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Potential of food by-products

Olive Stone as a Sustainable Agricultural By-Product: Valorization Pathways and Prospects in Food and Feed Industries

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ABSTRACT

Background: Olive stone (OS) has emerged as a promising by-product with potential applications in food and feed formulations, owing to its unique properties. Despite growing interest in recent years, research dedicated to the comprehensive evaluation of OS remains limited.

Aim: This review aimed to elucidate the structure, physical and chemical properties of OS, provide an overview of its diverse application areas, and highlight its potential utilization in food and feed formulations through case studies and recent advancements.

Methods: A systematic literature search was conducted using prominent databases, including Google Scholar, Web of Science, PubMed and Scopus, with a focus on studies published in recent years. The search strategy employed keywords such as olive, olive by-products, olive stone composition, valorization areas, use of agricultural wastes in food. Relevant publications in English or Turkish were considered, resulting in a reference list of 97 articles that were critically reviewed and cited.

Results: OSs are a significant by-product generated during the olive oil extraction and pitted table olive production, constituting approximately 18-22% of the olive fruit. OS possesses a lignocellulosic composed primarily of hemicellulose, cellulose and lignin. Although its current predominant use is as fuel due to its high calorific value, OS exhibits potential for diverse applications owing to its rich composition of fat, protein, bioactive phenolic compounds and dietary fiber. Potential valorization pathways include activated carbon production, oil extraction, furfural synthesis, plastic filling material, cosmetic formulations, biosorbents, resin production, and animal nutritional supplementation. Recent studies have increasingly explored the use of OSs as a functional food ingredient, with promising results demonstrating its efficacy as an antioxidant, nutraceutical and thickening agent in food formulations.

Conclusion: This review underscores the multifaceted potential of OS, particularly in food and feed applications. The valorization of OS aligns with sustainable waste management practices and offers innovative opportunities for enhancing food and feed formulations.

Keywords: Olive, olive stone, waste management, waste valorization, agricultural by-product.

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1 INTRODUCTION

Olive is one of the most significant agricultural products globally, with its cultivation being particularly widespread throughout the Mediterranean region. Olive farming plays a crucial role in the rural economy, local heritage, and environmental sustainability of this area. The Mediterranean and Middle East regions dominate olive production, accounting for 98% of the total cultivated surface area and 99% of global olive fruit production. Leading producers include Spain, Italy, Egypt, Türkiye, Greece, Algeria, and Morocco (International Olive Council, 2024a). Due to its bitter taste, primarily attributed to phenolic compounds such as oeuropein, the olive is not consumed directly but is instead integrated into diets in the form of olive oil or table olives.

The health benefits of olive oil and table olives have been widely documented throughout history, and increasing consumer awareness of these benefits has driven growing for these products. Traditionally, they have been utilized to address various health concerns, including muscle injuries, calcifications, fractures, wounds, burns, stomach disorders, and dietary needs. Popular beliefs also suggest that olive stones (OSs) are beneficial for stomach ailments (Kaplan & Arıhan, 2012). Olive oil and table olives are rich in essential nutrients, including monounsaturated fatty acids, antioxidants (e.g., α-tocopherol), and bioactive compounds

(e.g., phenolic substances), making them a vital component of a healthy diet and offering significant health benefits to consumers (Malheiro *et al.*, 2012; Owen *et al.*, 2000; Sakouhi *et al.*, 2008; Visioli *et al.*, 2002).

Olive oil production is primarily performed using either three-phase or two-phase continuous systems. The three-phase method involves the addition of large quantities of water, resulting in substantial wastewater that requires treatment. In contrast, the two-phase process separates olive paste into oil and wet pomace, with water expelled alongside the pomace. In both systems, OSs are present in the pomace, which exhibits varying moisture content and can be mechanically recovered through centrifugation. Although both production systems generate significant amounts of wastes, the two-phase system has gained popularity in recent years due to its reduced water consumption and lower waste output, producing hydrated pomace instead of olive mill wastewater.

Waste management in olive oil and table olive production faces several challenges that complicate the sustainability of the industry. Since various types of waste are generated, each type requires different management and recycling approaches, rendering the waste management process inherently complex. This substantial volume of solid residue presents a limited storage life, waste disposal difficulties, environmental concerns, and elevated transportation costs. Effective management, storage, and recycling of these wastes requires adequate infrastructure and resources, which often entail significant financial investments, creating considerable challenges for small-scale producers. Although several studies have been conducted and various solutions proposed, the technologies required for optimal waste management may not be widely adopted or accessible. Therefore, it is imperative to develop cost-effective, easy-to-implement solutions that facilitate the direct valorization of these waste materials to enhance sustainability.

Structurally, the olive fruit can be devided into three primary components (Figure 1):

- The **skin (epicarp)**, constituting 1.0–3.0% of the drupe's weight, contains chlorophyll, carotenoids and anthocyanins, which are responsible for the fruit's coloration.
- The **pulp or flesh (mesocarp)**, comprising 70–80% of the fruit, represents the largest portion of the olive.
- The **stone (endocarp)**, accounting for 18–25% of the olive weight, encloses the seed (Galanakis, 2011). According to Ruiz *et al.* (2017), the OS typically represents 8–15% of the whole olive's weight. OS is obtained in fragmented form through filtration during olive oil extraction and in whole form during the separation process in pitted table olive production.

OS represents the primary by-product of the olive industry, often discarded as a material with no apparent utility or value. The underutilization of this by-product for human consumption constitutes a significant economic loss, given its richness in dietary fiber, phenolic compounds, and antioxidants. Olive oil extraction and table olive represent an economic and social industrial activity that is highly relevant in the Mediterranean countries. During the 2023/2024 season, global olive oil and table olive production were estimated at 2.407 and 2.654 million tons per year, respectively (International Olive Council, 2024b). This substantial production volume generates a correspondingly massive quantity of by-products, including olive leaves, pomace, and stones. Given that OS constitutes a significant

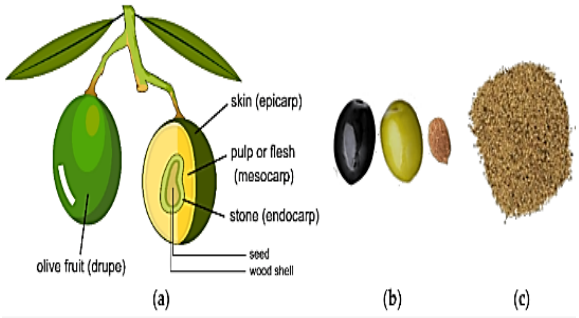


Figure 1. (a) Schematic of the Cross-Section of an Olive Fruit (b) images of olive fruits and stone; (c) image of OS granules mechanically grounded, sifted, de-oiled, and crushed from *Olea europaea* L. trees (Valvez *et al.*, 2021)

proportion of the olive fruit, a considerable amount of these by-products emerges. Therefore, OS represents the majority of the waste generated in the olive fruit industrial sector and holds significant commercial potential. Fortunately, advancements in technology have enabled the upcycling of solid residues. Modern separation, milling, and sieving techniques have facilitated the transformation of OS into distinct, value-added end products, offering promising opportunities for sustainable waste management and economic valorization.

2 METHODS

Although OS has numerous potential applications, its widespread use is primarily directed toward serving as a solid fuel or a derivative fuel source, leveraging its renewable energy potential. While the utilization of OS as biomass offers environmental benefits, certain challenges persist, including air pollution caused by the emission of carbon monoxide, nitrogen oxides, and particulate matter such as soot and ash during combustion. Beyond its utility as a fuel, whole OS is a valuable resource due to its chemicals and physical properties, as well as its high combustion heat. OS is

also recognized for its health-promoting properties, attributed to its high fiber content, including hemicellulose, cellulose, and lignin. Furthermore, its rich composition of essential nutritional and bioactive components—such as phenolics, protein, free sugars, and fat—enhances its potential economic value as a product (Rodríguez *et al.*, 2008).

Several studies have demonstrated the efficacy of OS extracts as a potent anti-inflammatory and antioxidant agents, further increasing its value. Additionally, OS has exhibited beneficial effects on various organs and organ systems, including central nervous system, cardiovascular system, liver, kidneys, and skin (Bartolomei *et al.*, 2022; Batçıoğlu *et al.*, 2023; Ben Saad *et al.*, 2021; Gouvinhas *et al.*, 2022; Samba Garba & Bouderbala, 2022; Vasquez-Villanueva *et al.*, 2018). Reported health benefits of OS in include its potential use in treating obesity, hypertension, cancer, and diabetes (Ben Saad *et al.*, 2021; Vasquez-Villanueva *et al.*, 2018; Veciana-Galindo *et al.*, 2015).

Despite these advantages, olive stones have traditionally been discarded as waste, often deemed unsuitable for consumption or further utilization. However, OS can be used directly or in powdered form through grinding, and its components can be extracted to enhance its value. Recently, there has been growing interest in OS research, particularly in the recovery of bioactive compounds and their applications in cosmetics, pharmaceuticals, food and feed industries.

2.1 OS composition

The olive stone (OS) comprises two main components: the woody shell (stone) and the seed. The stone is typically recovered in fragmented form during the filtration of solid waste in olive oil processing, while the whole stone is obtained from the table olive industry. During olive oil extraction, the olive fruit is processed through a mill, which breaks down its structure, resulting in fragmented endocarps — comprising the endocarp and the seed (Kiritsakis, 1998). In the production of pitted table olives, whole seeds are mechanically removed using a pitting machine.

OS is a value-added product with significant potential as a source of edible oil, protein, or meal, serving as a supplement in food and feed applications due to its rich nutritional profile and nutraceutical components. The concentration of these bioactive substances in OS varies depending on factors such as geographical origin, genotype, fruit maturity stage, agricultural practices, and extraction methods (Ben Saad *et al.*, 2021; Maestri *et al.*, 2019; Vasquez-Villanuevá *et al.*, 2018; Veciana-Galindo *et al.*, 2015). The primary components of OS are summarized in Table 1 and discussed in details in the following sections.

Fiber is a major component of the olive fruit, influencing its texture and digestibility. OSs contain high levels of dietary fiber, exceeding even that of chia seeds which are renowned for their fiber content (38–43% dry weight basis) (Galanakis, 2011). The fiber in OS is nearly equally distributed between insoluble and soluble forms. OS is composed of lignocellulosic biomass, primarily consisting of hemicellulose, cellulose, and lignin, with trace amounts of pectin, and sugars. The fiber composition of the whole OS has been studied across various olive cultivars using the neutral detergent fiber method (Heredia *et al.*, 1987). The fiber content varies among cultivars, with significant variability observed in the seed and moderate variability in the stone. Cellulose is the predominant component in the stone, while hemicellulose is more abundant in the seed. Hemicellulose, a common component of plant cell walls, is composed of sugars such as D-mannose, D-galactose, and D-xylose. Together with cellulose, it accounts for over 60% of the total dietary fiber in OS. Compared to cellulose, hemicellulose exhibits lower chemical and thermal stability due to its lower degree of polymerization and crystallinity (Valvez *et al.*, 2021). Lignin, a natural biopolymer, differs from cellulose and hemicellulose in its chemical structure, being primarily composed of phenylpropane units instead of glucose units. It forms through the oxidation of cinnamoyl alcohols, including p-coumaric, sinapoyl, and coniferyl alcohol, which are cross-linked via β-O-4 linkages and other bonds such as C–C, α-O-4, and β-1 (Demir, 2021). Lignin constitutes approximately 25% of the total lignocellulosic pool in OSa and the hard part of the stone forming a

Table 1. Composition of OS (Heredia-Moreno *et al.*, 1987; Ryan *et al.*, 2003)

Component	Whole stone (%, w/w)
Hemicellulose	21.9
Cellulose	31.9
Lignin	26.5
Protein	3.20
Fat	5.53
Free sugar	0.48
Neutral detergent fiber (NDF)	80.1
Acid detergent fiber (ADF)	58.2
Phenolics	
▪ Tyrosol	0.1 – 0.8
▪ Hydroxytyrosol	0.4 – 1.9
▪ 3,4 DHFEA-EDA	0.3 – 1.0
▪ Oleuropein	0.1 – 0.2
▪ Verbascoside	0.4 – 0.8
▪ Nüzhenide	2.8 – 7.6
Ash	0.01 – 0.68
Moisture	9.79

protective barrier network along with cellulose and hemicellulose that resists enzymatic degradation, thereby safeguarding the plant cell wall.

OS contains a considerable amount of oil, representing 22–27% of its weight. Triacylglycerols constitute the primary fraction (90–95%), with the remaining portion comprising fatty acids (FAs), phytosterols, and phospholipids (Ben Mansour *et al.*, 2015; Esteve *et al.*, 2012). Generally, both olive pulp and seed have similar FAs composition but differ in fatty acid unsaturation profile. Both unsaturated and saturated fatty acids (SFA) are reported in the lipid portion of olive seeds, with polyunsaturated fatty acids (PUFAs) being the dominant group (Maestri *et al.*, 2019). PUFAs and monounsaturated fatty acids (MUFAs) account for approximately 69.1% and 16.1% of the total FAs in seeds, respectively. OS oil is particularly rich in PUFAs due to its high linoleic acid content (Ranalli *et al.*, 2002), which exceeds that of its wild counterparts (Eromosele & Eromosele, 2002). Among saturated FAs, palmitic acid is the most abundant in both seeds (13.9%) and pulp (16.6%). In contrast, olive pulp is richer in MUFAs (approximately 74.7%) and lower in PUFAs (16.67%) (Maestri *et al.*, 2019; Ranalli *et al.*, 2002). Oleic acid, a MUFA, is the predominant fatty acid in pulp (55–70%), while linoleic acid (17–24%) and linolenic acid (0.5–5%), both PUFAs, are more abundant in seeds (Rahman *et al.*, 2024).

Several studies have highlighted variations in the lipid profiles of seeds and pulp, influenced by factors such as genotype, geographical origin, climatic conditions, maturation stage, and agronomic practices. Ripening generally increases levels of unsaturated FAs concurrent with a decrease in saturated FAs. For instance, a study examining the effect of maturation index on FAs composition in the pulp of two Tunisian cultivars Chemlali and Oueslati revealed different profiles at the same maturation stage. At an early stage Chemlali seeds exhibited lower levels of palmitic acid (14.2%) and higher levels of linoleic acid in the pulp (17.3%), compared to Oueslati, which showed 10.6, and 14.4% respectively. As maturation progressed, oleic acid levels in seeds increased to 68.7%, surpassing those in the pulp (57.5%) (Ben Mansour *et al.*, 2015). Another study comparing oil extracted from both seeds and pulp from two cultivars, Shengeh, and Arbequina, revealed that Shengeh pulp was richer in oleic acid (70%) compared to its seeds (62%), whereas linolenic acid was more abundant in seeds (90%). Similar trends were observed in Arbequina (D'Angeli & Altamura, 2016). These findings align with existing literature, confirming the predominance of PUFAs in seeds and PUFAs in pulp (D'Angeli & Altamura, 2016; Maestri *et al.*, 2019).

Tocopherols, commonly referred to as vitamin E, are classified into α, β, γ and δ isomers based on the number and position of methyl substituents on the phenolic ring. Olive seeds are notably rich in tocopherols, including α-tocopherol (7.5 mg/g), β-tocopherol (0.1 mg/g), γ-tocopherol (0.15 mg/g), and γ-tocotrienol 0.1 mg/g). In comparison, crude wheat germ seed oil contains significantly lower levels of α -tocopherol, reaching only 2.25 mg/g (Demir, 2021; Reboredo-Rodríguez *et al.*, 2013). A separate study on olive seed oil, derived from the residual flesh of *O. europea* L. species during the canning industry in the Mediterranean region, detected α-tocopherol at much lower concentrations, up to 79.8 mg/kg (Mallamaci *et al.*, 2021).

Phytosterols, a class of triterpene alcohols primarily composed of 4-demethyl alcohol, are biosynthesized from squalene and constitute the unsaponifiable fraction of most vegetable oils. The predominant sterols in olive seeds include β-sitosterol (1675 mg/kg), campesterol (70.7 mg/kg), and stigmasterol (53.9 mg/kg), all derived from 4-demethylsterols. Minor 4-demethylsterols such as cholesterol, stigmastenol, and stigmastol are also present (Maestri *et al.*, 2019; Ranalli *et al.*, 2002). In contrast, total triterpene di-alcohols, such as erythrodiol, were found at 3.5-fold higher levels in pulp (153.5 mg/kg) compared to seeds (40.5 mg/kg) (Ranalli *et al.*, 2002). However, this pattern was not observed for β-amyrin, a significant non-steroidal triterpene, which was detected at higher concentrations in olive seed oil (166.8 mg/kg oil) than in pulp (137.8 mg/kg) (Ghanbari *et al.*, 2012; Ranalli *et al.*, 2002).

Squalene, a major hydrocarbon in olive oil, serves as a biosynthetic precursor for both sterols and triterpenes. Virgin olive oil is considered the richest source of squalene among vegetable oils, with concentrations ranging from 700 to 1200 mg/kg. In two Tunisian cultivars, Oueslati (OU), and Chemlali (CH), squalene levels were significantly lower in seed compared to pulp and further declined with increasing maturation index (Ben Mansour *et al.*, 2015). Another study reported squalene levels in seed oil at 195 mg/kg, comparable to those found in edible vegetable oils (135–260 mg/kg) (Maestri *et al.*, 2019).

The sugars present in OS include sucrose, glucose, fructose, arabinose, xilose, mannitol, and myo-inositol. Glucose is the predominant sugar, accounting for 46–51% in the stone and 47–63% in the woody fraction, followed by galactose (25%) and xylose (14%). Arabinose and mannose were also detected (Heredia *et al.*, 1987). The reducing power of stone extracts has been attributed to the presence of glucose. Due to its lignocellulosic, OS is a promising candidate for sugar production, as it comprises up to 50% structural carbohydrates, including cellulose and hemicellulose. The cell wall polysaccharides of OS were isolated and characterized by Coimbra *et al.* (1995), revealing that 62% of the total carbohydrates are rich in xylose and glucose, derived from hemicellulose and cellulose, respectively. These

sugars can be biologically converted into high-value compounds such as ethanol, xylitol, and lactic acid through integrated process within a multi-feedstock and multi-product biorefinery framework.

OS contains a higher protein content compared to other parts of the olive fruit. The protein composition of OSs is approximately 17.5% of the seed dry weight, exceeding that of whole wheat (13.6%), corn (10.5%), and sorghum (12.4%), positioning it as a potential source of vegetable protein. Essential amino acids (EAAs) constitute about 45% of the total amino acids in OS, compared to 25% in whole wheat (Esteve *et al.*, 2012; Maestri *et al.*, 2019). The EAA to non-EAA ratio in olive seed protein (0.85) is significantly higher than FAO recommendation for adult humans (0.38) (Rahman *et al.*, 2024). The protein content is of OS is a critical component of its nutritional value, with adequate amounts of EAA meeting the requirements for both children and adults, except for lysine and phenylalanine which are limiting for children. However, valine levels in OS (165 mg/g protein) are twice those found in wheat and eggs (80 mg/g protein) (Maestri *et al.*, 2019). Among non-essential amino acids, glutamine (170 mg/g) and asparagine (89 mg/g), are the most abundant, while arginine, a semi-essential AA, is present at higher levels in OS (75 mg/g) compared to other cereal grains (Maestri *et al.*, 2019; Rahman *et al.*, 2024).

Recent studies have identified that the most abundant proteins in the mature OS belong to the 11S protein family (storage protein), accounting for approximately 70% of the total stone proteins. Olive seed storage proteins include albumins, globulins, prolamins, and glutelins, with globulins being the predominant form (Alché *et al.*, 2006). The high proteins content of OS underscores its potential as a valuable nutritional supplement for the animal feed formulations (Rodríguez *et al.*, 2008).

The OS is a rich source of bioactive compounds, particularly phenolic compounds, potent antioxidants that play a critical role in the chemical, organoleptic and nutritional properties of virgin olive oil and the table olives. Phenolic compounds are secondary metabolites produced by plants in response to pathogen and insect attacks, serving as protective agents against harmful factors. Numerous studies have demonstrated that phenolic components in olive products exhibit a wide range of pharmacological properties, including antioxidant, antifungal, antibacterial, antiviral, anti-inflammatory, antiallergic, antiatherogenic, and anticancer effects (Ahmad *et al.*, 2022; Cecchi *et al.*, 2023; Maestri *et al.*, 2019; Owen *et al.*, 2000; Tripoli *et al.*, 2005; Visioli *et al.*, 2002). However, although polyphenol levels in seeds are significantly lower than in pulp, their presence in OS remains noteworthy.

Three glucosides—salidroside (tirosol–glucose), nüzhenide (glucose–elenolic acid–glucose–tyrosol), and nüzhenide-oleoside—as well as two secoroiridoid glucosides containing tirosol, elenolic acid, and glucose moieties with differing sequences, were isolated from the OS (Maestro-Duran *et al.*, 1994; Servili *et al.* 1999). Nüzhenide, nüzhenide-oleoside, and salidroside are found exclusively in seeds, with nüzhenide being the predominant phenolic compound, while verbascoside appears in significant quantities only in OS (Ryan *et al.*, 2003). Tyrosol, hydroxytyrosol, oleuropein and diadehydic form of decarboxymethyl oleuropein (3,4 DHFEA-EDA) are present in all olive tissues including pulp, leaves, seeds, and stones. Tyrosol and hydroxytyrosol were identified for the first time in the OS by Fernández-Bolaños *et al.* (1998), who suggested their role as structural component. These compounds are found at much higher concentrations in olive pulp (19.48 and 76.73 mg/100g, respectively) compared to seeds (2.5–3 mg/100g), levels increasing throughout the ripening stages (Maestri *et al.*, 2019).

Fernández-Bolaños *et al.* (1998) evaluated the phenolic fraction of steam-exploded olive stones and found that hydroxytyrosol (105 mg/100 g) and tyrosol (49 mg/100 g) were the primary phenolic compounds in OS. A study of six olive seed cultivars (*O. europaea* L.) from Portugal reported nüzhenide and nüzhenide 11-methyl oleoside were the major components detected in olive seeds of all the cultivars studied (Silva *et al.*, 2010). The results also support the existence of di and tri (11-methyl oleosides) of nüzhenide (Silva *et al.*, 2010). Another important class of phenolics in olive seeds includes flavonoid glycosides, primarily flavanols, flavones, and anthocyanins exemplified by rutin (3.1 mg/g), luteolin 7-O-glucoside (2.9 mg/g), apigenin 7-O-glucoside, cyanidin 3-O-glucoside, and cyanidin 3-O-rutinoside (Ghanbari *et al.*, 2012). Compared to pulp, seeds exhibit lower levels of flavones, with concentrations varying among cultivars, ranging from 4.6 to 19.1 mg/100g (Ahmad *et al.*, 2022). Verbacoside, a hydroxytyrosol linked to a caffeic acid ester, is present in trace amounts in seeds but it concentrated in the pulp of cultivars such as Coratina, Moraiolo, and Leccino (21.2, 6.9, 0.23 mg/g dry weight, respectively) (Gouvinhas *et al.*, 2022; Maestri *et al.*, 2019). However, another study on the same cultivars reported higher verbascoside levels in Leccino (1745 mg/kg) compared to Moraiolo (787 mg/kg), with discrepancies attributed to differences in extraction methods, harvesting, or ripening stages (Cecchi *et al.*, 2023).

Free phenolic acids, including cinnamates and benzoates such as chlorogenic acid, p-hydroxybenzoic acid, caffeic acid, protocatechuic acid, ferulic acid, benzoic acid, sinapic acid, cinnamic acid, and gallic acid , have also been identified in seeds (Ahmad *et al.*, 2022; Maestri *et al.*, 2019). These

compounds likely contribute to the taste and health benefits of olive seeds.

Mineral's analysis of olive seeds reveals their richness in essential minerals required for several physiological functions. Potassium is the most abundant mineral (5578.1 mg/kg), followed by sodium (2758.2 mg/ kg), calcium (2615.4 mg/kg), and magnesium (1878.5 mg/kg) (Maestri *et al.*, 2019). Compared to the olive fruit, seeds contain higher levels of potassium (17955 mg/kg) but significantly lower concentrations of sodium, calcium, and magnesium (1090, 395, and 45 mg/kg, respectively) (Ahmad *et al.*, 2022; Fernandez-Hernandez *et al.*, 2010). Other essential microelements detected in seeds include nickel (3.8 mg/kg), chromium (6.3 mg/kg), barium (13.5 mg/kg), copper (23.5 mg/kg), manganese (31.9 mg/kg), zinc (16.5 mg/kg), and iron (12.5 mg/kg). Importantly, analysis of heavy metals such as arsenic, cadmium, and lead revealed no detectable levels in either fruits or seeds, underscoring their safety for consumption (Maestri *et al.*, 2019).

2.2 Valorization Fields

From both environmental and economic perspectives, OS can be regarded as a renewable energy source. Additionally, it offers the potential for the extraction of high-value compounds with diverse applications, depending on their specific chemical and physical properties. Table 2 outlines the most significant uses of OS. The primary application of OS is combustion for the generation of electrical energy or heat. Other notable uses include the production of activated carbon for the removal of unwanted colors, dyes, odors, tastes, and contaminants such as arsenic or aluminum; liquid and gas production; furfural production; olive seed oil extraction, plastic filling; bio-sorbent; animal feed; and resin formation (Carraro *et al.*, 2005; González *et al.*, 2003; Luaces *et al.*, 2003; Montané *et al.*, 2002; Pütün *et al.*, 2005; Siracusa *et al.*, 2001).

In the context of modern energy demands, it is essential to ensure that new biomass fuels have minimal environmental impact and cover new energy demands. From this viewpoint, the OS is particularly promising in this regard due its low nitrogen (N) and sulfur (S) content (González *et al.*, 2003).

Table 2. Key Applications and Uses of Olive Stones (OS)

Application	Material	Reference
Biscuit fortification and antioxidant	Olive stone	Bölek, 2020a
Biscuit's fortification	Olive pomace	Conterno *et al.*, 2019
Cereal foods fortification	Dried olive pomace	Cedola *et al.*, 2020
Pasta fortification	Olive pomace	Simonato *et al.*, 2020
Yogurt fortification	Olive stone	Bölek, 2020b
Feed supplement	Stoned or partly-destoned olive cake	Chiofalo *et al.*, 2020; Dal Bosco *et al.*, 2012; Luciano *et al.*, 2013; Vargas-Bello-Pérez *et al.*, 2013
Feed supplement	Olive stone	Carraro *et al.*, 2005
Feed supplement	Olive pomace	Iannaccone *et al.*, 2019; Nasopoulou, *et al.*, 2013; Nasopoulou *et al.*, 2014; Sioriki *et al.*, 2016
Combustion	Stone and seed	Durán, 1985; Gomez-Martin *et al.*, 2018; Gonzalez *et al.*, 2003; Mediavilla *et al.*, 2020
Pyrolysis and gasification	Stone and seed	Asimakidou and Chrissafis, 2022; Caballero *et al.*, 1997; Rios *et al.*, 2006; Skoulou *et al.*, 2009
Bio-oil	Stone and seed	Pütün *et al.*, 2005
Olive seed oil	Seed	Luaces *et al.*, 2003; Ranalli *et al.*, 2002
Furfural production	Stone and seed	Montané *et al.*, 2002; Riera *et al.*, 1990
Plastic filled	Stone	Cristofaro, 1997; Gülel and Güvenilir, 2024; Siracusa *et al.*, 2001
Activated carbon	Stone and seed	El-Sheikh *et al.*, 2004; Martinez *et al.*, 2006; Molina-Sabio *et al.*, 2006; Spahis *et al.*, 2008; Stavropoulos and Zabaniotou, 2005; Ubago-Pérez *et al.*, 2006; Uğurlu *et al.*, 2008
Bio-sorbent	Stone and seed	Aziz *et al.*, 2009; Blázquez *et al.*, 2009
Fractionation	Stone and seed	Fernández-Bolaños *et al.*, 2001
Ethanol and xylitol production	Olive stone	Saleh *et al.*, 2014
Oligosaccharides and sugar production	Olive stone	Cuevas *et al.*, 2015; Mateo *et al.*, 2013

Note. Estimation Method: Diagonally Weighted Least Squares; Model Fit Statistics: χ^2/df=1.50; RMSEA = 0.05; SRMR=0.08; NFI = 0.97; NNFI = 0.99; CFI = 0.99; GFI = 0.98; AGFI = 0.97; Hoelter's critical N (α = .05) =176.88.

This characteristic significantly reduces emissions of NOx and SO_2 which contribute to acid rain and ozone layer depletion. OS can also be mixed with concentrated vegetation water to create an efficient fuel, further mitigating the environmental impact of this waste (Vitolo *et al.*, 1999). With a high heating value (combustion heat of 4.075 kcal/kg), comparable to that of carbohydrates (4.10 kcal/kg) and exceeding that of dry olive pomace, OS is predominantly utilized in thermal processes. These include power generation in the electricity sector and space calefaction in residential and commercial buildings (Durán, 1985). Alternatively, OS can undergo thermal degradation through pyrolysis and gasification to produce syngas, , which serves as a fuel for electricity or steam generation, or as a chemical feedstock in the petrochemical and refining industries (Asimakidou and Chrissafis, 2022; Caballero *et al.*, 1997; Rios *et al.*, 2006; Skoulou *et al.*, 2009).

Activated carbon production constitutes another valuable application of OS, with uses in water purification, decontamination processes, and the removal of dyes, odors, tastes, and contaminants (Rodríguez *et al.*, 2008; Spahis *et al.*, 2008; Ubago-Pérez *et al.*, 2006; Uğurlu *et al.*, 2008). The adsorption properties of activated carbons have garnered significant interest across diverse industries, including food, chemical, petroleum, nuclear, mining, and pharmaceuticals (El-Sheikh *et al.*, 2004; Stavropoulos and Zabaniotou, 2005). Activated carbon is a microporous carbonaceous material characterized by a high surface area and porosity, which depends on the activation process. Several studies have explored the effects of chemical and physical activation techniques on OS-derived activated carbon, aiming to enhance its adsorption properties (El-Sheikh *et al.*, 2004; Martınez *et al.*, 2006; Molina-Sabio *et al.*, 2006; Stavropoulos and Zabaniotou, 2005; Ubago-Pérez *et al.*, 2006). Activated carbons produced from olive seed waste char were found to rival or even surpass commercial products in terms of adsorption capacity and surface area , particularly at high activation levels (Stavropoulos and Zabaniotou, 2005). In the literature, OS has been investigated as a bio-sorbent for heavy metal ions, such as as chromium (III) and (VI) and cadmium (Aziz *et al.*, 2009; Blázquez *et al.*, 2009).

Beyond its use in activated carbon production, pyrolysis of OS yields valuable liquid and gas products. For instance, bio-oil, a pyrolysis product, is a fuel with properties identical to petroleum, particularly its n-pentane fraction (Pütün *et al.*, 2005).

During the extraction of olive oil, a portion of the seed oil is incorporated into the final product. The seed is released through the mechanical breakdown of the whole stone. Comparative analyses between olive oil and olive seed oil were carried out by Ranalli *et al.*, (2002), revealing that seed oil is 2.3–fold higher in individual sterols, particularly in β-sitosterol, which plays a significant role in cholesterol and bile acid absorption (Hakala *et al.*, 1997). Seed oil also contains higher levels of total PUFAs, primarily due its elevated linoleic acid contents. In contrast, it has significantly lower concentrations of triterpene dialochols (3.5–fold less) compared to olive fruit oil.

Another notable application of OSs is in the production of furfural, a chemical compound with widespread industrial uses as a solvent or as a precursor for synthesizing derived solvents. Global furfural production is estimated at approximately 300.000 metric tons per year. Furfural is produced through dehydration of pentoses present in lignocellulosic materials. There are several processes to obtain furfural, some of which present the OS as a lignocellulosic biomass. OS, with its high xylose content (close to 20% on a dry basis), serves as a viable raw-material for furfural production, yielding up to 135 kg per ton of OS (Montané *et al.*, 2002). Additionally, studies explored the use of hydrolyzed OS residues from furfural production to develop humic fertilizers (Riera *et al.*, 1990).

Research has also focused on the biochemical conversion of OSs to produce xylitol and bioethanol. This process involves multiple stages, including pre-treatment, hydrolysis of polysaccharides, detoxification of hydrolysates, fermentation of sugars, and separation of bioproducts. utilizing microorganisms or enzymes. Xylitol and ethanol can be produced simultaneously during the fermentation stage using yeasts capable of converting D-xylose to xylitol and D-glucose to ethanol. Common pre-treatment methods for OS include liquid hot water, steam explosion, organosolv, and dilute-acid hydrolysis (García Martín *et al.*, 2020). However, the overall process is relatively costly, making the biochemical conversion of OS economically viable only if high-value products, such as antioxidants, oligosaccharides, or other bioactive molecules, are obtained alongside lower-value products like bioethanol or furfural.

In this context, the integrated production of xylitol, furfural, ethanol and poly-3-hydroxybutyrate from OS within a biorefinery framework has been proposed. This approach includes a cogeneration system to produce bioenergy from the solid residues generated during the production of these bioproducts (García Martín *et al.*, 2020).

OS also exhibits significant potential for use as plastic filler material. To minimize the adverse environmental effects associated with conventional plastic structures, the promotion of clean technologies and the utilization of recycled products have been prioritized. In this context, the application of OS as a plastic filler has been investigated. The widespread use of non-biodegradable, petroleum-derived polymers in industrial applications exacerbates environmental challenges, including plastic waste

accumulation and the depletion of fossil resources. A promising solution to address this issue lies in the substitution of these polymers with biodegradable and bio-based alternatives. Within the polymer industry, OS powder has been employed as a biofiller and reinforcing agent in various thermoplastic polymers to develop polymer composites with enhanced physical, mechanical, and thermal properties (Valvez *et al.*, 2021). Several studies have explored the preparation of composite samples using solid-phase methods derived from an olive oil mill by-products (Gülel and Güvenilir, 2024; Pardalis *et al.*, 2024; Siracusa *et al.*, 2001; Valvez *et al.*, 2021). Furthermore, industrial applications have successfully developed homogeneous polymer compounds incorporating OS as a natural and biodegradable raw material. Products such as panels, pipes, tubes, and profiles, amongst others, have been manufactured using extrusion and injection molding technologies (Cristofaro, 1997).

According to Badiu *et al.* (2010), olive oil possesses antioxidant properties and contains several essential FAs required for the synthesis of phospholipids, such as alpha-linolenic acid and gamma-linolenic acid. Although olive oil has been used in skincare for millennia, the mechanisms underlying its beneficial effects remain largely unexplored (Badiu *et al.*, 2010). OSs are also emerging as a promising candidate for moderating the effects of the skin aging by reducing the biochemical consequences of oxidative stress owing to its bioactive compounds. Given the compositional attributes of olive by-products, they represent a viable and sustainable source of cosmetic ingredient from both environmental and economic perspectives.

2.3 Food and feed application

The presence of numerous valuable components in in the structure of olive stone, coupled with its lack of allergens such as gluten, underscores its potential as a viable material for incorporation into food and feed formulations (Table 3). While OSs can be directly utilized in food formulations, it it also offers the possibility of extracting and utilizing its various constituent components. Although the literature on this subject remains limited it is anticipated that research into the application of OS in food and feed formulations will garner increased attention in the near future. Owing to its favorable properties, OS holds promise as a functional ingredient in a wide range of food products.

For instance, OS powder can partially replace wheat flour in the production of bread and bakery products to enhance functional properties and nutritional value. In a study by Bölek (2020a), wheat flour was substituted with OS powder at concentrations of 0%, 5%, 10% and 15% for biscuit production. The study aimed to enrich bakery products with OS powder and evaluated its impact on biscuit quality. The substitution of wheat flour with OS powder resulted in increased antioxidant activity, fat content, and fiber content in the biscuit samples. Sensory analysis revealed that wheat flour could be substituted with OS powder at levels of up to 15% without compromising the sensory acceptability of the final product. In another study by Bölek (2020b), the potential utilization of OS in yogurt was investigated. OS was added to yogurt in varying proportions, and its effects on protein, fiber, ash, fat, total phenolic, and antioxidant properties were examined. The incorporation of OS powder significantly increased the fiber and total phenolic content of the yoghurt samples, leading to the conclusion that OS could serve as a health-promoting ingredient in yogurt production. Similarly, Jahanbakhshi and Ansari, (2020) demonstrated that replacing 25% of wheat flour with OS powder in a sponge cake recipe yielded a product with acceptable dietary fiber content and antioxidant phenolic compounds, without adversely affecting its sensory properties.

In addition to OS, olive pomace (OP) has been explored as a functional ingredient in bakery products. OP can be incorporated into bread and pasta at a concentration of 10% (w/w) in bread and pasta. Cedola *et al.* (2020) demonstrated that while both olive wastewater and OP enhanced the nutraceutical value of the final product, OP was identified as the more suitable ingredient despite its more pronounced impact on sensory attributes, such as imparting a bitter and spicy taste. Simonato *et al.* (2020) fortified pasta by replacing

Table 3. Primary Bioactive Compounds with Potential Food and Feed Technological Functions of OS (Rodríguez *et al.*, 2008)

Bioactive compounds	Technological functions
Phenolic compounds	Antioxidant properties
Hemicellulose	Gelling agents
Cellulose	Emulsifying properties
Lignin	Thickeners
Bioactive peptides	Dispersing agents
	Sugar-alcohol production
	Source of mono- and oligosaccharides
	Food and feed fortification (increase fiber and total phenolic content)
	Fewer calories
	Lowered glycemic index

durum wheat semolina with OP at concentrations of 0.5% and 10% (w/w). This fortification significantly increased the phenolic content and, consequently, the antioxidant activity of the pasta, both before and after cooking. Furthermore, the fiber content of OP altered the in vitro digestibility of starch, reducing the rapidly digestible fraction while increasing the slowly digestible starch fraction.

OP flour has also been utilized in the development of functional biscuits. Lin *et al.*, (2017) incorporated OP flour into biscuits at a 15% substitution level, resulting in products with higher fiber content, enhanced nutritional value, reduced caloric content, and a lower glycemic index. Consumption of OP-supplemented biscuits by volunteers was associated with a shift in intestinal microbiota composition. Metagenomic analysis of 16S rRNA profiles revealed an increase in the abundance of *Akkermansia* and *Bifidobacterium* genera known to be positively correlated with host physiology and protection against metabolic and cardiovascular diseases (Conterno *et al.*, 2019).

The production of fermentable sugars from OSs has been investigated by several researchers, employing methods such as enzymatic hydrolysis and steam explosion as pretreatment. Enzymatic hydrolysis of cellulose derived from OSs releases glucose; a fermentable sugar suitable for alcohol production. The remaining lignin was limited to the enzymatic action and only when it was treated with sodium chlorite a complete saccharification was obtained. The highest sugar yield obtained from cellulose hydrolysis was 87% of the theoretical yield, increasing to 100% after sodium chlorite treatment. Cuevas *et al.* (2015) explored the pretreatment of OSs using hot water (autohydrolysis) at temperatures up to 225 °C, achieving a high oligosaccharide yield of 14.7 kg/100 kg OS at 190 °C for 300 seconds. The solid residues from this process were further hydrolyzed using cellulases to assess enzymatic digestibility. Results indicated that enzymatic saccharification of solids pretreated at 225 °C for 600 seconds yielded 54.3% D-glucose (12.6 kg/100 kg of OS), representing a 28-fold improvement compared to solids pretreated at 150 °C for 600 seconds. These findings underscore the potential of OS as a raw material for fermentable sugar production. Combining autohydrolysis and enzymatic processes, the total yield of fermentable sugars reached 27.3 kg/100 kg of OS.

Doménech *et al.* (2020) investigated the pretreatment of OS biomass using reactive extrusion technology with NaOH as the chemical agent. This process serves as a preliminary step in the biological conversion of carbohydrates within the material into bio-based products. OS biomass was pretreated in a twin-screw extruder at temperatures of 100, 125, and 150 °C, with NaOH/biomass ratios of 5% and 15% (dry weight basis), to evaluate the effectiveness of the process in enhancing sugar release through enzymatic hydrolysis. This result demonstrated that alkaline extrusion significantly increased sugar release compared to untreated raw material. Optimal conditions of 15% NaOH/biomass ratio and 125 ◦C yielded carbohydrate conversion rates of 55.5% for cellulose and 57.7% for xylan, relative to the maximum theoretical achievable. Under these conditions, 31.57 g of total sugars were obtained from 100 g of raw OS.

Additional applications of OS

Beyond sugar production, OS offers a range of valuable applications. For instance, vanillin, a lignin-derived monomer, can be extracted from OS and utilized in the food and pharmaceutical industries. The cellulose fraction of OS, in addition to its role in saccharification for ethanol production, has diverse industrial applications. These include its use as an anticaking agent, emulsifier, stabilizer, dispersing agent, thickener, gelling agent, and moisture-retaining agent, depending on the crystalline degree of the cellulose (Fernández-Bolaños *et al.*, 2001). Furthermore, various reaction studies have been conducted to explore new applications for this cellulose source (Rodríguez *et al.*, 2008; Vaca-Garcia & Borredon, 1999).

In the food industry, OS has been utilized in innovative ways. For example, solid polyphenol extracts from olive seeds, combined with grape seed extracts, have been incorporated into in emulsion gels as animal fat replacers in the production of Frankfurters. These fat replacers enhance the nutritional composition of meat products and improve oxidative stability, offering significant benefits to the meat industry (Pintado *et al.*, 2021). Additionally, OS has been explored as a source of bioactive peptides with potential health benefits. Bartolomei *et al.*, (2022) extracted proteins from OSs and produced two protein hydrolysates using the enzymes alcalase and papain. These hydrolysates demonstrated antioxidant and anti-diabetic properties, suggesting their potential as functional ingredients in nutraceuticals and foods aimed at preventing metabolic syndrome.

The use of OP for animal feed is a well-established practice, offering a sustainable means of reusing this by-product. To be commercially viable, feed must provide both adequate nutritional value and cost-effectiveness to compete with conventional feed options. The incorporation of olive by-products into feed for both aquaculture and livestock has been shown to have no adverse effects on animal growth. Moreover, it enhances the fatty acid profile by reducing the proportion of SFA (Tzamaloukas *et al.*, 2021). Sioriki *et al.* (2016) demonstrated that the addition of 8% OP to sea bream feed improved the cardioprotective properties of the fish by enriching its lipid profile with specific cardioprotective compounds of vegetable origin. In addition, other studies have integrated OP in the diets of sea bass and

sea bream, revealing a presumed cardioprotective effect characterized by increased production of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the lipid fraction of the fish (Nasopoulou *et al.*, 2013; Nasopoulou *et al.*, 2014).

OP has also been incorporated into the feed of rabbits and lambs. Specifically, the inclusion of OP in lamb feed increased the oxidative stability of the meat (Dal Bosco *et al.*, 2012). Comparable results were observed in lambs by Luciano *et al.*, (2013). The addition of 35% OP and linseed to animal feed demonstrated a synergistic effect, increasing the level of PUFAs and vitamin E while reducing the level of peroxides, thiobarbituric acid reactive substances (TBARS), and conjugated dienes. Furthermore, the oxidative stability of pork has been monitored with similar positive outcomes. Several studies have highlighted the benefits of incorporating OP into animal feed, not only for the animals themselves but also for improving the nutritional quality and quantity of derived products, such as dog, food, milk, cheese, and eggs.

Carraro *et al.* (2005) investigated the effects of OS on the prevention of digestive disorders in growing rabbits, aiming to achieve a better balance of dietary fiber fractions. The inclusion of OS increased the proportion of large dietary particles without adversely affecting growth performance, digestive physiology, or carcass and meat quality. Although the use of this low-digestibility fiber source did not yield negative effects, no significant differences were observed between trials conducted with and without OS. In a related study, Chiofalo *et al.* (2020) demonstrated that the addition of OP at concentrations of 7.5 to 15% positively influenced animal performance, enhancing meat tenderness and improving meat quality indices such as intramuscular fat content and the proportion of unsaturated FAs. Furthermore, milk produced from cows fed OP-fortified feed exhibited an increase in unsaturated FAs, including oleic acid, vaccenic acid, and conjugated linoleic acid CLA), alongside a reduction in short- and medium-chain saturated FAs. These findings suggest that OP can improve the nutritional properties of milk and cheese without compromising their sensory attributes (Chiofalo *et al.*, 2020). Vargas-Bello-Pérez *et al.* (2013) also reported that the inclusion of OP in feed improved the nutritional characteristics of sheep milk. Similarly, Chiofalo *et al.* (2004) evaluated the yield and composition of ewe's milk, noting that OP supplementation positively influenced milk yield and enhanced nutritional value by increasing the ratio of unsaturated to saturated FAs. Finally, the use of OP at 10% inclusion rate in laying hen feed was found to positively impact egg quality. Transcriptomics analysis revealed a reduction in egg cholesterol content compared to controls, likely attributable to the modulatory effects of phenolic compounds on genes involved in cholesterol biosynthesis pathways (Iannaccone *et al.*, 2019).

The valorization of OSs in food and feed formulations is associated with several limitations and challenges. A significant gap remains in the understanding of the health benefits and bioactive components of OS, necessitating further research and development in this field. While the management of OS waste presents considerable challenges, it also offers opportunities for innovation and the advancement of sustainable practices. Addressing these challenges will require concerted efforts and collaboration among producers, researchers, and policymakers.

One notable limitation is the presence of phenolic compounds in Oss, which can impart a bitter taste, thereby reducing the palatability of formulations. Additionally, the inherent hardness of OSs complicates their direct use as a ingredient in food applications. Consequently, processing techniques, such as grinding or milling, may be required to reduce OS to a finer consistency, such as flour, to facilitate their incorporation. Prior to integration into food or feed products, further processing may be necessary to achieve an appropriate texture.

Moreover, there is a lack of comprehensive studies evaluating the nutritional value, safety, and functional properties of OSs as food and feed ingredients. It is imperative to conduct rigorous research to establish evidence-based guidelines for their effective utilization. Overall, while there is potential for the valorization of OSs in food and feed formulations, overcoming these limitations will require targeted research, innovation, and collaborative efforts among stakeholders.

3 Conclusions

In conclusion, OSs represent a significant by-product generated in substantial quantities during olive oil extraction and pitted table olive production. OS possesses a lignocellulosic structure, primarily composed of hemicellulose, cellulose and lignin, which confers high fiber properties. As consumer preferences increasingly shift toward healthier foods with reduced caloric and fat content, dietary fibers have become a priority in food formulations. Beyond their high fiber content, OS contains notable amounts of fat, protein, and bioactive phenolic compounds, making them a versatile material for evaluation across various applications.

OS is characterized by a diverse range of phytochemicals, including a balanced fatty acids profile rich in MUFA, moderate levels of PUFA, and low content of SFA. Additionally, OS is abundant in sterols and tocopherols. The protein content of OS is notable for its richness in essential amino acids, especially valine and arginine, while appreciable quantities of phenolic compounds, such as tyrosol and

hydroxytyrosol, further enhance its nutritional profile. Compared to other edible nuts and seeds, OS contains a sufficient mineral composition to meet the recommended daily allowance (RDA) of macroelements (potassium, calcium, magnesium, sodium, and phosphorus) and microelements (zinc, manganese, and copper).

OS also presents a potential source of cellulose used in ethanol production and as a base material for gelling agents, emulsifiers, thickeners, and dispersing agents. Furthermore, OS-derived oligosaccharides can serve as low-calorie natural sweeteners, while bioactive peptides extracted from OS offer protective effects against oxidative stress. Considering its overall chemical profile, OS emerges are a valuable source of functional substances with potential for use in food and feed formulations. To fully harness the potential of OS, considerable efforts should be directed toward its incorporation into food and feed products, as well as the development of methods to isolate, purify, and recover the valuable chemical constituents at optimal levels. Such advancements would not only enhance the utilization of this by-product but also contribute to the development of sustainable and innovative applications in the food and feed industries.

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