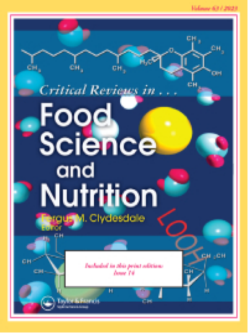

Taylor & Francis
Taylor & Francis Group

Critical Reviews in Food Science and Nutrition

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/bfsn20

In vivo evidences of the health-promoting properties of bioactive compounds obtained from olive by-products and their use as food ingredient

Fernando Rivero-Pino, Maria C. Millan-Linares, Alvaro Villanueva-Lazo, África Fernandez-Prior & Sergio Montserrat-de-la-Paz

To cite this article: Fernando Rivero-Pino, Maria C. Millan-Linares, Alvaro Villanueva-Lazo, África Fernandez-Prior & Sergio Montserrat-de-la-Paz (2023): *In vivo* evidences of the health-promoting properties of bioactive compounds obtained from olive by-products and their use as food ingredient, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2023.2203229

To link to this article: https://doi.org/10.1080/10408398.2023.2203229

CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION
https://doi.org/10.1080/10408398.2023.2203229

REVIEW

In vivo evidences of the health-promoting properties of bioactive compounds obtained from olive by-products and their use as food ingredient

Fernando Rivero-Pino[a], Maria C. Millan-Linares[a], Alvaro Villanueva-Lazo[b], África Fernandez-Prior[a] and Sergio Montserrat-de-la-Paz[a]

[a]Department of Medical Biochemistry, Molecular Biology, and Immunology, School of Medicine, University of Seville, Seville, Spain; [b]Department of Food & Health, Instituto de la Grasa, Spanish National Research Council (IG-CSIC), Seville, Spain

ABSTRACT
Olea europaea L. is the source of virgin olive oil (VOO). During its extraction, a high amount of by-products (pomace, mill wastewaters, leaves, stones, and seeds) is originated, which possess an environmental problem. If the generation of waste cannot be prevented, its economic value must be recovered and its effects on the environment and climate change must be avoided or minimized. The bioactive compounds (e.g., phenols, pectins, peptides) of these by-product fractions are being investigated as nutraceutical due to the beneficial properties it might have. In this review, the aim is to summarize the *in vivo* studies carried out in animals and humans with bioactive compounds exclusively obtained from olive by-products, aiming to demonstrate the potential health benefits these products can exert, as well as to describe its use in the food industry as bioactive ingredient. Several food matrices have been fortified with olive by-products fractions, leading to an improvement of properties. Animal and human studies suggest the benefits of ingesting olive-derived products to promote health. However, the investigation until now is scarce and consequently, well-designed human studies are required in order to fully address and confirm the safety and health-promoting properties of olive oil by-products.

KEYWORDS
By-products; bioeconomy; circular food; olive oil; immunonutrition

Introduction

Olives and health

The oil collected from the extrusion of the olive tree's (*Olea europea* L.) fruit by mechanical procedures or other physical treatments under circumstances that do not change the end product is known as virgin olive oil (VOO). VOO has a clear history of consumption in many societies, and is of special interest in Mediterranean diets. VOO, in fact, is associated with numerous beneficial properties for humans, generally associated with its phenol content (Basdeki, Salis, and Hagidimitriou 2020; Estruch et al. 2018; Gavahian et al. 2019; Millman et al. 2021; Romani et al. 2019). The health claim of olive oil polyphenols was included in the Commission Regulation (EU) No 432/2012 of 16 May 2012 establishing a list of permitted health claims made on foods, after the evaluation carried out by the European Food Safety Authority (EFSA). The claim refers to the protection of blood lipids from oxidative stress, related to olive oil that contains at least 5 mg of hydroxytyrosol (HTyr) and its derivatives (e.g., tyrosol (Tyr) and oleuropein complex) per 20 g of olive oil, with a daily intake of 20 g of olive oil.

The International Olive Council estimates that there are 11 million hectares of olive groves planted worldwide, of which 2.5 million are on Spanish soil. This assumes that the global average production of olive oil was around 3106 t/year from 2012 to 2021. 73.5% of the total is attributable to production in Europe, primarily from the top three countries that produce olive oil: Spain (41.7%), Italy (18.1%), and Greece (11.4%). According to Mordor Intelligente, the demand for olive has been growing year over year. In 2021, the import value was estimated to be USD 90.8 million, up nearly 40% from the previous year. In addition, the export value increased by nearly 26% from 2020 to USD 79.1 million. This trend is anticipated to continue during the anticipated period due to the growing demand. In fact, The olive market is anticipated to record a compound annual growth rate of 4.5% over the forecast period.

The steps for the production of VOO are: washing, milling, malaxation, centrifugation, decantation, and filtration. Depending on how the oil is separated in the final stages of the continuous process, a distinction can be made between two- or three-phase extraction systems, Figure 1).

Table olives have a significant nutritional value due to their abundance in monounsaturated fatty acids (MUFAs), particularly oleic acid, fiber, and vitamin E, as well as the presence of a number of phytochemicals. Among the components found, exclusively found in Oleaceae plants,

CONTACT Maria C. Millan-Linares mmlinares@us.es

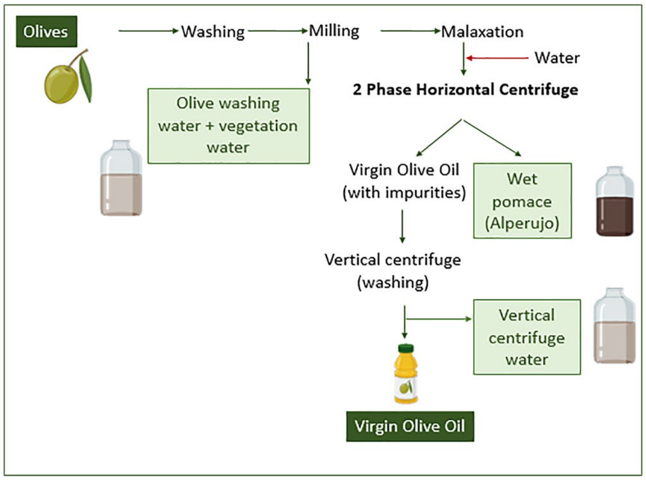

Figure 1. Scheme of the process for obtaining VOO by horizontal centrifugation of 2 phases and the by-products generated: "*alperujo*" and wastewater (olive washing water, vegetation water, and production process water).

including *Olea europaea* L, secoiridoids are a group of compounds that has gained the interest from scientists due to their structure and potential health-promoting properties. During the table olive processing these compounds are transformed into their constituents like alcoholic phenolics and elenolic acid derivatives. Secoiridoids are accumulated mainly during fruit ripening, and their biosynthesis and biotransformation depends on agronomic and processing factors (El Riachy et al. 2011). Among the phenolic compounds, the most prevalent substance is HTyr, which is found in all varieties of table olives. Studies on table olives conducted *in vitro*, with animals and/or on humans are few (Rocha, Borges, and Pinho 2020).

The relevant significance of nutrition in the prevention of non-communicable chronic diseases, where the immune-inflammatory response plays a crucial role, has been highlighted by the World Health Organization (WHO). As people live longer in society, age-related degenerative disorders like Alzheimer's disease (AD), Parkinson's disease, cancer, stroke, and osteoporosis are becoming more common. In this context, the promotion of diets in which health-promoting ingredients are an important player are becoming more relevant, in order to prevent or pre-treat the development of diseases in humans. Olives and products thereof are a promising food to tackle this, based on the unique composition they have. Natural resource consumption has significantly increased in recent decades as a result of the rising worldwide demand for resources, which is mostly attributable to the expanding global population and the development of emerging economies. A food system simply based on a linear economy is not sustainable in the mid and long-term to sustain our current way of life. The circular economy signifies a paradigm shift in how we use resources naturally and, consequently, how we interact with the environment. It plays an important role in achieving an efficient use of resources, promoting that materials and products remain in the economy for as long as possible and in which the generation of waste is minimized. In this sense, the use of the currently considered "by-products" obtained after olive oil extraction has been recently appointed as another interesting product derives from olives.

Olive oil extraction – by-products generation

During the harvesting, the first olive by-product that can be recuperated would be the olive leaves. In fact, the leaves can represent around 10% in weight of the raw material arriving at the mill (Nunes et al. 2016). Several compounds such as hydroxybenzoic acid, cinnamic acid, HTyr, elenolic acid, chlorogenic acid, verbacoside or tocopherols have been detected in leaves, depending on the extraction method employed, but mostly being oleuropein (a coumarin-like compounds classified as secoiridoids) the major one. However, even when it has been reported that the most abundant component of all the constituents of olive leaf extract is oleuropein (ranging from 17 to 23%), this compound can lead to the formation of HTyr during fruit maturation and olive oil production, achieving a variable amount depending on the conditions (Sorrenti et al. 2023). On the other hand, the wood shell (stone) and the seed make up the entire olive fruit stone. While the full stone is sourced from the olive table industries, the stone is recovered through the filtration of solid waste during the production of olive oil (Nunes et al. 2016).

In the case of olive oil extraction using a three-phase system, a horizontal centrifuge allows the separation of the paste into oil, pomace (ca. 35–40 kg/100 kg of olive, solid) and "*alpechín*" (= mill wastewater, liquid, vegetable water plus the added water during the malaxation). This olive mill wastewater (OMWW) has been evaluated after being subjected to different processes such as fermentation, spray drying, and encapsulation (Nunes et al. 2016) and represent one of the most interesting fractions of the by-products, based on the interesting composition enriched in bioactive compounds. Bioactive compounds can be defined as nutrients and non-nutrients components of which a food matrix is composed that can exert physiological effects beyond their classical nutritional properties, thus, implying a health benefit in the organism (Cazarin et al. 2022)

The two-phase extraction results in oil and *"alperujo"* (wet, semi-solid pomace). The *"alperujo"* obtained by the two-phase extraction method (700-800 kg/t olive) presents a variable humidity over 62%. It is a rich source of polysaccharides, proteins, fatty acids, pigments, and polyphenols. The recovery of compounds of interest such as proteins, pectins, or polyphenols supposes an added value of this by-product (Abbattista et al. 2021).

The alpechín is the liquid waste in the olive washing machines (Figure 1). This mixture coming from the different effluents of the process is generally characterized by having an intense dark purple/brown to black color with a specific olive odor. This residue has a high organic content, with high levels of suspended solids, slightly acidic or basic, and *per se*, electrical conductivity. In addition, OMWW is known to contain tannins, lignins, long-chain fatty acids, reduced sugars, phenolic compounds, that at high concentrations can be toxic to microorganisms and plants (Gerasopoulos et al. 2015), as well as proteins.

During the virgin olive oil production process, 98% of bioactive compounds such as phenols, proteins, and pectins pass into the *"alperujo"*, leaving only 2% of its total weight in the oil. These compounds have innumerable biological properties acting as antioxidants, anti-inflammatories, antibacterials, and anticancer agents, among others. However, at high concentrations, they are considered phytotoxic agents that, if spilled, can generate true natural disasters. In this sense, the recovery, isolation, and purification of products with high added value is a natural, economical, and ecological alternative that would be of great help to the olive oil sector (Tufariello et al. 2019).

According to Directive 2010/75/EU and its transposition through Law 5/2013, it is determined that olive oil wastewater is considered one of the most polluting residues from the food industry. The efforts of olive oil producers focus on reducing its production or on the minimization or revaluation of its organic load. At the same time, a low-quality diet contributes to the development of highly prevalent diseases such as obesity or cardiovascular or neurodegenerative diseases. Due to all of these factors, the agri-food business is actively searching for technologies that may effectively recover revalued components from generated by-products while remaining technically and economically feasible, in order to include them too in the food system as bioactive ingredient.

Based on this context, the presence of compounds such as phenols, proteins, and pectins in by-products of the olive industry, *"alperujo"* and wastewater, can be revalued as functional food ingredients potentially applied to modulate several physiological processes of human beings, in order to prevent or pre-treat diseases. In addition to these major compounds, there are also reports of the presence of tocopherols, phytosterols, triterpenoids, among others (Otero et al. 2021). In light of the background described, it is clear that one of the greatest problems in olive growing is the excess of by-products during the olive oil production process. With an olive fruit production between 17 and 22 million tons, the olive sector produces an amount of *"alperujo"* located between 13 and 18 million tons and close to 1 million m^3 of wastewater worldwide, according to a study made for the National Association of Orujo Oil Companies.

Concerning these bioactive compounds and their presence in the olive by-products, Exposito-Diaz (2022) quantified the amount of phenols and terpenic acids from olive pomace from 43 international olive cultivars during three consecutive seasons, in order to evaluate the influence of genetic and interannual factors on bioactive compounds of olive pomace. According to the authors, cultivar was confirmed as the most determinant factor modulating the relative concentration of these compounds. Currently available published literature using *in vitro* and cell culture suggests beneficial activity of olive by-products. However, the limited scope of these studies makes them not be enough as confirmation to establish health-promoting properties, thus, are not in the scope of this review. For instance, olives can lessen oxidative stress and the inflammatory response in activated microglia, which are cells of the central nervous system's innate immune system (Martin et al. 2019; Millan-Linares, Montserrat-de la Paz, and Martin 2021; Toscano et al. 2019). On the other hand, bioactive peptides can exert antioxidant and/or antihypertensive activity (Esteve, Marina, and García 2015). In addition, *in vitro* studies have also shown the effects of olive paste in human microbiota, reporting an increase of *Lactobacillaceae* and *Bifidobacteriaceae* populations after nine-day administration (Giuliani et al. 2019), among other reports. The scope of this review focuses on the *in vivo* evidences, described in the section 4, since they provide more biologically relevant outcomes.

The history of use of olives provides an argument for safety of the by-products, considering that these by-products of the Virgin Olive Oil (VOO) extraction are being consumed when eating olives, though the yield of the extraction and the concentration of these compounds have to be taking into consideration if these by-products are used to fortify commodities or are used to produce food supplements.

Olive by-products main constituents

Proteins

Proteins are one of the macronutrients widely found in food. Regarding olives, the fruit, particularly the seed, contains the most well-characterized olive proteins, including several oleosins and storage proteins, and contains all the essential amino acids (Nunes et al. 2016). During the process of extracting olive oil, some of these proteins can be transported from the fruit to the oil (Montealegre et al. 2014). The presence of proteins in the by-products derived from VOO extraction is not widely reported in literature (Chebaibi et al. 2019). Sar et al. (2020) reported a methodology to recover the protein from OMWW through bioconversion with edible filamentous fungi (*Aspergillus oryzae, Neurospora intermedia,* and *Rhizopus delemar*). These authors describe the optimization of the process, up to obtaining a biomass protein content of 29.5%. Recently, low molecular weight peptides in VOO have been identified and the sequences VCGEAFGKA, NALLCSNS, CPANGFY, CCYSVY, and DCHYFL were proposed as highly bioactive by *in vitro* assays (Lopez-Huertas and Alcaide-Hidalgo 2022) and RDGGYCC and CCGNAVPQ in animal studies, showing the antihypertensive activity in spontaneously hypertensive rats (Alcaide-Hidalgo et al. 2020). Further research concerning isolation and purification of olive proteins, in order to fully exploit their potential is needed, since the literature published until now is scarce but promising. Proteins and bioactive peptides are one of the most relevant research fields in the food industry, and it could be a promising way to revalorize olive by-products.

Pectin

Pectin is a carbohydrate found in fruits (main structural component of plant cell walls) - complex heteropolysaccharides, which include at least 17 kinds of monosaccharides and over 20 types of linkage (Millan-Linares, Montserrat-de la Paz, and Martin 2021). In the wet olive pomace, it represents circa 39% - depending on the cultivar, maturation stage, and other parameters - with a degree of methylesterification and acetylation of 48 and 11%, respectively. Research on the bioactivity of this fraction is not as extended as the phenols, but some studies have reported the *in vitro* bile acid and glucose binding (Rubio-Senent et al. 2015). On top of that, pectin might have a role in reducing the development of liver injury in nonalcoholic fatty liver disease and alcoholic liver disease through its impact on gut bacteria (Hu, Cassard, and Ciocan 2023), and cancer preventive potential (Bermudez-Oria et al. 2019; Emran et al. 2022). However, specific research will olive pectin is lacking, especially with *in vivo* models, and opens new opportunities and lines of research for the near future.

Phenols

Phenols are a class of organic compounds containing a hydroxyl group and a benzene ring. Based on the content and unique composition of olives in terms of phenols, they represent one of the most interesting fractions of this foodstuff. Rigacci and Stefani (2016) reviewed the literature available on olive oil polyphenols, including culture cells analyses, as well as animal and human studies. The main conclusions were that i) these compounds act as signaling molecules, ii) participate to the redox balance of the cell iii) can interact with other molecules through different types of links.

The fate of phenols in human body is still unclear, since the relatively low amount of these components in the oil makes it hard to detect or to follow the metabolic route they might follow. However, it has been reported that these phenols can be found in tissues, including the brain, further supporting their ability to exert a biological relevant change in human physiological status (Rigacci and Stefani 2016). Fernandez-Prior et al. (2022) reported the stability of the main phenolic components from *"alperujo"* (i.e., HTyr, HTyr-4-β-D-glucoside, 3,4-dihydroxyphenylglycol, and Tyr), over two seasons and in ten oil mills under similar climatological and agronomic conditions. Authors reported that HTyr and Tyr increase, whereas 3,4-dihydroxyphenylglycol decreases only in the last month of the season and HTyr-4-β-D-glucoside decreases drastically from the beginning. This is important when recovering phenols, since their content is highly variable over the time (e.g., if the aim is to recover HTyr-4-β-D-glucoside, it should better be done at the beginning), it is, depending on the type of phenolics sought, one time of the season is more interesting than another.

Several authors have reported the effect of individual phenols in cell culture, evaluating different parameters such as mRNA expression of numerous catabolic and pro-inflammatory factors and pathways mediators (Núñez-Carro et al. 2022; Rivero-Pino et al. 2023; Scotece et al. 2018). The individual bioactivity of each phenol in different metabolic ways and the synergy that can occur among them is still to be unraveled, but the results published until now indicates that they represent a relevant fraction of olive and products thereof in terms of bioactivity.

Use of olive by-products as ingredient

One research field gaining relevance in these recents years is the fortification of oils with phenolic extract from olive by-products, as means to revalue this fraction and improve the quality and properties of (usually low-quality) oils. For instance, Romeo et al. (2020) fortified a sunflower oil with a phenolic extract obtained from OMWW derived from the extraction of olive oil from Ottobratica cultivar olive. The product manufactured exhibited an enhancement of oxidative stability of 50% in the fortified oil during a 90-day storage study, reported by differences in physicochemical and antioxidant parameters. The total polyphenol content was reported to be 788.96 mg/100 mL.

Overall, literature shows that the addition of polyphenols recovered from olive by-products into olive oil, extends its shelf life without changing its organoleptic properties (Athanasiadis et al. 2023; Sanchez De Medina et al. 2012; Vidal et al. 2022). However, it has been reported that specific phenols (e.g., oleuropein aglycone and ligstroside aglycone isomers) tend to provide more oxidative stability to olive oils, compared to samples with the same amount but different phenols in their composition (Miho et al. 2020). It has been reported that storage, even in darkness, at room temperature, led to a decrease of the phenol content (average decrease of 42% in 160 VOO samples evaluated). This decrease is shown to depend on the phenol profile at the initial stage (Castillo-Luna et al. 2021). However, a minimum decrease of the secoiridoids content was reported at 4 and −18 °C during 3 years by Mousavi et al. (2021).

On top of that, there are also studies reporting the use of olive by-products in other products. Alvarez et al. (2022) evaluated how olive-pomace oil could modify the physic-chemical (rheology and texture) properties of puff pastry margarines, as well as the microstructure and fatty acid profile, aiming to develop a healthier product. Differences compared to the control (without olive-pomace oil) were found in viscosity, plasticity, crystallization and melting temperature, having a healthier fatty acids profile as well, indicating the technological viability of the ingredient to be incorporated in this food matrix. In terms of bakery products, recently the fortification of biscuits has been reported (Trindade et al. 2023), by substituting wheat flour with olive pomace flour (0 to 20%). Based on the parameters assessed (chemical composition, lipid profile, and sensory aspects), authors indicated that substitution of 10% was adequate as it showed an increase of the contents of lipids, minerals, and dietary fiber, without highly affecting the sensorial properties. Similarly, Giannoutsos et al. (2023) aimed to replace wheat flour with olive stone flour (10 and 30%) in crackers, and to evaluate the effect on texture, color, moisture and sensory aspects. The main changes reported by the authors were a sensorial perception of the cracker negatively affected by the addition of the test item, as well as, a softer product, with reduction in hardness and toughness, when the addition was of 30% of the sample. These results are relevant in the understanding of which products would be accepted by the consumers.

Foti et al. (2023) aimed to produce a functional beverage by subjecting OMWW to fermentation with strains belonging to *Lactiplantibacillus plantarum, Candida boidinii,* and *Wickerhamomyces anomalus*. This processing led to an increase of phenol and organic acid contents, and also higher *in vitro* antioxidant and antiinflammatory activity. Fermentation is a widely used processing technique in the food industry that shows also a promising potential to increase the value of olive by-products to be used as bioactive ingredients. In addition, the potential protective effect of phenolics from olive by-products in the Maillard reaction has been investigated in dairy products. By incorporating phenols from OMWW in raw milk before ultra-pasteurization, an inhibition of the formation of off-flavour compounds during the heat treatment, improving both the nutritional and sensorial properties was reported (Troise et al. 2014).

On the other hand, there are reports on the antioxidant and antimicrobial capacity of phenols from olive by-products to increase the shelf life of meat products, especially those obtained mechanically such as sausages or hamburger. For instance, a phenolic extract effectively reduced primary and secondary lipid oxidation, as well as cholesterol oxidation products, during the shelf life of raw hamburgers (87.5 and 175 mg of phenols/kg meat, of which 61.5% was oleacein, 20.6% HTyr, 13.2% verbascoside, and 4.7% Tyr). The sensory analysis also confirmed the efficacy of the phenolic extract addition, which resulted in a positive effect on the red color intensity (raw product) and thus reduced browning during storage (Barbieri et al. 2021). In the case of sausages, the modulation of lipolysis and the reduction of microbial targets (*Listeria monocytogenes* and *Staphylococcus aureus*) in a naturally contaminated product suggests that the phenolics can contribute to improve food safety and quality (Fasolato et al. 2016). In the same line, it has been reported that an extract enriched in phenols obtained from olive vegetation water, when added to fresh pork sausages (0.075 and 0.15 g/100 g) had a positive impact during a two weeks aerobic storage at refrigerated temperature, related to a decrease in diacylglycerols, peroxide value, thiobarbituric acid reactive species and cholesterol oxidation products. Regarding the sensorial properties, these products were not considered disagreeable to the panelists, although differences were reported (Balzan et al. 2017).

Similarly, Miraglia et al. (2021) demonstrated the efficacy of olive by-products for shrimps, both hygienically and in terms of quality. Specifically, the phenolic extracts demonstrated bactericidal and antioxidant activity, as well as a pleasant taste (proportional to phenolic compound concentration, major compound being dialdehydic form of elenolic acid linked to 3,4-dihydroxyphenylethanol), without altering the sensory characteristics of enriched shrimps. The authors also reported that sulfites, when combined with extract, appear to protect phenolic compounds from oxidation during cooking and storage, despite being the least effective in antibacterial action. These authors also reported the efficacy of these phenolic extracts (2 g/L of phenols) from OMWW, during eight days. These bioactive compounds led to a reduction in bacterial counts, without implying variations in the color of the shrimps (Miraglia et al. 2020). The incorporation of olive by-products in other food matrices has been also proved to exert positive effects at nutritional level without implying a negative sensory shift, including mayonnaise (Menchetti et al. 2020) or tomato sauce (Taticchi, Bartocci, et al. 2017).

Finally, the heat treatment might also affect the content of phenols, as reported by Cicerale et al. (2009), who subjected an oil to temperatures ranging from 100 to 240 °C and time periods up to 90 min, leading to a decrease on phenols content. Similarly, Cecchi et al. (2023) evaluated how industrial drying process would have an impact of the composition (i.e., phenols and polysaccharides) of olive pomace. This by-product was subjected to freeze-drying and oven dried at 50–110 °C, at lab and industrial scale. According to the authors oven drying resulted in a decrease of the phenolic content and negatively influenced the sensory properties, whereas the application of industrial drying (150 °C, in an atomizer) led to a powder similar to the one obtained in the freeze-drying process, avoiding browning and off-flavour developing.

The literature published until now concerning the use of olive by-products as food ingredient, including its processing, is not widely extended. The temperature and pH stability of the components, the potential interactions with the food matrix in which is incorporated as well as storage studies in the long term, are still missing. However, the studies published until know provide evidence of their potential to be used, once their safety is demonstrated under human consumption conditions.

In vivo health-promoting effects exerted by olive by-products

Animal studies

Research is currently trying to elucidate the mechanisms by which olive oil by-products components can modulate different physiological processes in humans, exerting a beneficial effect. The main limitation when comparing studies is the differences in composition among test items employed in the published literature since, as indicated previously, there are many factors determining the content of components that will be retained in the olive by-products, from the cultivar to the extraction method.

Animal studies, usually carried out with rodents, are useful to unravel the mechanisms by which different components have an impact on the physiology of living organisms. In addition to that, there are also performance studies in which poultry are feed with specific substances intended to be used as additives in their diets (El-Damrawy, Khalifah, and Fares 2013) or studies evaluating how the inclusion of olive cake in animals' diet affect the properties of the products derived from them (Leite et al. 2023; Branciari et al. 2017; Taticchi, Bartocci, et al. 2017). In terms of performance studies, for instance, De-Cara et al. (2023) reported how an olive leaf and grape-based by-product supplementation (2 g/kg, for 40 days) modified productive performance, antioxidant status and meat quality in broilers. Among other changes, authors reported an increase in the plasma superoxide dismutase (SOD), which is related to protective effects against oxidative stress, and potentially have an effect on the immune response (Lee, Lin, and Lee 2019).

Haloui et al. (2011) evaluated the potential analgesic and antiinflammatory properties of two extracts obtained from olive leaves (cv. Chemlali) enriched in polyphenols, oleuropein, and Htyr. Doses of 100, 200, and 300 mg/kg led to inhibition of writhing and reduced carrageenan-induced hind paw edema, being Htyr more effective. This report also indicates that a dose of 1000 mg/kg (intraperitoneal injection) in an acute toxicity test did not led to any adverse effects.

The effects of polyphenols from VOO in managing inflammation and the diseases that can result from that, including neurological illneses such as Alzheimer diseases have been widely studied *in vitro* (Millman et al. 2021; Rivero-Pino et al. 2023). *In vivo* studies carried out with transgenic TgCRND8 (Tg) mice, orally supplemented in a low fat diet (during eight weeks) a mix of polyphenols from OMWW (50 mg/kg) also gave promising results. According to the authors, there was an improvement in cognitive functions, and the A$ß_{42}$ and pE-3Aß plaque area and number decreased both in the cortex and hippocampus by the mix of polyphenols (Pantano et al. 2017). These results *in vivo* suggest the potential of olive phenols in managing neuropathologies and open the way to new strategies to prevent these diseases.

Also recently, the first study on the promotion of abdominal pain relief (related to inflammatory bowel diseases and irritable bowel syndrome) in a dinitrobenzene sulfonic acid-induced colitis rat model using two derived by-products was described. The oral administration of 300 mg/kg of OMWW retentate and dry olive pomace during 14 days led to a reduction of the abdominal viscero-motor response to colon-rectal distension at 3 mL of balloon distension volume, as well as a reduction of intestinal damage (evaluated at macro and microscopic level) (Parisio et al. 2020), suggesting the potential protective role of the by-products as inflammatory modulators in the intestine.

The hypocholesterolmic effect of aqueous and ethanol extracts from leaves (200 mg/kg), by measuring serum total cholesterol, triacylglycerides, high-density lipoproteins (HDL), low-density lipoproteins (LDL), and very low-density lipoproteins (VLDL) in mice has been also described. A reduction of the parameters was reported in the mice treated with the test items, except for HDL, which increased. The study support the potential properties of olive leaves extracts to prevent hypercholesterolemia and atherosclerosis (Cheurfa et al. 2019). The effect on glucose homeostatis has also been evaluated in normal, streptozotocin diabetic, and sand rats (Wainstein et al. 2012). As indicated by the authors, the animals showed reduced starch digestion and absorption following the treatment with the olive leaf extract in both the mucosal and serosal sides of the intestine. These authors also carried out a human study that it is described in the following section, supporting these results. Similarly, Hadrich et al. (2023) also evaluated the anti-insulin resistant effect of olive (cv. Chemlali) leaves ethanol-extracts on 3T3-L1 Cell (showing a reduction of triacylglyceride accumulation, apoptosis of preadipocytes cells, and a reduction of the mRNA levels of adipogenesis and proinflammatory genes) as well as the effect of oleuropein in high-fat diet fed rats. The oral adminiistration (50 mg/kg) for 8 wk resulted in a reduction of the inflammation in liver and adipose tissues, improved glucose intolerance, and decreased hyperinsulinemia.

On the other hand, it has also been shown how olive leaf extract may be used as adjuvant to mitigate disorders related to a reduction of the calcium sensing receptor expression as well as signaling and affecting renal water permeability. Using adult male Wistar rats, it was reported an impairment of vasopressin-induced aquaporin-2 trafficking through the activation of the calcium-sensing receptor (Ranieri et al. 2021) by the olive (cv. Coraline) leaf extract. This is related to several diseases inlcuding hypertension and inappropriate antidiuretic hormone secretion.

Durante et al. (2020) extracted oil from the patè olive cake and tested it in animals as health-promoting agent. The authors reported that, in the optimal parameters, there was an increased amount of phytosterols, tocopherols, and squalene. The intake of 20–40 μL/die by BALB/c mice did not modify the redox homeostasis of liver, but implied a decrease of the amount of triacyglycerides, as well as an improvement of NAD(P)H:Quinone oxidoreductase 1, carnitinepalmitoyl-CoA transferase, and mitochondrial respiratory complexes activity, indicating a positive response in liver fatty acid oxidation.

For its part, Contreras et al. (2022) employed two extract (aqueous and aqueous/acetonic) from olive pomace, obtained by hydrothermal and ultrasound-assisted extraction, respectively. The analyses revealed that major compounds were HTyr and mannitol. For the animal study, rats received the extracts (50 mg/kg bw) by gavage during 8 wk, without or with also being administrarted with CCl_4 to prompt hepatoxicity. Results following the treatment with the test items showed preventive effects against DNA damage and a counteraction of hepatic biomarker levels and the lipids profile, to basal levels. In addition, antioxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase) content increased, and oxidate stress in the liver tissue was decreased.

As it can be seen from this revision of the literature, the number of animal studies where the test item is derived from olive by-products is scarce at this moment. However, there are several reports evaluating the effect of VOO (usually enriched in polyphenols) or polyphenol extract is more common (Karampetsou et al. 2022; Montoya et al. 2021). The most studied by-product are olive leaves (extracts), and it shows the high potential that this raw material can have. On top of that, studies with wastewaters or seeds are also needed in order to confirm and elucidate their potential to be used as nutraceuticals.

Human studies

Human studies are required in order to establish a correlation between the consumption of bioactive compounds to health-promoting properties. Most of the studies published in literature focus on the intake of VOO, and not in the by-products obtained after the extraction of the oil.

In a randomized, placebo-controlled, crossover trial, de Bock et al. (2013) demonstrated the effect of polyphenols from olive leaves on glucose homeostasis in humans. During 12 wk, overweight men (*n* = 47, average age 46 years old) were orally supplemented with capsules containing the equivalent to intake per day 51.1 mg oleuropein and 9.7 mg HTyr per day). The purpose of the study was to evaluate insulin action and cardiovascular disease risk factors. According to the authors, an improvement in insulin sensitivity (ca. 15%) and pancreatic beta-cell responsiveness (28%) was found in the group supplemented with the capsules, while no effects were found for the interleukin-8, TNF-α, lipid profile, ambulatory blood pressure, body composition, carotid intima-media thickness, or liver function. These results were in line at some extent with the study of Crespo et al. (2015) in healthy volunteers (*n* = 22, between 20 and 40 years old). In this investigation, the supplementation with OMWW extract (5 and 25 mg of HTyr/day for 1 week) had no appreciable effects on blood lipid parameters or inflammation markers. Recently, Meireles et al. (2023) reported that the acute intake of olive leaf tea (dose of 250 mL) on postprandial glycemia in healthy volunteers when ingested with a high-carbohydrate meal (double-blind, randomized, placebo-controlled, and crossover design trial, *n* = 18) receiving a high-rich carbohydrate meal did not have an impact on the glycemic curve. However, long-term studies are required in order to clearly see potential health benefits in terms of glucose metabolism, as reported by Wainstein et al. (2012). These authors evaluated whether the ingestion of olive leaf extract daily (during 14 wk) in tablet (dose of 0.5 g) could have an impact on glucose homeostasis in adults (*n* = 79) with type 2 diabetes (T2DM). A decrease in HbA1c (related to glucose levels in bloods) and fasting plasma insulin levels was reported, but no effects were observed in postprandial plasma insulin levels, suggesting an effect in glucose homeostasis.

Lopez-Huertas and Fonolla (2017) isolated HTyr (purity 99.5%) from or OMWW, to evaluate the safety and the effects on volunteers suffering from mild hyperlipidemia (*n* = 14, average of 34 years old). During eight weeks, a daily dose of 45 mg (equivalent to 10–15 olives from variety rich in polyphenol) was administered to the subjects, and markers of cardiovascular disease risk, enzyme markers of several clinical conditions, hematology, antioxidant parameters, vitamins, and minerals were measured at the half and at the end of the treatment. Most of the parameters assesed did not change as consequence of the treatment, indicating safety of the intake of this dose of the test item. A decrease in ferritin, serum folate, and red blood cell folate levels were reported, as well as an increase of vitamin, though authors indicate that the limitation of the study was the lack of a control group consuming a placebo.

The effect of an olive leaf extract on vascular function and inflammation in a postprandial setting by means of a randomized, double-blind, placebo-controlled, crossover intervention trial. An acute intervention with healthy volunteers (*n* = 18, 18 to 40 years old) was carried out, who orally ingested the extract (containing 51 mg oleuropein and 10 mg of HTyr, as major components). According to the authors, digital volume pulse-stiffness index and *ex vivo* IL-8 production significantly decreased in the group ingesting the test item, who also excreted several phenols in the urine after 8–24 h. The study suggest that oleuropein from leaves positively regulates vascular health in humans (Lockyer et al. 2015). Lockyer et al. (2017) evaluated the impact of a phenolic-rich olive leaf extract on blood pressure, plasma lipids, and inflammatory markers. The study was double-blind, randomized, controlled, crossover trial. Patients with pre-hypertension (*n* = 60, average of 45 years old) consumed the supplement (dose of 10 mL) twice per day (6 wk), including a 4-week wash out period. The major phenolic in the supplement was oleuropein. Authors reported decrease in daytime and 24-h systolic and distolic blood pressure, as well as a decrease in plasma total cholesterol, LDL cholesterol, triacylglycerides, and in IL-8. The treatment did not have an effect on other markers inflammation, vascular function, and glucose metabolism such as TNF-α, IL-10, or C-reactive protein, pulse wave velocity and insulin or glucose. These results suggest that oleuropein might be useful as hypotensive and lipid-lowering agent. Regarding hypolipidemic and hypertensive effects, it has been also reported that an olive leaf extract was as effective as captopril (antihypertensive agent) in a double-blind, randomized, parallel and active-controlled clinical study (patients with stage-1 hypertension). It means that, after the treatment period of 8 wk, ingesting 500 mg/twice/daily of the extract, a reduction in systolic and dyastolic blood pressure was reported. In addition, the levels of triacylglycerides decreased in the group orally administred with the extract, supporting the beneficial effects at different endpoints of olive by-products components (Susalit et al. 2011).

Dinu et al. (2021) reported the effects of an olive by-product on cardiovascular risk factors. For that purpose, they carried out a crossover trial (*n* = 19, average of 38 years old), where participants ingested 4 tables/day during 2 months, followed a 2 month of washout and finally, 2 months of crossed over treatment. The dose contained 30 mg of HTyr/day. These authors reported that leukocyte response to exogenous oxidative stress decreased by almost 13% and levels of the antioxidant transcription factor Nrf-2 increased ca. 90%. In addition to this, plasma levels of the pro-inflammatory protein MCP-1 (one of the key chemokines that regulate migration and infiltration of monocytes/macrophages) were significantly reduced. Monocyte migration across the vascular endothelium from the blood stream is necessary for both normal immunological monitoring of tissues and in response to inflammation. These results suggest that olive by-products can have an impact on the immune system of humans. Similarly, Gonzalez-Ramila et al. (2022) aimed to unravel how dietary consumption of olive-pomace oil would have an impact on cardiovascular disease risk factors (endpoints: blood lipids, blood pressure, inflammation, and endothelial function). For this purpose, a randomized, controlled, blind, crossover intervention in healthy and hypercholesterolemic subjects ($n = 72$, average of 35 and 44 years old) was carried out. During 4 wk, volunteers consumed 45 g of the olive-pomace oil, or a control consisting of high-oleic acid sunflower oil. The participants ingesting the test item showerd a decrease in LDL-cholesterol and apolipoprotein B serum concentrations, and LDL/HDL ratio, whereas blood pressure, peripheral artery tonometry, endothelial function, and inflammation biomarkers were not affected. These results support the results obtained in previous reports on the hypolipidemic actions in subjects at cardiovascular disease risk as consequence of olive by-products consumption, contributing to cardiovascular disease prevention. In a similar study by these authors, they reported that the consumption of this olive-pomace oil also had an effect on eNOS levels, as well as a decrease in visceral fat in both groups and an increment of leptin in the hypercholesterolemic group (González-Rámila, Sarriá, Seguido, García-Cordero, Bravo-Clemente, et al. 2022).

The number of clinical trials aiming to demonstrate health benefits of bioactive compounds from olive by-products is limited, hampering to draw conclusions on the effect on the different organs and physiological processes. It is worth noting the limitation of these test items not being comparable among studies and within the same production unit, considering all the agronomical factors determining the composition of the olives and therefore, the by-products. In spite of that, the existing data offers evidence for the role of olive by-products compounds as a promising health-promoting ingredient. However, standardization and assessment with isolated compounds in order to unravel the contribution of each molecule is needed. On top of that, there are several reports evaluating the effect of VOO (usually enriched in polyphenols), isolated phenols or tea derived from leaves (Agrawal et al. 2017; Colica et al. 2017; Noce et al. 2021; Patti et al. 2020; Rojas Gil et al. 2022; Romani et al. 2020), which are also useful in the benefit assessment of olive by-products potential if the composition is comparable. However, usually some of these reports present limitations, such as the sample size, protocol, or duration.

Challenges and future perspectives

Olive by-products contain numerous bioactive molecules, including phenols that have shown to exert pleiotropic effects in both *in vitro* and *in vivo* studies (Centrone et al. 2021). At the same time, one of the most efficient and affordable strategies to lessen the burden of many diseases and their risk factors is through sufficient nutrition, especially during prolonged treatments. The employment of these by-products as bioactive ingredients is described in this review, highlighting their importance in preventing diseases and promoting a circular economy. However, there are still many challenges to be addressed.

There is a growing interest in the food industry to isolate polyphenols in order to formulate products enriched with these compounds and/or to have a sufficient amount to carry out animal or human studies to demonstrate health benefits in well-designed studies. However, this is still a challenge since the technologies to extract specific components at pilot scale are not widely developed. In addition, the characterization of cultivars and the development cross-breeding programmes, combined with genetic characterization, in order to unravel and discover unexplored genes related to the synthesis of specific bioactive compound could be useful to continue with the research, to increase the content of specific phenols. Another factor to consider is that recovering by-products in the food industry may necessitate the implementation of new procedures and equipment, which may be impractically expensive for small businesses. It appears necessary to encourage inter-sectoral dialogue and collaboration in order to raise awareness of the applications of by-products in various fields. In addition, while the development of high-value and innovative products from olive by-products is an urgent goal in many agri-food strategies, policy implementation is primarily focused on the application of biomass or materials to bioenergy (Otero et al. 2021). In terms of scaling up the process, studies on how much is the effective cost of the technology to recover the bioactive compounds are also required, to minimize them and to optimize the process in the most sustainable way.

In terms of fortifying products, some technological challenges have been identified such as the dispersability and stability of phenols in the matrix meant to be incorporated in, or the bitterness that the product might develop based on the high amount of phenols. On top of that, human bioavailability studies revealed that, while the absorption of olive oil phenols is approximately 55-66%, the final phenol content might be too low to produce beneficial effects. To face this latter challenge, encapsulation is currently seen as an alternative in the food industry to overcome these problems. Recently, Paulo, Tavares, and Santos (2022) evaluated the *in vitro* digestion, bioaccessibility, and release kinetics studies of encapsulated (in ethylcellulose microparticles by 5 and 1o % W/O/W double emulsion) bioactive compounds obtained from olive mill pomace. The encapsulation efficiency was found to be higher than 83%, containing mostly phenols, and were protected during salivary and gastric processing, and then released in the simulated intestinal tract. This shows that when extraction and encapsulation techniques are used together, bioactive compounds may be effectively protected and delivered to a specific site—the intestinal lumen—for potential uptake by intestinal epithelial cells. The bioavailability of bioactive compounds was improved compared to their respective non-encapsulated patterns. Similarly, Bellumori et al. (2021) reported the coating (with maltodextrin DE19, octenyl succinic anhydride, starch and sunflower lecithin) of olive pomace (pate) by fluid bed coating, in order to evaluate the phenolic profile during storage and after *in vitro* gastrointestinal digestion. The storage test was carried out at 40 °C and 75% (accelerated conditions) during 75 days, no decrease of the phenol content was reported at the end, though free Tyr and HTyr content was higher because of hydrolytic reaction of secoiridoids. On the other hand, the simulated digestion released 15% of the phenols. These results suggest that the coating process was adequate at least to improve the storage stability of phenols. However, further research could be done employing other coating materials, to improve the bioaccessibility, as in this case there was a decrease of this parameter.

In terms of bioactivity, as seen in the reported results, in some cases, there seem to be contradictory results in terms of which parameters are modified. In fact, these differences are likely due to the differences in the study design, the administered doses of test items, the type of animal (race, model, wild-type, or transgenic) and mostly because of the composition and concentration of the components in the by-products. The identification of the effect of every single compound, as well as the synergy among them is key to fully understand why olive by-products compounds are health-promoting for humans, the requirements of the products and the target population. It should be noted that, depending on the analysis (e.g., bioavailability analysis), some limitations can hinder the proper analysis due to the artifact derivatives and metabolites that can be formed.

The positive impacts' underlying molecular signals are still unknown (Centrone et al. 2021). Furthermore, it must be taken into consideration that most of the aforementioned studies do not necessarily explore how these potential bioactive compounds might interact with a regular diet, and other macro and micronutrients, where numerous macronutrients and micronutrients are involved in the digestion process. Further research should be conducted in order to explore how this compound is metabolized under normal conditions in humans, and the physiological effects derived from it.

In terms of commodity to be sold, labeling exact phenol content in ingredients derived from by-products can be a problem considering the difficulty to accurately determine the amount of bioactive compounds over long-term for a brand since these levels can change by time based on storage conditions, added to the unfamiliarity of the consumer with phenols properties. One other challenge that has to be addressed is to understand if the consumer would accept, in terms of sensorial properties, these functional foods, considering the bitterness and odors associated to the phenols mostly. In this sense, for instance, it has been reported that, besides encapsulation as already described, the addition of proteins such as egg yolk or whey can suppresses both bitterness and pungency (Peyrot Des Gachons et al. 2021).

Beyond the olive phenolic compounds, which represent the most interesting and investigated fraction, further research is also required in the scope of the protein and pectin fractions. As it has been discussed previously, scarce information can be currently found in literature concerning the proteins from olives. The *alperujo* protein could be considered as an alternative source of vegetable protein to that of traditional crops, and can be useful in numerous fields of application. In order to achieve this, investigation on extraction methods and implementation at the pilot plant level are still missing. In addition, research on the bioavailability of these proteins, the bioactive peptides encrypted in the native sequences, and their effect on modulating different physiological processes has to be carried out in order to confirm this source as adequate to produce protein hydrolysates. On the other side, the pectins from olive by-products have not been investigated in depth, in spite of the multiple applications that these could serve for. An adequate characterization and extraction of this fraction from the residues and investigation on how these could be used as functional component in different fields is still required. In terms of bioactivity, research on how olive pectin might have an effect on gut microbiota also could be of interest for the scientific community, considering the relevance of microbiota on human health.

On top of the use of olive by-products in the food industry as health-promoting components of the diet, there are other fields in which these can be used are. For instance, to enhance the functional properties of packaging systems (Khwaldia et al. 2022), in agriculture as fertilizers (Abu Salem et al. 2023; Campani et al. 2017), as medicaments in the pharmaceutical (Otero et al. 2021), or as bioactive ingredient in cosmetic formulations (Cádiz-Gurrea et al. 2021).

Conclusions

Olive by-products are a source of extremely interesting compounds to be potentially used in functional nutrition. The amount and profile of bioactive compounds in the by-products depends on several factors, including the cultivar. The minor phenolic secoiridoids exclusively found in VOO are a very interesting target research should go toward, due to its potential health benefits. The literature published until now suggest the promising potential of olive by-products to be used in the food industry, in line with the circular economy policies, to prevent the development of diseases based on the bioactivity their components exert. Further studies concerning purification of components, safety, bioavailability and well-defined human studies are needed in order to declare these by-products as safe and bioactive ingredients. On the other hand, it is also needed to evaluate their behavior when used as ingredient (subjected to processing, interaction with other nutrients, storage at different temperature, etc). Standardized and harmonized studies should be carried out, to clearly unravel the benefits that olive by-products have in modulating physiological status of humans.

Acknowledgments

Maria C. Millan-Linares acknowledges the financial grant US-1381492 supported by Andalusian Plan for Research, Development, and Innovation 2020 from the European Regional Development Fund (ERDF). Africa Fernandez-Prior has the benefit of a Juan de la Cierva postdoctoral fellowship (FJC2021-047485-I) from the Spanish Ministry of Science and Innovation. Fernando Rivero-Pino acknowledges his postdoctoral contract supported by TED2021-130521A-I00.

Disclosure statement

The authors declare no conflicts of interest.

Funding

This study was supported by the TED2021-130521A-I00 research project (Ministry of Science and Innovation, Government of Spain) into the Recovery, Transformation, and Resilience Plan funding by NextGenerationEU.

References

Abbattista, R., G. Ventura, C. D. Calvano, T. R. I. Cataldi, and I. Losito. 2021. Bioactive compounds in waste by-products from olive oil production: Applications and structural characterization by mass spectrometry techniques. *Foods* 10 (6):1236. doi: 10.3390/foods10061236.

Abu Salem, Z., K. Alzboon, J. Al-Tabbal, M. A. Haddad, and N. M. Bani-Hani. 2023. The effects of fermented olive mill wastewater mixtures on the growth and development of bean (*Phaseolus vulgaris*). *Water Resources and Industry* 29:100201. doi: 10.1016/j.wri.2023.100201.

Agrawal, K., E. Melliou, X. Li, T. L. Pedersen, S. C. Wang, P. Magiatis, J. W. Newman, and R. R. Holt. 2017. Oleocanthal-rich extra virgin olive oil demonstrates acute anti- platelet effects in healthy men in a randomized trial. *Journal of Functional Foods* 36:84–93. doi: 10.1016/j.jff.2017.06.046.

Alcaide-Hidalgo, J. M., M. Romero, J. Duarte, and E. Lopez-Huertas. 2020. Antihypertensive effects of virgin olive oil (unfiltered) low molecular weight peptides with ace inhibitory activity in spontaneously hypertensive rats. *Nutrients* 12 (1):271. doi: 10.3390/nu12010271.

Álvarez, M. D., S. Cofrades, M. Pérez-Mateos, A. Saiz, and B. Herranz. 2022. Development and physico-chemical characterization of healthy puff pastry margarines made from olive-pomace oil. *Foods* 11 (24):4054. doi: 10.3390/foods11244054.

Athanasiadis, V., A. Voulgaris, K. Katsoulis, S. I. Lalas, I. G. Roussis, and O. Gortzi. 2023. Development of enriched oil with polyphenols extracted from olive mill wastewater. *Foods* 12 (3):497. doi: 10.3390/foods12030497.

Balzan, S., A. Taticchi, B. Cardazzo, S. Urbani, M. Servili, G. Di Lecce, I. B. Zabalza, M. T. Rodriguez-Estrada, E. Novelli, and L. Fasolato. 2017. Effect of phenols extracted from a by-product of the oil mill on the shelf-life of raw and cooked fresh pork sausages in the absence of chemical additives. *LWT - Food Science and Technology* 85:89–95. doi: 10.1016/j.lwt.2017.07.001.

Barbieri, S., D. Mercatante, S. Balzan, S. Esposto, V. Cardenia, M. Servili, E. Novelli, A. Taticchi, and M. T. Rodriguez-Estrada. 2021. Improved oxidative stability and sensory quality of beef hamburgers enriched with a phenolic extract from olive vegetation water. *Antioxidants* 10 (12):1969. doi: 10.3390/antiox10121969.

Basdeki, E., C. Salis, and M. Hagidimitriou. 2020. The effects of Mediterranean diet and EVOO consumption in relation to human health. *Notulae Scientia Biologicae* 12 (3):466–85. doi: 10.15835/nsb12310784.

Bellumori, M., L. De Marchi, F. Mainente, F. Zanoni, L. Cecchi, M. Innocenti, G. Zoccatelli, and N. Mulinacci. 2021. A by-product from virgin olive oil production (pate) encapsulated by fluid bed coating: Evaluation of the phenolic profile after shelf-life test and in vitro gastrointestinal digestion. *International Journal of Food Science & Technology* 56 (8):3773–83. doi: 10.1111/ijfs.15068.

Bermúdez-Oria, A., G. Rodríguez-Gutiérrez, M. Alaiz, J. Vioque, J. Girón-Calle, and J. Fernández-Bolaños. 2019. Pectin-rich extracts from olives inhibit proliferation of Caco-2 and THP-1 cells. *Food & Function* 10 (8):4844–53. doi: 10.1039/c9fo00917e.

Branciari, R., R. Galarini, D. Giusepponi, M. Trabalza-Marinucci, C. Forte, R. Roila, D. Miraglia, M. Servili, G. Acuti, and A. Valiani. 2017. Oxidative status and presence of bioactive compounds in meat from chickens fed polyphenols extracted from olive oil industry waste. *Sustainability* 9 (9):1566. doi: 10.3390/su9091566.

Cádiz-Gurrea, M., L. de la, D. Pinto, C. Delerue-Matos, and F. Rodrigues. 2021. Olive fruit and leaf wastes as bioactive ingredients for cosmetics—A preliminary study. *Antioxidants* 10 (2):245. doi: 10.3390/antiox10020245.

Campani, T., I. Caliani, C. Pozzuoli, M. Romi, M. Cristina, and S. Casini. 2017. Assessment of toxicological effects of raw and bioremediated olive mill waste in the earthworm *Eisenia fetida*: A biomarker approach for sustainable agriculture. *Applied Soil Ecology* 119:18–25. doi: 10.1016/j.apsoil.2017.05.016.

Castillo-Luna, A., I. Criado-Navarro, C. A. Ledesma-Escobar, M. A. López-Bascón, and F. Priego-Capote. 2021. The decrease in the health benefits of extra virgin olive oil during storage is conditioned by the initial phenolic profile. *Food Chemistry* 336:127730. doi: 10.1016/j.foodchem.2020.127730.

Cazarin, C. B. B., J. L. Bicas, G. M. Pastore, and M. R. M. Junior (Eds.). 2022. *Bioactive Food Components Activity in Mechanistic Approach*. Academic Press. doi: 10.1016/B978-0-12-823569-0.00004-7.

Cecchi, L., M. Khatib, M. Bellumori, V. Civa, P. Domizio, M. Innocenti, D. Balli, and N. Mulinacci. 2023. Industrial drying for agrifood by-products re-use: Cases studies on pomegranate peel (*Punica granatum* L.) and stoned olive pomace (pâtè, *Olea europaea* L.). *Food Chemistry* 403:134338. doi: 10.1016/j.foodchem.2022.134338.

Centrone, M., M. Ranieri, A. Di Mise, M. D'Agostino, M. Venneri, G. Valenti, and G. Tamma. 2021. Health benefits of olive oil and by-products and possible innovative applications for industrial processes. *Functional Foods in Health and Disease* 11 (7):295–309. doi: 10.31989/ffhd.v11i7.800.

Chebaibi, S., M. Leriche Grandchamp, G. Burgé, T. Clément, F. Allais, and F. Laziri. 2019. Improvement of protein content and decrease of anti-nutritional factors in olive cake by solid-state fermentation: A way to valorize this industrial by-product in animal feed. *Journal of Bioscience and Bioengineering* 128 (3):384–90. doi: 10.1016/j.jbiosc.2019.03.010.

Cheurfa, M., H. H. Abdallah, R. Allem, A. Noui, C. M. N. Picot-Allain, and F. Mahomoodally. 2019. Hypocholesterolaemic and antioxidant properties of *Olea europaea* L. leaves from Chlef province, Algeria using in vitro, in vivo and in silico approaches. *Food and Chemical Toxicology : An International Journal Published for the British Industrial Biological Research Association* 123:98–105. doi: 10.1016/j.fct.2018.10.002.

Cicerale, S., X. A. Conlan, N. W. Barnett, A. J. Sinclair, and R. S. J. Keast. 2009. Influence of heat on biological activity and concentration of oleocanthal – A natural anti-inflammatory agent in virgin olive oil. *Journal of Agricultural and Food Chemistry* 57 (4):1326–30. doi: 10.1021/jf803154w.

Colica, C., L. Di Renzo, D. Trombetta, A. Smeriglio, S. Bernardini, G. Cioccoloni, R. Costa De Miranda, P. Gualtieri, P. Sinibaldi Salimei, and A. De Lorenzo. 2017. Antioxidant effects of a hydroxytyrosol-based pharmaceutical formulation on body composition, metabolic state, and gene expression: A randomized double-blinded, placebo-controlled crossover trial. *Oxidative Medicine and Cellular Longevity* 2017:2473495. doi: 10.1155/2017/2473495.

Contreras, M. d M., I. Gómez-Cruz, A. Feriani, S. Alwasel, A. H. Harrath, I. Romero, E. Castro, and N. Tlili. 2022. Hepatopreventive properties of hydroxytyrosol and mannitol-rich extracts obtained from exhausted olive pomace using green extraction methods. *Food & Function* 13 (22):11915–28. doi: 10.1039/d2fo00888b.

Crespo, M. C., J. Tomé-Carneiro, E. Burgos-Ramos, V. Loria Kohen, M. I. Espinosa, J. Herranz, and F. Visioli. 2015. One-week administration of hydroxytyrosol to humans does not activate Phase II enzymes. *Pharmacological Research* 95–96:132–7. doi: 10.1016/j.phrs.2015.03.018.

De-Cara, A., B. Saldaña, P. Vazquez, and A. I. Rey. 2023. Dietary protected sodium butyrate and/or olive leaf and grape-based by-product supplementation modifies productive performance, antioxidant status and meat quality in broilers. *Antioxidants* 12 (1):201. doi: 10.3390/antiox12010201.

de Bock, M., J. G. B. Derraik, C. M. Brennan, J. B. Biggs, P. E. Morgan, S. C. Hodgkinson, P. L. Hofman, and W. S. Cutfield. 2013. Olive (*Olea europaea* L.) leaf polyphenols improve insulin sensitivity in middle-aged overweight men: A randomized, placebo-controlled, crossover trial. *PLoS One*. 8 (3):e57622. doi: 10.1371/journal.pone.0057622.

Dinu, M., G. Pagliai, F. Scavone, M. Bellumori, L. Cecchi, C. Nediani, N. Maggini, F. Sofi, L. Giovannelli, and N. Mulinacci. 2021. Effects of an olive by-product called pâté on cardiovascular risk factors. *Journal of the American College of Nutrition* 40 (7):617–23. doi: 10.1080/07315724.2020.1813060.

Durante, M., A. Ferramosca, L. Treppiccione, M. Di Giacomo, V. Zara, A. Montefusco, G. Piro, G. Mita, P. Bergamo, and M. S. Lenucci. 2020. Application of response surface methodology (RSM) for the optimization of supercritical CO2 extraction of oil from patè olive cake: Yield, content of bioactive molecules and biological effects in vivo. *Food Chemistry* 332:127405. doi: 10.1016/j.foodchem.2020.127405.

El-Damrawy, S. Z., M. M. Khalifah, and W. A. Fares. 2013. Dietary olive leaf and antioxidative status in chickens "performance, some physiological traits and immunological responses of mandarah chicks supplemented olive leaves powder in their diets". *Egyptian Poultry Science* 33 (1):279–87.

El Riachy, M., F. Priego-Capote, L. León, L. Rallo, and M. D. Luque de Castro. 2011. Hydrophilic antioxidants of virgin olive oil. Part 2: Biosynthesis and biotransformation of phenolic compounds in virgin olive oil as affected by agronomic and processing factors. *European Journal of Lipid Science and Technology* 113 (6):692–707. doi: 10.1002/ejlt.201100096.

Emran, T., Bin Islam, F. Mitra, S. Paul, S. Nath, N. Khan, Z. Das, R. Chandran, D. Sharma, R. Lima, C. M. G, et al. 2022. Pectin: A bioactive food polysaccharide with cancer preventive potential. *Molecules* 27 (21):7405. doi: 10.3390/molecules27217405.

Esteve, C., M. L. Marina, and M. C. García. 2015. Novel strategy for the revalorization of olive (*Olea europaea*) residues based on the extraction of bioactive peptides. *Food Chemistry* 167:272–80. doi: 10.1016/j.foodchem.2014.06.090.

Estruch, R., E. Ros, J. Salas-Salvadó, M.-I. Covas, D. Corella, F. Arós, E. Gómez-Gracia, V. Ruiz-Gutiérrez, M. Fiol, J. Lapetra, et al. 2018. Primary prevention of cardiovascular disease with a mediterranean diet supplemented with extra-virgin olive oil or nuts. *The New England Journal of Medicine* 378 (25):e34. doi: 10.1056/nejmoa1800389.

Expósito-Díaz, A., H. Miho, C. A. Ledesma-Escobar, J. Moral, C. M. Díez, and F. Priego-Capote. 2022. Influence of genetic and interannual factors on bioactive compounds of olive pomace determined through a germplasm survey. *Food Chemistry* 378:132107. doi: 10.1016/j.foodchem.2022.132107.

Fasolato, L., L. Carraro, P. Facco, B. Cardazzo, S. Balzan, A. Taticchi, N. A. Andreani, F. Montemurro, M. E. Martino, G. Di Lecce, et al. 2016. Agricultural by-products with bioactive effects: A multivariate approach to evaluate microbial and physicochemical changes in a fresh pork sausage enriched with phenolic compounds from olive vegetation water. *International Journal of Food Microbiology* 228:34–43. doi: 10.1016/j.ijfoodmicro.2016.04.003.

Fernandez-Prior, Á., A. Bermudez-Oria, J. Fernandez-Bolaños, J. A. Espejo-Calvo, F. Lopez-Maestro, and G. Rodriguez-Gutierrez. 2022. Evolution of hydroxytyrosol, hydroxytyrosol 4-β-D-glucoside, 3,4-dihydroxyphenylglycol and tyrosol in olive oil solid waste or "alperujo". *Molecules* 27 (23):8380. doi: 10.3390/molecules27238380.

Foti, P., P. S. Occhipinti, N. Russo, A. Scilimati, M. Miciaccia, C. Caggia, M. G. Perrone, C. L. Randazzo, and F. V. Romeo. 2023. Olive mill wastewater fermented with microbial pools as a new potential functional beverage. *Molecules* 28 (2):646. doi: 10.3390/molecules28020646.

Gavahian, M., A. Mousavi Khaneghah, J. M. Lorenzo, P. E. S. Munekata, I. Garcia-Mantrana, M. C. Collado, A. J. Meléndez-Martínez, and F. J. Barba. 2019. Health benefits of olive oil and its components: Impacts on gut microbiota antioxidant activities, and prevention of noncommunicable diseases. *Trends in Food Science & Technology* 88:220–7. doi: 10.1016/j.tifs.2019.03.008.

Gerasopoulos, K., D. Stagos, K. Petrotos, S. Kokkas, D. Kantas, P. Goulas, and D. Kouretas. 2015. Feed supplemented with polyphenolic byproduct from olive mill wastewater processing improves the redox status in blood and tissues of piglets. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association* 86:319–27. doi: 10.1016/j.fct.2015.11.007.

Giannoutsos, K., A. Panagiotis, D. Ioanna, G. Menexes, I. Mourtzinos, D. Sarris, and K. Gkatzionis. 2023. Production of functional crackers based on non-conventional flours. Study of the physicochemical and sensory properties. *Food Chemistry Advances* 2:100194. doi: 10.1016/j.focha.2023.100194.

Giuliani, C., Marzorati, M. Daghio M., Franzetti A., Innocenti M., Van De Wiele T., and Mulinacci, N. 2019. Effects of olive and pomegrate by-products on human microbiota: A study using the shime® in vitro simulator. *Molecules* 24 (20):3791. doi: 10.3390/molecules24203791.

González-Rámila, S., B. Sarriá, M. Á. Seguido, J. García-Cordero, L. Bravo-Clemente, and R. Mateos. 2022. Effect of olive pomace oil on cardiovascular health and associated pathologies. *Nutrients* 14 (19):3927. doi: 10.3390/nu14193927.

González-Rámila, S., B. Sarriá, M. A. Seguido, J. García-Cordero, R. Mateos, and L. Bravo. 2022. Olive pomace oil can improve blood lipid profile: A randomized, blind, crossover, controlled clinical trial in healthy and at-risk volunteers. *European Journal of Nutrition* 62: 589–603. doi: 10.1007/s00394-022-03001-y.

Hadrich, F., A. Mahmoudi, M. Chamkha, H. Isoda, and S. Sayadi. 2023. Olive leaves extract and oleuropein improve insulin sensitivity in 3T3-L1 cells and in high-fat diet-treated rats via PI3K/AkT signaling pathway. *Oxidative Medicine and Cellular Longevity* 2023:6828230. doi: 10.1155/2023/6828230.

Haloui, E., B. Marzouk, Z. Marzouk, A. Bouraoui, and N. Fenina. 2011. Hydroxytyrosol and oleuropein from olive leaves: Potent anti-inflammatory and analgesic activities. *Journal of Food, Agriculture and Environment* 9 (2):128–33.

Hu, W., A. M. Cassard, and D. Ciocan. 2023. Pectin in metabolic liver disease. *Nutrients* 15 (1):157. doi: 10.3390/nu15010157.

Karampetsou, K., O. S. Koutsoni, F. Badounas, A. Angelis, G. Gogou, L. A. Skaltsounis, M. Halabalaki, and E. Dotsika. 2022. Exploring the immunotherapeutic potential of oleocanthal against Murine cutaneous leishmaniasis # authors. *Planta Medica* 88 (09/10):783–93. doi: 10.1055/a-1843-9788.

Khwaldia, K., N. Attour, J. Matthes, L. Beck, and M. Schmid. 2022. Olive byproducts and their bioactive compounds as a valuable source for food packaging applications. *Comprehensive Reviews in Food Science and Food Safety* 21 (2):1218–53. doi: 10.1111/1541-4337.12882.

Lee, M. T., W. C. Lin, and T. T. Lee. 2019. Potential crosstalk of oxidative stress and immune response in poultry through phytochemicals—A review. *Asian-Australasian Journal of Animal Sciences* 32 (3):309–19. doi: 10.5713/ajas.18.0538.

Leite, A., L. Vasconcelos, I. Ferreira, R. Domínguez, E. Pereira, S. Rodrigues, J. M. Lorenzo, and A. Teixeira. 2023. Effect of the inclusion of olive cake in the diet on the physicochemical characteristics of dry-cured loin and dry-cured "cachaço" of bísaro pig. *Applied Sciences* 13 (3):1439. doi: 10.3390/app13031439.

Lockyer, S., G. Corona, P. Yaqoob, J. P. E. Spencer, and I. Rowland. 2015. Secoiridoids delivered as olive leaf extract induce acute improvements in human vascular function and reduction of an inflammatory cytokine: A randomised, double-blind, placebo-controlled, cross-over trial. *The British Journal of Nutrition* 114 (1):75–83. doi: 10.1017/S0007114515001269.

Lockyer, S., I. Rowland, J. P. E. Spencer, P. Yaqoob, and W. Stonehouse. 2017. Impact of phenolic-rich olive leaf extract on blood pressure, plasma lipids and inflammatory markers: A randomised controlled trial. *European Journal of Nutrition* 56 (4):1421–32. doi: 10.1007/s00394-016-1188-y.

Lopez-Huertas, E., and J. M. Alcaide-Hidalgo. 2022. Characterisation of endogenous peptides present in virgin olive oil. *International Journal of Molecular Sciences* 23 (3):1712. doi: 10.3390/ijms23031712.

Lopez-Huertas, E., and J. Fonolla. 2017. Hydroxytyrosol supplementation increases vitamin C levels in vivo. A human volunteer trial. *Redox Biology* 11:384–9. doi: 10.1016/j.redox.2016.12.014.

Martin, M. E., M. C. Millan-Linares, M. C. Naranjo, R. Toscano, R. Abia, F. J. G. Muriana, B. Bermudez, and S. Montserrat-de la Paz. 2019. Minor compounds from virgin olive oil attenuate LPS-induced inflammation via visfatin-related gene modulation on primary human monocytes. *Journal of Food Biochemistry* 43 (8):e12941. doi: 10.1111/jfbc.12941.

Meireles, M., A. C. Cortez-Ribeiro, D. Polck, J. Almeida-de-Souza, and V. Ferro-Lebres. 2023. Olive leaf tea impact on postprandial glycemia: A randomized cross-over trial. *Foods* 12 (3):528. doi: 10.3390/foods12030528.

Menchetti, L., A. Taticchi, S. Esposto, M. Servili, D. Ranucci, R. Branciari, and D. Miraglia. 2020. The influence of phenolic extract from olive vegetation water and storage temperature on the survival of Salmonella Enteritidis inoculated on mayonnaise. *LWT* 129:109648. doi: 10.1016/j.lwt.2020.109648.

Miho, H., J. Moral, M. A. López-González, C. M. Díez, and F. Priego-Capote. 2020. The phenolic profile of virgin olive oil is influenced by malaxation conditions and determines the oxidative stability. *Food Chemistry* 314:126183. doi: 10.1016/j.foodchem.2020.126183.

Millan-Linares, M. C., S. Montserrat-de la Paz, and M. E. Martin. 2021. Pectins and olive pectins: From biotechnology to human health. *Biology* 10 (9):860. doi: 10.3390/biology10090860.

Millman, J. F., S. Okamoto, T. Teruya, T. Uema, S. Ikematsu, M. Shimabukuro, and H. Masuzaki. 2021. Extra-virgin olive oil and the gut-brain axis: Influence on gut microbiota, mucosal immunity, and cardiometabolic and cognitive health. *Nutrition Reviews* 79 (12):1362–74. doi: 10.1093/nutrit/nuaa148.

Miraglia, D., M. Castrica, S. Esposto, R. Roila, R. Selvaggini, S. Urbani, A. Taticchi, B. Sordini, G. Veneziani, and M. Servili. 2021. Quality evaluation of shrimp (*Parapenaeus longirostris*) treated with phenolic extract from olive vegetation water during shelf-life, before and after cooking. *Foods* 10 (9):2116. doi: 10.3390/foods10092116.

Miraglia, D., M. Castrica, L. Menchetti, S. Esposto, R. Branciari, D. Ranucci, S. Urbani, B. Sordini, G. Veneziani, and M. Servili. 2020. Effect of an olive vegetation water phenolic extract on the physico-chemical, microbiological and sensory traits of shrimp (*Parapenaeus longirostris*) during the shelf-life. *Foods* 9 (11):1647. doi: 10.3390/foods9111647.

Montealegre, C., C. Esteve, M. C. García, C. García-Ruiz, and M. L. Marina. 2014. Proteins in olive fruit and oil. *Critical Reviews in Food Science and Nutrition* 54 (5):611–24. doi: 10.1080/10408398.2011.598639.

Montoya, T., M. Sánchez-Hidalgo, M. L. Castejón, M. Á. Rosillo, A. González-Benjumea, and C. Alarcón-De-la-Lastra. 2021. Dietary oleocanthal supplementation prevents inflammation and oxidative stress in collagen-induced arthritis in mice. *Antioxidants* 10 (5):650. doi: 10.3390/antiox10050650.

Mousavi, S., R. Mariotti, V. Stanzione, S. Pandolfi, V. Mastio, L. Baldoni, and N. G. M. Cultrera. 2021. Evolution of extra virgin olive oil quality under different storage conditions. *Foods* 10 (8):1945. doi: 10.3390/foods10081945.

Noce, A., Marrone, G. Urciuoli, S. D. Daniele, F. Di Lauro, M. Zaitseva, A. P. D. Daniele, N, and Romani, A. 2021. Usefulness of extra virgin olive oil minor polar compounds in the management of chronic kidney disease patients. *Nutrients* 13 (2):581. doi: 10.3390/nu13020581.

Nunes, M. A., F. B. Pimentel, A. S. G. Costa, R. C. Alves, and M. B. P. P. Oliveira. 2016. Olive by-products for functional and food applications: Challenging opportunities to face environmental constraints. *Innovative Food Science & Emerging Technologies* 35:139–48. doi: 10.1016/j.ifset.2016.04.016.

Núñez-Carro, C., T. Montoya, M. Blanco-Blanco, K. M. Villagrán-Andrade, J. G. Fernández-Bolaños, F. J. Blanco, and M. C. de Andrés. 2022. Epigenetic interplay between H3K27Me3 and Mir-146a/B on the protective role of the nutraceutical oleocanthal in osteoarthritis. *Osteoarthritis and Cartilage* 30 (2022):S342. doi: 10.1016/j.joca.2022.02.460.

Otero, P., P. Garcia-Oliveira, M. Carpena, M. Barral-Martinez, F. Chamorro, J. Echave, P. Garcia-Perez, H. Cao, J. Xiao, J. Simal-Gandara, et al. 2021. Applications of by-products from the olive oil processing: Revalorization strategies based on target molecules and green extraction technologies. *Trends in Food Science & Technology* 116:1084–104. doi: 10.1016/j.tifs.2021.09.007.

Pantano, D., I. Luccarini, P. Nardiello, M. Servili, M. Stefani, and F. Casamenti. 2017. Oleuropein aglycone and polyphenols from olive mill waste water ameliorate cognitive deficits and neuropathology. *British Journal of Clinical Pharmacology* 83 (1):54–62. doi: 10.1111/bcp.12993.

Parisio, C., E. Lucarini, L. Micheli, A. Toti, M. Bellumori, L. Cecchi, L. Calosi, D. Bani, L. Di Cesare Mannelli, N. Mulinacci, et al. 2020. Extra virgin olive oil and related by-products (: *Olea europaea* L.) as natural sources of phenolic compounds for abdominal pain relief in gastrointestinal disorders in rats. *Food & Function* 11 (12):10423–35. doi: 10.1039/d0fo02293d.

Patti, A. M., G. Carruba, A. F. G. Cicero, M. Banach, D. Nikolic, R. V. Giglio, A. Terranova, M. Soresi, L. Giannitrapani, G. Montalto, et al. 2020. Daily use of extra virgin olive oil with high oleocanthal concentration reduced body weight, waist circumference, alanine transaminase, inflammatory cytokines and hepatic steatosis in subjects with the metabolic syndrome: A 2-month intervention study. *Metabolites* 10 (10):392. doi: 10.3390/metabo10100392.

Paulo, F., L. Tavares, and L. Santos. 2022. In vitro digestion, bioaccessibility, and release kinetics studies of encapsulated bioactive compounds obtained from olive mill pomace. *Journal of Food Measurement and Characterization* 16 (6):4880–95. doi: 10.1007/s11694-022-01579-9.

Peyrot Des Gachons, C., A. J. O'Keefe, L. Slade, and G. K. Beauchamp. 2021. Protein suppresses both bitterness and oleocanthal-elicited pungency of extra virgin olive oil. *Scientific Reports* 11 (1):11851. doi: 10.1038/s41598-021-91046-0.

Ranieri, M., A. Di Mise, M. Centrone, M. D'Agostino, S. J. Tingskov, M. Venneri, T. Pellegrino, G. Difonzo, F. Caponio, R. Norregaard, et al. 2021. Olive Leaf Extract (OLE) impaired vasopressin-induced aquaporin-2 trafficking through the activation of the calcium-sensing receptor. *Scientific Reports* 11 (1):1–14. doi: 10.1038/s41598-021-83850-5.

Rigacci, S., and M. Stefani. 2016. Nutraceutical properties of olive oil polyphenols. An itinerary from cultured cells through animal models to humans. *International Journal of Molecular Sciences* 17 (6):843. doi: 10.3390/ijms17060843.

Rivero-Pino, F., E. Grao-Cruces, S. Lopez-Enriquez, G. Alba, E. Marquez-Paradas, C. M. Claro-Cala, C. Santa-Maria, and S. Montserrat-de la Paz. 2023. Modulation of beta-amyloid-activated primary human neutrophils by dietary phenols from virgin olive oil. *Nutrients* 15 (4):941. doi: 10.3390/nu15040941.

Rocha, J., N. Borges, and O. Pinho. 2020. Table olives and health: A review. *Journal of Nutritional Science* 9. doi: 10.1017/jns.2020.50.

Rojas Gil, A. P., I. Kodonis, A. Ioannidis, T. Nomikos, I. Dimopoulos, G. Kosmidis, M. E. Katsa, E. Melliou, and P. Magiatis. 2022. The effect of dietary intervention with high-oleocanthal and oleacein olive oil in patients with early-stage chronic lymphocytic leukemia: A pilot randomized trial. *Frontiers in Oncology* 11:810249. doi: 10.3389/fonc.2021.810249.

Romani, A., Bernini, R., Noce A., Urciuoli S., Lauro M. D., Zaitseva A. P., Marrone G., and N. Di Daniele. 2020. Potential beneficial effects of extra virgin olive oils characterized by high content in minor polar compounds in nephropathic patients: A pilot study. *Molecules* 25 (20):4757. doi: 10.3390/molecules25204757.

Romani, A., F. Ieri, S. Urciuoli, A. Noce, G. Marrone, C. Nediani, and R. Bernini. 2019. Health effects of phenolic compounds found in extra-virgin olive oil, by-products, and leaf of *Olea europaea* L. *Nutrients* 11 (8):1776. doi: 10.3390/nu11081776.

Romeo, R., A. De Bruno, V. Imeneo, A. Piscopo, and M. Poiana. 2020. Impact of stability of enriched oil with phenolic extract from olive mill wasterwaters. *Foods* 9 (7):856. doi: 10.3390/foods9070856.

Rubio-Senent, F., G. Rodríguez-Gutiérrez, A. Lama-Muñoz, and J. Fernández-Bolaños. 2015. Pectin extracted from thermally treated olive oil by-products: Characterization, physico-chemical properties, invitro bile acid andglucose binding. *Food Hydrocolloids*. 43:311–21. doi: 10.1016/j.foodhyd.2014.06.001.

Sánchez De Medina, V., F. Priego-Capote, and M. D. Luque De Castro. 2012. Characterization of refined edible oils enriched with phenolic extracts from olive leaves and pomace. *Journal of Agricultural and Food Chemistry* 60 (23):5866–73. doi: 10.1021/jf301161v.

Sar, T., Murat, O. J. Taherzadeh, M, and Ferreira, A. J. 2020. New insights on protein recovery from olive oil mill wastewater through bioconversion with edible filamentous fungi. *Processes* 8 (10):1210. doi: 10.3390/pr8101210.

Scotece, M., J. Conde, V. Abella, V. López, V. Francisco, C. Ruiz, V. Campos, F. Lago, R. Gomez, J. Pino, et al. 2018. Oleocanthal inhibits catabolic and inflammatory mediators in LPS-activated human primary osteoarthritis (OA) chondrocytes through MAPKs/NF-κB pathways. *Cellular Physiology and Biochemistry: International Journal of Experimental Cellular Physiology, Biochemistry, and Pharmacology* 49 (6):2414–26. doi: 10.1159/000493840.

Sorrenti, V., I. Burò, V. Consoli, and L. Vanella. 2023. Recent advances in health benefits of bioactive compounds from food wastes and by-products: Biochemical aspects. *International Journal of Molecular Sciences* 24 (3):2019. doi: 10.3390/ijms24032019.

Susalit, E., N. Agus, I. Effendi, R. R. Tjandrawinata, D. Nofiarny, T. Perrinjaquet-Moccetti, and M. Verbruggen. 2011. Olive (*Olea europaea*) leaf extract effective in patients with stage-1 hypertension: Comparison with Captopril. *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology* 18 (4):251–8. doi: 10.1016/j.phymed.2010.08.016.

Taticchi, A., S. Bartocci, M. Servili, S. Di Giovanni, M. Pauselli, E. Mourvaki, D. M. Zilio, and S. Terramoccia. 2017. Effect on quanti-quality milk and mozzarella cheese characteristics with further increasing the level of dried stoned olive pomace in diet for lactating buffalo. *Asian-Australasian Journal of Animal Sciences* 30 (11):1605–11. doi: 10.5713/ajas.16.0767.

Taticchi, A., S. Esposto, S. Urbani, G. Veneziani, R. Selvaggini, B. Sordini, and M. Servili. 2017. Effect of an olive phenolic extract added to the oily phase of a tomato sauce, on the preservation of phenols and carotenoids during domestic cooking. *LWT* 84:572–8. doi: 10.1016/j.lwt.2017.06.028.

Toscano, R., M. C. Millan-Linares, M. C. Naranjo, A. Lemus-Conejo, C. Claro, and S. Montserrat-de la Paz. 2019. Unsaponifiable and phenolic fractions from virgin olive oil prevent neuroinflammation skewing microglia polarization toward M2 phenotype. *Journal of Functional Foods* 62:103543. doi: 10.1016/j.jff.2019.103543.

Trindade, P. C. O., Dalfolo, A. D. C. Monteiro C. S., Wagner R., Santos B. A., dos Nora, F. M. D., Verruck S, and Rosa, C. S. d. 2023. Development and characterization of biscuits with olive pomace. *Food Science and Technology* 43:e99922. doi: 10.1590/fst.99922.

Troise, A. D., A. Fiore, A. Colantuono, S. Kokkinidou, D. G. Peterson, and V. Fogliano. 2014. Effect of olive mill wastewater phenol compounds on reactive carbonyl species and maillard reaction end-products in ultrahigh-temperature-treated milk. *Journal of Agricultural and Food Chemistry* 62 (41):10092–100. doi: 10.1021/jf503329d.

Tufariello, M., M. Durante, G. Veneziani, A. Taticchi, M. Servili, G. Bleve, and G. Mita. 2019. Patè olive cake: Possible exploitation of a by-product for food applications. *Frontiers in Nutrition* 6:3. doi: 10.3389/fnut.2019.00003.

Vidal, A. M., M. Moya, S. Alcalá, I. Romero, and F. Espínola. 2022. Enrichment of refined olive oils with phenolic extracts of olive leaf and exhausted olive pomace. *Antioxidants* 11 (2):204. doi: 10.3390/antiox11020204.

Wainstein, J., T. Ganz, M. Boaz, Y. Bar Dayan, E. Dolev, Z. Kerem, and Z. Madar. 2012. Olive leaf extract as a hypoglycemic agent in both human diabetic subjects and in rats. *Journal of Medicinal Food* 15 (7):605–10. doi: 10.1089/jmf.2011.0243.