, S.; Richard, T.; Garcia-Parrilla, M. C.; Troncoso, A. M. Phenolic compounds characteristic of the Mediterranean diet in mitigating microglia-mediated neuroinflammation. *Front. Cell. Neurosci.* **2018**, *12*, 373.
(87) Leri, M.; Vasarri, M.; Carnemolla, F.; Oriente, F.; Cabaro, S.; Stio, M.; Degl'Innocenti, D.; Stefani, M.; Bucciantini, M. EVOO polyphenols exert anti-inflammatory effects on the microglia cell through TREM2 signaling pathway. *Pharmaceuticals.* **2023**, *16*, 933.
(88) Musolino, V.; Macrì, R.; Cardamone, A.; Serra, M.; Coppoletta, A. R.; Tucci, L.; Maiuolo, J.; Lupia, C.; Scarano, F.; Carresi, C.; et al. Nocellara Del Belice (*Olea europaea* L. Cultivar): leaf extract concentrated in phenolic compounds and its anti-inflammatory and radical scavenging activity. *Plants.* **2023**, *12*, 27.
(89) Fayez, N.; Khalil, W.; Abdel-Sattar, E.; Abdel-Fattah, A.-F. M. In vitro and in vivo assessment of the anti-inflammatory activity of olive leaf extract in rats. *Inflammopharmacol.* **2023**, *31*, 1529−1538.
(90) Romani, A.; Bernini, R.; Noce, A.; Urciuoli, S.; Di Lauro, M.; Pietroboni Zaitseva, A.; Marrone, G.; Di Daniele, N. Potential beneficial effects of extra virgin olive oils characterized by high content in minor polar compounds in nephropathic patients: a pilot study. *Molecules.* **2020**, *25*, 4757.
(91) Oliveras-Ferraros, C.; Fernández-Arroyo, S.; Vazquez-Martin, A.; Lozano-Sánchez, J.; Cufí, S.; Joven, J.; Micol, V.; Fernández-

Gutiérrez, A.; Segura-Carretero, A.; Menendez, J. A. Crude phenolic extracts from extra virgin olive oil circumvent de novo breast cancer resistance to HER1/ HER2-targeting drugs by inducing GADD45-sensed cellular stress, G2/M arrest and hyperacetylation of Histone H3. *Int. J. Oncol.* **2011**, *38*, 1533−1547.
(92) Coccia, A.; Bastianelli, D.; Mosca, L.; Monticolo, R.; Panuccio, I.; Carbone, A.; Calogero, A.; Lendaro, E. Extra virgin olive oil phenols suppress migration and invasion of T24 human bladder cancer cells through modulation of matrix Metalloproteinase-2. *Nutr. Cancer.* **2014**, *66* (6), 946−954.
(93) Polini, B.; Digiacomo, M.; Carpi, S.; Bertini, S.; Gado, F.; Saccomanni, G.; Macchia, M.; Nieri, P.; Manera, C.; Fogli, S. Oleocanthal and oleacein contribute to the in vitro therapeutic potential of extra virgin oil-derived extracts in non-melanoma skin cancer. *Toxicol. In Vitro.* **2018**, *52*, 243−250.
(94) De Stefanis, D.; Scimè, S.; Accomazzo, S.; Catti, A.; Occhipinti, A.; Bertea, C. M.; Costelli, P. Anti-proliferative effects of an extra-virgin olive oil extract enriched in ligstroside aglycone and oleocanthal on human liver cancer cell lines. *Cancers.* **2019**, *11*, 1640.
(95) D'Archivio, M.; Santangelo, C.; Scazzocchio, B.; Varì, R.; Filesi, C.; Masella, R.; Giovannini, C. Modulatory effects of polyphenols on apoptosis induction: relevance for cancer prevention. *Int. J. Mol. Sci.* **2008**, *9*, 213−228.
(96) Sun, L.; Luo, C.; Liu, J. Hydroxytyrosol induces apoptosis in human colon cancer cells through ROS generation. *Food Funct.* **2014**, *5*, 1909−1914.
(97) Laghezza Masci, V.; Bernini, R.; Villanova, N.; Clemente, M.; Cicaloni, V.; Tinti, L.; Salvini, L.; Taddei, A. R.; Tiezzi, A.; Ovidi, E. In vitro anti-proliferative and apoptotic effects of hydroxytyrosyl oleate on SH-SY5Y human neuroblastoma cells. *Int. J. Mol. Sci.* **2022**, *23*, 12348.
(98) Ruzzolini, J.; Peppicelli, S.; Bianchini, F.; Andreucci, E.; Urciuoli, S.; Romani, A.; Tortora, K.; Caderni, G.; Nediani, C.; Calorini, L. Cancer glycolytic dependence as a new target of olive leaf extract. *Cancers* **2020**, *12*, 317.
(99) Rosignoli, P.; Fuccelli, R.; Fabiani, R.; Servili, M.; Morozzi, G. Effect of olive oil phenols on the production of inflammatory mediators in freshly isolated human monocytes. *J. Nutr Biochem.* **2013**, *24*, 1513−1519.
(100) Abuznait, A. H.; Qosa, H.; Busnena, B. A.; El Sayed, K. A.; Kaddoumi, A. Olive oil-derived oleocanthal enhances b-amyloid clearance as a potential neuroprotective mechanism against Alzheimer's disease: In vitro and in vivo studies. *ACS Chem. Neurosci.* **2013**, *4*, 973−982.
(101) Akl, M. R.; Ayoub, N. M.; Mohyeldin, M. M.; Busnena, B. A.; Foudah, A. I.; Liu, Y.-Y.; El Sayed, K. A. Olive phenolics as c-Met inhibitors: (−)-oleocanthal attenuates cell proliferation, invasiveness, and tumor growth in breast cancer models. *PLoS One* **2014**, *9*, No. e97622.
(102) Peri, S.; Ruzzolini, J.; Urciuoli, S.; Versienti, G.; Biagioni, A.; Andreucci, E.; Peppicelli, S.; Bianchini, F.; Bottari, A.; Calorini, L.; et al. An oleocanthal-enriched EVO oil extract induces the ROS oroduction in gastric cancer cells and potentiates the effect of chemotherapy. *Antioxidants* **2022**, *11*, 1762.
(103) Bagli, E.; Stefaniotou, M.; Morbidelli, L.; Ziche, M.; Psillas, K.; Murphy, C.; Fotsis, T. Luteolin inhibits vascular endothelial growth factor-induced angiogenesis; inhibition of endothelial cell survival and proliferation by targeting phosphatidylinositol 3′-kinase activity. *Cancer Res.* **2004**, *64*, 7936−7946.
(104) Liman, R.; Çoban, F.; Ciğerci, I.; Bulduk, I.; Bozkurt, S. Antiangiogenic and apoptotic effects of oleuropein on breast cancer cells. *Br J. Pharm. Res.* **2017**, *16*, 1−10.
(105) Scoditti, E.; Calabriso, N.; Massaro, M.; Pellegrino, M.; Storelli, C.; Martines, G.; De Caterina, R.; Carluccio, M. A. Mediterranean diet polyphenols reduce inflammatory angiogenesis through MMP-9 and COX-2 inhibition in human vascular endothelial cells: A potentially protective mechanism in atherosclerotic vascular disease and cancer. *Arch. Biochem. Biophys.* **2012**, *527* (2), 81−89.
(106) Lamy, S.; Akla, N.; Ouanouki, A.; Lord-Dufour, S.; Beliveau, R. Diet-derived polyphenols inhibit angiogenesis by modulating the interleukin-6/STAT3 pathway. *Exp Cell. Res.* **2012**, *318*, 1586−1596.
(107) Gordon, M. H.; Paiva-Martins; Almeida, M. Antioxidant activity of hydroxytyrosol acetate compared with that of other olive oil polyphenols. *J. Agric. Food Chem.* **2001**, *49*, 2480.
(108) Chakraborti, A. K.; Gulhane, R. Perchloric acid adsorbed on silica gel as a new, highly efficient, and versatile catalyst for acetylation of phenols, thiols, alcohols, and amines. *Chem. Commun.* **2003**, *2003*, 1896−1897.
(109) Capasso, R.; Sannino, F.; De Martino, A.; Manna, C. Production of triacetylhydroxytyrosol from olive mill waste waters for use as stabilized bioantioxidant. *J. Agric. Food Chem.* **2006**, *54*, 9063.
(110) Bernini, R.; Mincione, E.; Barontini, M.; Crisante, F. Convenient synthesis of hydroxytyrosol and its lipophilic derivatives from tyrosol or homovanillyl alcohol. *J. Agric. Food Chem.* **2008**, *56*, 8897−8904.
(111) Bernini, R.; Carastro, I.; Santoni, F.; Clemente, M. Synthesis of lipophilic esters of tyrosol, homovanillyl alcohol and hydroxytyrosol. *Antioxidants.* **2019**, *8* (6), 174.
(112) Fernandez-Pastor, I.; Martínez-García, M.; Medina-O'Donnell, M.; Rivas, F.; Martinez, A.; Pérez-Victoria, J. M.; Parra, A. Semisynthesis of ω-hydroxyalkylcarbonate derivatives of hydroxytyrosol as antitrypanosome agents. *J. Nat. Prod.* **2018**, *81* (9), 2075−2082.
(113) Appendino, G.; Minassi, A.; Daddario, N.; Bianchi, F.; Tron, G. C. Chemoselective esterification of phenolic acids and alcohols. *Org. Lett.* **2002**, *4*, 3839.
(114) Torres de Pinedo, A.; Peñalver, P.; Rondón, D.; Morales, J. C. Efficient lipase-catalyzed synthesis of new lipid antioxidants based on a catechol structure. *Tetrahedron.* **2005**, *61*, 7654.
(115) Takao, K.; Toda, K.; Saito, T.; Sugita, Y. Synthesis of amide and ester derivatives of cinnamic acid and its analogs: evaluation of their free radical scavenging and monoamine oxidase and cholinesterase inhibitory activities. *Chem. Pharm. Bull.* **2017**, *65* (11), 1020−1027.
(116) Mohyeldin, M. M.; Busnena, B. A.; Akl, M. R.; Dragoi, A. M.; Cardelli, J. A.; El Sayed, K. A. Novel c-Met inhibitory olive secoiridoid semisynthetic analogs for the control of invasive breast cancer. *Eur. J. Med. Chem.* **2016**, *118*, 299−315.
(117) Trujillo, M.; Gallardo, E.; Madrona, A.; Bravo, L.; Sarriá, B.; González-Correa, J. A.; Mateos, R.; Espartero, J. L. Synthesis and antioxidant activity of nitrohydroxytyrosol and its acyl derivatives. *J. Agric. Food Chem.* **2014**, *62*, 10297−10303.
(118) Romanucci, V.; Giordano, M.; De Tommaso, G.; Iuliano, M.; Bernini, R.; Clemente, M.; Garcia-Viñuales, S.; Milardi, D.; Zarrelli, A.; Di Fabio, G. Synthesis of new tyrosol-based phosphodiester derivatives: effect on amyloid β aggregation and metal chelation ability. *Chem. Med. Chem.* **2021**, *16*, 1172−1183.
(119) Bernini, R.; Cacchi, S.; Fabrizi, G.; Filisti, E. 2-Arylhydroxytyrosol derivatives via Suzuki-Miyaura cross-coupling. *Org. Lett.* **2008**, *10*, 3457−3460.
(120) Pereira-Caro, G.; Mateos, R.; Traka, M. H.; Bacon, J. R.; Bongaerts, R.; Sarriá, B.; Bravo, L.; Kroon, P. A. Hydroxytyrosyl ethyl ether exhibits stronger intestinal anticarcinogenic potency and effects on transcript profiles compared to hydroxytyrosol. *Food Chem.* **2013**, *138*, 1172−1182.
(121) Marrero, A. D.; Castilla, L.; Espartero, J. L.; Madrona, A.; Quesada, A. R.; Medina, M. A.; Martínez-Poveda, B. A comparative study of the antiangiogenic activity of hydroxytyrosyl alkyl ethers. *Food Chem.* **2020**, *333*, 127476.
(122) Muñoz-Marín, J.; De La Cruz, J. P.; Reyes, J. J.; López-Villodres, J. A.; Guerrero, A.; López-Leiva, I.; Espartero, J. L.; Labajos, M. T.; González-Correa, J. A. Hydroxytyrosyl alkyl ether derivatives inhibit platelet activation after oral administration to rats. *Food Chem. Toxicol.* **2013**, *58*, 295−300.
(123) Larghi, E. L.; Kaufman, T. S. The Oxa-Pictet-Spengler cyclization: synthesis of isochromans and related pyran-type heterocycles. *Synthesis.* **2006**, *2006*, 187−220.

(124) Guiso, M.; Bianco, A.; Marra, C.; Cavarischia, C. One-Pot synthesis of 6-hydroxyisochromans: the example of demethyl-oxacoclaurine. *Eur. J. Org. Chem.* **2003**, *2003*, 3407.
(125) Bernini, R.; Crisante, F.; Fabrizi, G.; Gentili, P. Convenient synthesis of 1-aryl-dihydroxyisochromans exhibiting antioxidant activity. *Curr. Org. Chem.* **2012**, *16*, 1051−1057.
(126) Begines, P.; Martos, S.; Lagunes, I.; Maya, I.; Padrón, J. M.; López, Ó.; Fernández-Bolaños, J. G. Chemoselective preparation of new families of phenolic-organoselenium hybrids—a biological assessment. *Molecules.* **2022**, *27*, 1315.
(127) Jerbi, A.; Mosset, P.; Grée, R.; Kammoun, M. Selective modification of oleuropein, a multifunctional bioactive natural product. *J. Saudi Chem. Soc.* **2019**, *23*, 1049−1059.
(128) Vougogiannopoulou, K.; Lemus, C.; Halabalaki, M.; Pergola, C.; Werz, O.; Smith, A. B.; Michel, S.; Skaltsounis, L.; Deguin, B. One-step semisynthesis of oleacein and the determination as a 5-lipoxygenase inhibitor. *J. Nat. Prod.* **2014**, *77*, 441−445.
(129) Castillo-Luna, A.; Criado-Navarro, I.; Ledesma-Escobar, C. A.; Lopez-Bascon, M. A.; Priego-Capote, F. The decrease in the health benefits of extra virgin olive oil during storage is conditioned by the initial phenolic profile. *Food Chem.* **2021**, *336*, 127730.
(130) Bernini, R.; Carastro, I.; Santoni, F.; Clemente, M. Synthesis of Lipophilic Esters of Tyrosol, Homovanillyl Alcohol and Hydroxytyrosol. *Antioxidants* **2019**, *8*, 174.
(131) Bonechi, C.; Donati, A.; Tamasi, G.; Pardini, A.; Rostom, H.; Leone, G.; Lamponi, S.; Consumi, M.; Magnani, A.; Rossi, C. Chemical characterization of liposomes containing nutraceutical compounds: Tyrosol, hydroxytyrosol and oleuropein. *Biophys. Chem.* **2019**, *246*, 25−34.
(132) Asghar, H. B.; Hassan, R. K. A.; Barakat, L. A. A.; Alharbi, A.; El Behery, M.; Elshaarawy, R. F. M.; Hassan, Y. A. Cross-linked quaternized chitosan nanoparticles for effective delivery and controllable release of O. europaea phenolic extract targeting cancer therapy. *J. Drug Delivery Sci. Technol.* **2023**, *83*, 104388.
(133) Chen, Y.; Xia, G.; Zhao, Z.; Xue, F.; Gu, Y.; Chen, C.; Zhang, Y. 7, 8-Dihydroxyflavone nano-liposomes decorated by crosslinked and glycosylated lactoferrin: Storage stability, antioxidant activity, in vitro release, gastrointestinal digestion and transport in Caco-2 cell monolayers. *J. Funct. Foods* **2020**, *65*, 103742.
(134) Liu, Y.; Liu, D.; Zhu, L.; Gan, Q.; Le, X. Temperature-dependent structure stability and in vitro release of chitosan-coated curcumin liposome. *Food Research International* **2015**, *74*, 97−105.
(135) Mirafzali, Z.; Thompson, C. S.; Tallua, K. Application of Liposomes in the Food Industry. In *Microencapsulation in the Food Industry*; Elsevier, 2014; pp 139−150.
(136) Burgalassi, S.; Zucchetti, E.; Birindelli, E.; Tampucci, S.; Chetoni, P.; Monti, D. Ocular Application of Oleuropein in Dry Eye Treatment: Formulation Studies and Biological Evaluation. *Pharmaceuticals.* **2021**, *14*, 1151.
(137) Paulo, F.; Santos, L. Inclusion of hydroxytyrosol in ethyl cellulose microparticles: in vitro release studies under digestion conditions. *Food Hydrocoll.* **2018**, *84*, 104−116.
(138) Paulo, F.; Santos, L. Microencapsulation of caffeic acid and its release using a w/o/w double emulsion method: assessment of formulation parameters. *Dry. Technol.* **2019**, *37*, 950−961.
(139) Paulo, F.; Santos, L. Encapsulation of the antioxidant tyrosol and characterization of loaded microparticles: an integrative approach on the study of the polymer carriers and loading contents. *Food Bioprocess Technol.* **2020**, *13*, 764−785.
(140) Muzzalupo, I.; Badolati, G.; Chiappetta, A.; Picci, N.; Muzzalupo, R. In vitro antifungal activity of olive (Olea europaea) leaf extracts loaded in chitosan nanoparticles. *Front. Bioeng. Biotechnol.* **2020**, *8*, 151.
(141) Paulo, F.; Tavares, L.; Santos, L. Olive Mill Pomace Extract Loaded Ethylcellulose Microparticles as a Delivery System to Improve Olive Oils Oxidative Stability. *Resources* **2023**, *12*, 6.
(142) Nobari Azar, F. A.; Pezeshki, A.; Ghanbarzadeh, B.; Hamishehkar, H.; Mohammadi, M.; Hamdipour, S.; Daliri, H. Pectin-sodium caseinat hydrogel containing olive leaf extract-nano lipid carrier: Preparation, characterization and rheological properties. *LWT.* **2021**, *148*, 111757.
(143) Alongi, M.; Lucci, P.; Clodoveo, M. L.; Schena, F. P.; Calligaris, S. Oleogelation of extra virgin olive oil by different oleogelators affects the physical properties and the stability of bioactive compounds. *Food Chem.* **2022**, *368*, 130779.
(144) Pintado, T.; Muñoz-González, I.; Salvador, M.; Ruiz-Capillas, C.; Herrero, A. M. Phenolic compounds in emulsion gel-based delivery systems applied as animal fat replacers in frankfurters: Physico-chemical, structural and microbiological approach. *Food Chem.* **2021**, *340*, 128095.
(145) Melgarejo, P.; Núñez-Gómez, D.; Legua, P.; Martínez-Nicolás, J. J.; Almansa, M. S. Pomegranate (*Punica granatum* L.) a dry pericarp fruit with fleshy seeds. *Trends Food Sci. Technol.* **2020**, *102*, 232−236.
(146) Hussein, L.; Gouda, M. E. L. Pomegranate: cultivation, pomological properties, processing, global market and health benefits. Pomegranate: cultivation, antioxidant and health benefits. *Food Science and Technology*; Nova Science: New York, 2018; pp 267−302.
(147) Moga, M. A.; Dimienescu, O. G.; Balan, A.; Dima, L.; Toma, S. I.; Bîgiu, N. F.; Blidaru, A. Pharmacological and therapeutic properties of punica granatum phytochemicals: possible roles in breast cancer. *Molecules.* **2021**, *26*, 1054.
(148) Puneeth, H. R.; Chandra, S. S. P. A Review on potential therapeutic properties of pomegranate (*Punica granatum* L.). *Plant Sci. Today.* **2020**, *7*, 9−16.
(149) Borochov-Neori, H.; Judeinstein, S.; Harari, M.; Bar-Ya'akov, I.; Patil, B. S.; Lurie, S.; Holland, D. Climate effects on anthocyanin accumulation and composition in the pomegranate (*Punica granatum* L.) fruit arils. *J. Agric. Food Chem.* **2011**, *59*, 5325−5334.
(150) Fischer, U. A.; Carle, R.; Kammerer, D. R. Identification and quantification of phenolic compounds from pomegranate (Punica granatum L.) peel, mesocarp, aril and differently produced juices by HPLC-DAD−ESI/MSn. *Food Chem.* **2011**, *127*, 807−821.
(151) Mena, P.; Calani, L.; Dall'Asta, C.; Galaverna, G.; García-Viguera, C.; Bruni, R.; Crozier, A.; Del Río, D. Rapid and comprehensive evaluation of (poly)phenolic compounds in pomegranate (Punica granatum L.) juice by UHPLC-MSn. *Molecules.* **2012**, *17*, 14821−14840.
(152) Balli, D.; Cecchi, L.; Khatib, M.; Bellumori, M.; Cairone, F.; Carradori, S.; Zengin, G.; Cesa, S.; Innocenti, M.; Mulinacci, N. Characterization of arils juice and peel decoction of fifteen varieties of *Punica granatum* L.: a focus on anthocyanins, ellagitannins and polysaccharides. *Antioxidants.* **2020**, *9*, 238.
(153) Gómez-Caravaca, A. M.; Verardo, V.; Toselli, M.; Segura-Carretero, A.; Fernández-Gutiérrez, A.; Caboni, M. F. Determination of the major phenolic compounds in pomegranate Juices by HPLC-DAD−ESI-MS. *J. Agric. Food Chem.* **2013**, *61*, 5328−5337.
(154) Sentandreu, E.; Cerdán-Calero, M.; Sendra, J. M. Phenolic profile characterization of pomegranate (Punica granatum) juice by high-performance liquid chromatography with diode array detection coupled to an electrospray ion trap mass analyzer. *J. Food Compos. Anal.* **2013**, *30*, 32−40.
(155) Thembekile Fakudze, N.; Chekwube Aniogo, E.; George, B. P.; Abrahamse, H. The therapeutic efficacy of Punica granatum and its bioactive constituents with special reference to photodynamic therapy. *Plants* **2022**, *11*, 2820.
(156) Montefusco, A.; Durante, M.; Migoni, D.; De Caroli, M.; Ilahy, R.; Pek, Z.; Helyes, L.; Fanizzi, F. P.; Mita, G.; Piro, G.; Lenucci, M. S. Analysis of the phytochemical composition of pomegranate fruit juices, peels and kernels: a comparative study on four cultivars grown in southern Italy. *Plants* **2021**, *10* (11), 2521.
(157) Middha, S. K.; Usha, T.; Pande, V. A review on antihyperglycemic and antihepatoprotective activity of eco-friendly Punica granatum peel waste. *Evidence-Based Complementary and Alternative Medicine* **2013**, *2013*, 1−10.
(158) Romani, A.; Campo, M.; Pinelli, P. HPLC/DAD/ESI-MS analyses and anti-radical activity of hydrolyzable tannins from different vegetal species. *Food Chem.* **2012**, *130* (1), 214−221.

(159) Amakura, Y.; Okada, M.; Tsuji, S.; Tonogai, Y. High-performance liquid chromatographic determination with photodiode array detection of ellagic acid in fresh and processed fruits. *J. Chromatogr. A* **2000**, *896*, 87−93.
(160) Ambigaipalan, P.; Costa de Camargo, A. C.; Shahidi, F. Phenolic compounds of pomegranate byproducts (outer skin, mesocarp, divider membrane) and their antioxidant activities. *J. Agric. Food Chem.* **2016**, *64*, 6584−6604.
(161) Fischer, U. A.; Jaksch, A. V.; Carle, R.; Kammerer, D. R. Determination of lignans in edible and nonedible parts of Pomegranate (*Punica granatum* L.) and products derived therefrom, particularly focusing on the quantitation of isolariciresinol using HPLC-DAD-ESI/MS. *J. Agric. Food Chem.* **2012**, *60*, 283−292.
(162) Ocieczek, A.; Pukszta, T.; Kaizer, A.; Korzeniowska-Ginter, R. The Optimisation of Storage Conditions for Pomegranate Juice during Its Maritime Transport. *Sustainability* **2024**, *16*, 375.
(163) Benedetti, G.; Zabini, F.; Tagliavento, M.; Meneguzzo, M.; Calderone, V.; Testai, L. An overview of the health benefits, extraction methods and improving the properties of pomegranate. *Antioxidants* **2023**, *12* (7), 1351.
(164) Fazaeli, M.; Yousefi, S.; Emam-Djomeh, Z. Investigation on the effects of microwave and conventional heating methods on the phytochemicals of pomegranate (*Punica granatum* L.) and black mulberry juices. *Food Res. Int.* **2013**, *50*, 568−573.
(165) Onsekizoglu, P. Production of high-quality clarified pomegranate juice concentrate by membrane processes. *J. Membr. Sci.* **2013**, *442*, 264−271.
(166) Conidi, C.; Cassano, A.; Caiazzo, F.; Drioli, E. Separation and purification of phenolic compounds from pomegranate juice by ultrafiltration and nanofiltration membranes. *J. Food Eng.* **2017**, *195*, 1−13.
(167) Conidi, C.; Drioli, E.; Cassano, A. Perspective of membrane technology in pomegranate juice processing: a review. *Foods* **2020**, *9* (7), 889.
(168) Fawole, O. A.; Makunga, N. P.; Opara, U. L. Antibacterial, antioxidant and tyrosinase-inhibition activities of pomegranate fruit peel methanolic extract. *BMC Complement Altern. Med.* **2012**, *12*, 200.
(169) Cacace, J. E.; Mazza, G. Optimization of extraction of anthocyanins from black currants with aqueous ethanol. *J. Food Sci.* **2003**, *68*, 240−248.
(170) Lang, G. H.; Lindemann, I. D. S.; Ferreira, C. D.; Hoffmann, J. F.; Vanier, N. L.; de Oliveira, M. Effects of drying temperature and long-term storage conditions on black rice phenolic compounds. *Food Chem.* **2019**, *287*, 197−204.
(171) Belwal, T.; Chemat, F.; Venskutonis, P. R.; Cravotto, G.; Jaiswal, D. K.; Bhatt, I. D.; Devkota, H. P.; Luo, Z. Recent advances in scaling-up of non-conventional extraction techniques: Learning from successes and failures. *Trac. Trends Anal. Chem.* **2020**, *127*, 115895.
(172) Mushtaq, M.; Sultana, B.; Anwar, F.; Adnan, A.; Rizvi, S. S. H. Enzyme-assisted supercritical fluid extraction of phenolic antioxidants from pomegranate peel. *J. Supercrit. Fluids* **2015**, *104*, 122−131.
(173) Lampakis, D.; Skenderidis, P.; Leontopoulos, S. Technologies and extraction methods of polyphenolic compounds derived from pomegranate (Punica granatum) peels. A mini review. *Processes* **2021**, *9*, 236.
(174) Cantao Freitas, L.; Rodrigues Barbosa, J.; Caldas da Costa, A. L.; Figueiredo Bezerra, F. W.; Holanda Pinto, R. H.; Nunes de Carvalho, R. N., Jr. From waste to sustainable industry: How can agro-industrial wastes help in the development of new products? *Resour Conserv Recycl.* **2021**, *169*, 105466.
(175) Rajha, H. N.; Abi-Khattar, A.; El Kantar, S.; Boussetta, N.; Lebovka, N.; Maroun, R. G.; Louka, N.; Vorobiev, E. Comparison of aqueous extraction efficiency and biological activities of polyphenols from pomegranate peels assisted by infrared, ultrasound, pulsed electric fields and high-voltage electrical discharges. *Innovative Food Sci. Emerging Technol.* **2019**, *58*, 102212.
(176) Liu, Y.; Kong, K. W.; Wu, D. T.; Liu, H. Y.; Li, H. B.; Zhang, J. R.; Gan, R. Y. Pomegranate peel-derived punicalagin: Ultrasonic-assisted extraction, purification, and its α-glucosidase inhibitory mechanism. *Food Chem.* **2022**, *374*, 131635.
(177) Cano-Lamadrid, M.; Martinez-Zamora, L.; Castillejo, N.; Bueso, M. C.; Kessler, M.; Artes-Hernandez, F. Francisco Artés-Hernandez. F. Ultrasound-assisted ethanolic extraction of punicalagin from pomegranate by-products influenced by cultivar, pre-drying treatment, particle size, and temperature. *LWT - Food Sci. Technol.* **2023**, *186*, 115236.
(178) Wu, W.; Jiang, S.; Liu, M.; Tian, S. Simultaneous process optimization of ultrasound-assisted extraction of polyphenols and ellagic acid from pomegranate (*Punica granatum* L.) flowers and its biological activities. *Ultrason. Sonochem.* **2021**, *80*, 105833.
(179) Kaderides, K.; Papaoikonomou, L.; Serafim, M.; Goula, A. M. Microwave-assisted extraction of phenolics from pomegranate peels: Optimization, kinetics, and comparison with ultrasounds extraction. *Chem. Eng. Process.: Process Intensif.* **2019**, *137*, 1−11.
(180) Kumar, M.; Tomar, M.; Punia, S.; Amarowicz, R.; Kaur, C. Evaluation of cellulolytic enzyme-assisted microwave extraction of *Punica granatum* peel phenolics and antioxidant activity. *Plant Foods Hum. Nutr.* **2020**, *75*, 614−620.
(181) Garcia-Villalba, R.; Espìn, J. C.; Aaby, K.; Alasalvar, C.; Heinonen, M.; Jacobs, G.; Voorspoels, S.; Koivumäki, T.; Kroon, P. A.; Pelvan, E.; Saha, S.; Tomàs-Barberàn, F. A. Validated method for the characterization and quantification of extractable and nonextractable ellagitannins after acid hydrolysis in pomegranate fruits, juices, and extracts. *J. Agric. Food Chem.* **2015**, *63* (29), 6555−6566.
(182) Sun, S.; Huang, S.; Shi, Y.; Shao, Y.; Qiu, J.; Sedjoah, R. C. A. A.; Yan, Z.; Ding, L.; Zou, D.; Xin, Z. Extraction, isolation, characterization and antimicrobial activities of non-extractable polyphenols from pomegranate peel. *Food Chem.* **2021**, *351*, 129232.
(183) Clementi, M. E.; Pani, G.; Sampaolese, B.; Tringali, G. Punicalagin reduces H2O2- induced cytotoxicity and apoptosis in PC12 cells by modulating the levels of reactive oxygen species. *Nutr. Neurosci.* **2018**, *21*, 447−454.
(184) Gaharwar, S. S.; Kumar, A.; Mandavgane, S. A.; Rahagude, R.; Gokhale, S.; Yadav, K.; Borua, A. P. Valorization of *Punica granatum* (pomegranate) peels: a case study of circular bioeconomy. *Biomass Conv. Bioref.* **2024**, *14*, 7707−7724.
(185) Zhao, R.; Long, X.; Yang, J.; Du, L.; Zhang, X.; Li, J.; Hou, C. Pomegranate peel polyphenols reduce chronic low-grade inflammatory responses by modulating gut microbiota and decreasing colonic tissue damage in rats fed a high-fat diet. *Food Funct.* **2019**, *10*, 8273−8285.
(186) Zhang, Y.; Cao, Y.; Chen, J.; Qin, H.; Yang, L. A new possible mechanism by which punicalagin protects against liver injury induced by type 2 diabetes mellitus: upregulation of autophagy via the Akt/FoxO3a signaling pathway. *J. Agric. Food Chem.* **2019**, *67*, 13948−13959.
(187) Zhang, L.; Chinnathambi, A.; Alharbi, S. A.; Veeraraghavan, V. P.; Mohan, S. K.; Zhang, G. Punicalagin promotes the apoptosis in human cervical cancer (ME-180) cells through mitochondrial pathway and by inhibiting the NF-kB signaling pathway. *Saudi J. Biol. Sci.* **2020**, *27*, 1100−1106.
(188) Ramlagan, P.; Labib, R. M.; Farag, M. A.; Neergheen, V. S. Advances towards the analysis, metabolism and health benefits of punicalagin, one of the largest ellagitannin from plants, with future perspectives. *Phytomed. Plus* **2022**, *2*, 100313.
(189) Cam, M.; Hışıl, Y. Pressurised water extraction of polyphenols from pomegranate peels. *Food Chem.* **2010**, *123*, 878−885.
(190) Kazemi, M.; Karim, R.; Mirhosseini, H.; Abdul Hamid, A. Optimization of pulsed ultrasound-assisted technique for extraction of phenolics from pomegranate peel of Malas variety: punicalagin and hydroxybenzoic acids. *Food Chem.* **2016**, *206*, 156−166.
(191) Talekar, S.; Patti, A. F.; Vijayraghavan, R.; Arora, A. Rapid, enhanced and ecofriendly recovery of punicalagin from fresh waste pomegranate peels via aqueous ball milling. *J. Clean. Prod.* **2019**, *228*, 1238−1247.

(192) Swilam, N.; Nematallah, K. A. Polyphenols profile of pomegranate leaves and their role in green synthesis of silver nanoparticles. *Sci. Rep.* **2020**, *10*, 14851.
(193) Longtin, R. The pomegranate: nature's power fruit? *J. Nat. Cancer Instit.* **2003**, *95* (5), 346−348.
(194) Jourdes, M.; Pouységu, L.; Quideau, S.; Mattivi, F.; Truchado, P.; Tomas-Barberan, F. A. Hydrolyzable tannins: gallotannins, ellagitannins, and ellagic acid. *Handbook of Analysis of Active Compounds in Functional Foods.* **2012**, 435−459.
(195) Lansky, E.; Shubert, S.; Neeman, I. Pharmacological and therapeutic properties of pomegranate. In *Production, Processing and Marketing of Pomegranate in the Mediterranean Region: Advances in Research and Technology*; Melgarejo, P., Martínez-Nicolás, J. J., Martínez-Tomé, J., Ed.; CIHEAM: Zaragoza, 2000; pp 231−235.
(196) Bhowmik, D.; Gopinath, H.; Kumar, B. P.; Kumar, K. S. Medicinal uses of Punica granatum and its health benefits. *J. Pharmacogn. Phytochem* **2013**, *1*, 28−35.
(197) Valero-Mendoza, A. G.; Meléndez-Rentería, N. P.; Chávez-González, M. L.; Flores-Gallegos, A. C.; Wong-Paz, J. E.; Govea-Salas, M.; Zugasti-Cruz, A.; Ascacio-Valdés, J. A. The whole pomegranate (Punica granatum. L), biological properties and important findings: A review. *Food Chem. Adv.* **2023**, *2*, 100153.
(198) Gil, M. I.; Tomás-Barberán, F. A.; Hess-Pierce, B.; Holcroft, D. M.; Kader, A. A. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *J. Agric. Food Chem.* **2000**, *48*, 4581−4589.
(199) Machado, J. C. B.; Ferreira, M. R. A.; Soares, L. A. L. Punica granatum leaves as a source of active compounds: A review of biological activities, bioactive compounds, food, and technological application. *Food Biosci.* **2023**, *51*, 102220.
(200) Ökmen, G.; Giannetto, D.; Fazio, F.; Arslan, K. Investigation of pomegranate (Punica granatum L.) flowers' antioxidant properties and antibacterial activities against different Staphylococcus species associated with bovine mastitis. *Vet. Sci.* **2023**, *10*, 394.
(201) Bekir, J.; Mars, M.; Vicendo, P.; Fterrich, A.; Bouajila, J. Chemical composition and antioxidant, anti-inflammatory, and antiproliferation activities of pomegranate (Punica granatum) flowers. *J. Med. Food* **2013**, *16*, 544−550.
(202) Li, Y.; Guo, C.; Yang, J.; Wei, J.; Xu, J.; Cheng, S. Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. *Food Chem.* **2006**, *96*, 254−260.
(203) Yasoubi, P.; Barzegar, M.; Sahari, M. A.; Azizi, M. H. Total phenolic contents and antioxidant activity of pomegranate (Punica granatum L.) peel extracts. *J. Agric. Sci. Technol.* **2007**, *9*, 35−44.
(204) Jalal, H.; Pal, M. A.; Hamdani, H.; Rovida, M.; Khan, N. N. Antioxidant activity of pomegranate peel and seed powder extracts. *J. Pharmacogn. Phytochem.* **2018**, *7*, 992−997.
(205) Shimizu, G. D.; Morais Vidal, T. C.; Aparecido Ribeiro, W. A.; Machado Ribeiro, L. T.; Menegon Castilho, I.; Goncalves, L. S. A.; Bonifacio Silva, J. Antioxidant activity and chemical composition from different parts of pomegranate (Punica granatum L.) cultivars. *Sci. Electron. Arch.* **2023**, *16*, 231675.
(206) Tzulker, R.; Glazer, I.; Bar-Ilan, I.; Holland, D.; Aviram, M.; Amir, R. Antioxidant activity, polyphenol content, and related compounds in different fruit juices and homogenates prepared from 29 different pomegranate accessions. *J. Agric. Food Chem.* **2007**, *55*, 9559−9570.
(207) Liu, C.; Guo, H.; DaSilva, N. A.; Li, D.; Zhang, K.; Wan, Y.; Gao, X.-H.; Chen, H.-D.; Seeram, N. P.; Ma, H. Pomegranate (Punica granatum) phenolics ameliorate hydrogen peroxide-induced oxidative stress and cytotoxicity in human keratinocytes. *J. Funct. Foods* **2019**, *54*, 559−567.
(208) Esposto, S.; Veneziani, G.; Taticchi, A.; Urbani, S.; Selvaggini, R.; Sordini, B.; Daidone, L.; Gironi, G.; Servili, M. Chemical composition, antioxidant activity, and sensory characterization of commercial pomegranate juices. *Antioxidants* **2021**, *10*, 1381.
(209) Aloqbi, A.; Omar, U.; Yousr, M.; Grace, M.; Lila, M. A.; Howell, N. Antioxidant activity of pomegranate juice and punicalagin. *Nat. Sci.* **2016**, *8*, 235.
(210) Sarker, M.; Das, S. C.; Saha, S. K.; Al Mahmud, Z.; Bachar, S. C. Analgesic and anti-inflammatory activities of flower extracts of Punica granatum Linn. (Punicaceae). *J. Appl. Pharm. Sci.* **2012**, *2*, 133−136.
(211) Xu, J.; Zhao, Y.; Aisa, H. A. Anti-inflammatory effect of pomegranate flower in lipopolysaccharide (LPS)-stimulated RAW264. 7 macrophages. *Pharm. Biol.* **2017**, *55*, 2095−2101.
(212) Ismail, T.; Sestili, P.; Akhtar, S. Pomegranate peel and fruit extracts: a review of potential anti-inflammatory and anti-infective effects. *J. Ethnopharmacol.* **2012**, *143*, 397−405.
(213) Mastrogiovanni, F.; Bernini, R.; Basiricò, L.; Bernabucci, U.; Campo, M.; Romani, A.; Santi, L.; Lacetera, N. Antioxidant and anti-inflammatory effects of pomegranate peel extracts on bovine mammary epithelial cells BME-UV1. *Nat. Prod. Res.* **2020**, *34*, 1465−1469.
(214) Mastrogiovanni, F.; Mukhopadhya, A.; Lacetera, N.; Ryan, M. T.; Romani, A.; Bernini, R.; Sweeney, T. Anti-inflammatory effects of pomegranate peel extracts on in vitro human intestinal Caco-2 cells and ex vivo porcine colonic tissue explants. *Nutrients* **2019**, *11*, 548−562.
(215) Salama, A. A.; Ismael, N. M.; Bedewy, M. The anti-inflammatory and antiatherogenic in vivo effects of pomegranate peel powder: from waste to medicinal food. *J. Med. Food* **2021**, *24* (2), 145−150.
(216) Singh, J.; Kaur, H. P.; Verma, A.; Chahal, A. S.; Jajoria, K.; Rasane, P.; Kaur, S.; Kaur, J.; Gunjal, M.; Ercisli, S.; Choudhary, R.; Bozhuyuk, M. R.; Sakar, E.; Karatas, N.; Durul, M. S. Pomegranate peel phytochemistry, pharmacological properties, methods of extraction, and its application: a comprehensive review. *ACS Omega* **2023**, *8*, 35452−35469.
(217) El Hosry, L.; Bou-Mitri, C.; Dargham, M. B.; Abou Jaoudeh, M.; Farhat, A.; El Hayek, J.; Bou Mosleh, J. M.; Bou-Maroun, E. Phytochemical composition, biological activities and antioxidant potential of pomegranate fruit, juice and molasses: a review. *Food Biosci.* **2023**, *55*, 103034.
(218) Alsataf, S.; Başyiğit, B.; Karaaslan, M. Multivariate analyses of the antioxidant, antidiabetic, antimicrobial activity of pomegranate tissues with respect to pomegranate juice. *Waste Biomass Valori.* **2021**, *12*, 5909−5921.
(219) Habib, H. M.; El-Gendi, H.; El-Fakharany, E. M.; El-Ziney, M. G.; El-Yazbi, A. F.; Al Meqbaali, F. T.; Ibrahim, W. H. Antioxidant, anti-inflammatory, antimicrobial, and anticancer activities of pomegranate juice concentrate. *Nutrients* **2023**, *15*, 2709.
(220) Alexova, R.; Alexandrova, S.; Dragomanova, S.; Kalfin, R.; Solak, A.; Mehan, S.; Petralia, M. C.; Fagone, P.; Mangano, K.; Nicoletti, F.; Tancheva, L. Anti-COVID-19 potential of ellagic acid and polyphenols of *Punica granatum* L. *Molecules* **2023**, *28*, 3772.
(221) Panth, N.; Manandhar, B.; Paudel, K. R. Anticancer activity of Punica granatum (pomegranate): a review. *Phytother. Res.* **2017**, *31*, 568−578.
(222) Baradaran Rahimi, V.; Ghadiri, M.; Ramezani, M.; Askari, V. R. Anti-inflammatory and anticancer activities of pomegranate and its constituent, ellagic acid: evidence from cellular, animal, and clinical studies. *Phytother. Res.* **2020**, *34*, 685−720.
(223) Cerdá, B.; Cerón, J. J.; Tomás-Barberán, F. A.; Espín, J. C. Repeated oral administration of high doses of the pomegranate ellagitannin punicalagin to rats for 37 days is not toxic. *J. Agric. Food Chem.* **2003**, *51*, 3493−3501.
(224) Aladaileh, S. H.; Al-Swailmi, F. K.; Abukhalil, M. H.; Ahmeda, A. F.; Mahmoud, A. M. Punicalagin prevents cisplatin-induced nephrotoxicity by attenuating oxidative stress, inflammatory response, and apoptosis in rats. *Life Sci.* **2021**, *286*, 120071.
(225) Wang, Y.; Han, D.; Huang, Y.; Dai, Y.; Wang, Y.; Liu, M.; Wang, N.; Yin, T.; Du, W.; He, K.; Zheng, Y. Oral administration of punicalagin attenuates imiquimod-induced psoriasis by reducing ROS generation and inflammation via MAPK/ERK and NF-κB signaling pathways. *Phytother. Res.* **2024**, *38*, 713−726.

(226) Venusova, E.; Kolesarova, A.; Horky, P.; Slama, P. Physiological and immune functions of punicalagin. *Nutrients* **2021**, *13*, 2150.
(227) Chen, P.; Guo, Z.; Zhou, B. Neuroprotective potential of punicalagin, a natural component of pomegranate polyphenols: A Review. *JIN* **2023**, *22*, 113.
(228) Savikin, K.; Nastic, N.; Jankovic, T.; Bigovic, D.; Milicevic, B.; Vidovic, S.; Menkovic, N.; Vladic, J. Effect of type and concentration of carrier material on the encapsulation of pomegranate peel using spray drying method. *Foods* **2021**, *10*, 1968.
(229) Cam, M.; İcyer, N. C.; Erdogan, F. Pomegranate peel phenolics: Microencapsulation, storage stability and potential ingredient for functional food development. *LWT Food Sci. Technol.* **2014**, *55*, 117−123.
(230) Vora, A.; Londhe, V.; Pandita, N. Herbosomes enhance the *in vivo* antioxidant activity and bioavailability of punicalagins from standardized pomegranate extract. *J. Funct. Foods* **2015**, *12*, 540−548.
(231) Sabzevari, A. G.; Sabahi, H. Montmorillonite an efficient oral nanocarrier for punicalagin-rich pomegranate peel extract: An *in vitro* study. *J. Drug Delivery Sci. Technol.* **2023**, *86*, 104713.
(232) Subash-Babu, P.; Al-Numair, N.; Almuzaini, T.; Athinarayanan, J.; Alshatwi, A. A. Punicalagin and ketogenic amino acids loaded organic lipid carriers enhance the bioavailability, mitochondrial *β*-oxidation, and ketogenesis in maturing adipocytes. *Nanomaterials* **2022**, *12*, 368.
(233) Karwasra, R.; Ahmad, S.; Bano, N.; Qazi, S.; Raza, K.; Singh, S.; Varma, S. Macrophage-targeted punicalagin nanoengineering to alleviate methotrexate-induced neutropenia: a molecular docking, DFT, and MD simulation analysis. *Molecules* **2022**, *27*, 6034.
(234) Enaime, G.; Dababat, S.; Wichern, M.; Lübken, M. Olive mill wastes: from wastes to resources. *Environ. Sci. Pollut. Res.* **2024**, *31*, 20853−20880.
(235) Lozano, P.; García-Verdugo, E. From green to circular chemistry paved by biocatalysis. *Green Chem.* **2023**, *25*, 7041−7057.

https://doi.org/10.1021/acs.jafc.4c00945
J. Agric. Food Chem. 2024, 72, 12871−12895