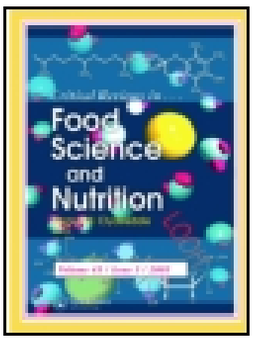

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REVIEW

Nutritional and other health properties of olive pomace oil

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ABSTRACT

Olive-pomace oil is rich in oleic acid, and thus it can be an interesting dietary fat alternative as it can allow reaching the recommendation of consuming 20% of total diet energy in the form of monounsaturated fatty acids. In addition, olive-pomace oil also contains a wide range of minor components that may contribute to its healthy properties. The major components identified with healthy properties are triterpenic dialcohols and acids, squalene, tocopherols, sterols, fatty alcohols and phenolic compounds. The refining process, that the crude pomace-oil must undergo for commercial purposes, significantly reduces the content of phenolic compounds, while the other minor components remain at concentrations which can induce positive health effects, especially on cardiovascular health, outstanding pentacyclic triterpenes and aliphatic fatty alcohols in olive-pomace oil. Numerous in vitro and preclinical studies support that mainly the pure compounds, or extracts isolated from plant sources, play an important role in preventing cardiovascular disease and risk factors. Likewise, tocopherols, squalene and phytosterols, in addition to the minor fraction of phenolic compounds, have shown high biological activity with particular association to the cardiovascular function. In the light of the foregoing, and taking into consideration the absence of clinical studies with olive-pomace oil, it would be of great interest to develop randomized, crossover, controlled, double-blind studies to extend the knowledge and understanding on the health effects of olive-pomace olive.

KEYWORDS

Olive-pomace oil; health; monounsaturated fat; minor components; pentacyclic triterpenes; aliphatic fatty alcohols

Introduction

Olive oil, the main fat in the Mediterranean diet, is one of the most appreciated dietary fats due to its proven health beneficial effects (Nocella et al. 2018; Schwingshackl et al. 2017). There are different types of olive oils, namely extra virgin olive oil (EVOO), virgin olive oil (VOO), olive oil (OO), and olive pomace oil (OPO) (CEE 1991; 2016), with similar lipid composition but different content of bioactive compounds, which would influence their health promoting potential.

During the process to obtain olive oil, olives are milled, subjected to malaxation and to two centrifugation steps (horizontal and vertical). The paste remaining after centrifugation is still rich in oil (approximately 12-15% of total oil), which is extracted through a second centrifugation process. This oil, together with that obtained by hexane extraction from the paste-like by-product called "alpeorujo" o "alperujo", corresponds to the crude olive pomace oil. Crude olive pomace oils require chemical refining to remove compounds responsible for undesirable colors and flavors and compounds affecting oil stability. Refined olive pomace oil is mixed with a proper amount of virgin olive oil (normally around 10%) hence resulting in the commercially available olive pomace oil.

Olive pomace oil shares with the rest of olive oil categories (EVOO, VOO and OO) some composition parameters that are widely recognized as healthy and are one of the pillars of the Mediterranean diet. Thus, olive pomace oil contains mostly monounsatured fatty acids, mainly oleic acid (C18:1), up to 85% of the total fatty acids. Furthermore, olive pomace oil also contains a wide range of minor components that may positively contribute to the potential health-promoting effects of this oil. Among the minor components are pentacyclic triterpenes, squalene, tocopherols, sterols, fatty alcohols and phenolic compounds.

Studies developed with olive pomace oil are scarce, consisting mostly on preclinical studies using animal models. These and other in vitro studies have been typically developed mainly with pure standards or extracts isolated from olive pomace oil; due to the scarcity of research with olive pomace oil, studies using extracts from other plant sources rich in the target bioactive compounds have also been included in the present review to highlight the potential actions of olive pomace oil minor components. On the other hand, most studies have been focused on the effect of olive pomace oil bioactive compounds and/or extracts on cardiovascular health, although the potential role of some minor components like pentacyclic triterpenes on other pathologies (e.g. diabetes, obesity, cancer, etc.) have been also extensively studied. In the present review, published data will be presented to understand the potential health benefits of olive pomace oil.

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Olive pomace oil is a rich source of oleic acid

Olive pomace oil, as a product obtained from olives, is mostly a monounsaturated fat, containing oleic acid which represents 56-85% of the total fatty acids, and linoleic and linolenic acid, which encounter 3-21% and 0.1-1.5%, respectively. Other relatively abundant fatty acids are palmitic acid, 6–20%, palmitoleic acid, 0.3–3.5% and stearic acid, 0.3–3.5%. In 2004, the United States Food and Drugs Administration (FDA) announced a claim about the health benefits of consuming 23 g (2 tablespoons) of olive oil daily to reduce the risk of coronary heart disease due to its content in monounsaturated fatty acids (MUFA), as long as it replaces a similar amount of saturated fatty acids (SFA) and the total daily intake of calories is not increased (FDA. Food and Drug Administration 2004). In spite of this health claim, the effects of MUFA on the prevention of coronary heart disease risk and coronary heart disease mortality are still unclear (Schwingshackl and Hoffmann 2012; Hooper et al. 2015). Schwingshackl and Hoffmann (2012) summarized systematic reviews and 16 meta-analyses of randomized clinical trials (RCT) and cohort studies investigating the effects of MUFA on cardiovascular and diabetic risk factors, cardiovascular events and cardiovascular death. Although major data supported the positive effects of MUFA minimizing the risk of suffering a cardiovascular event, one meta-analysis reported an increase in coronary heart diseases (Jakobsen et al. 2009). The negative effect was attributed to the MUFA supply in the Western diet being predominantly of animal origin, and thus resulting as a confounding factor that should be taken into account when comparing dietary fats (Jakobsen et al. 2009). On the other hand, Hooper et al. (2015) reviewed 15 RCT that suggested a small but potentially important decrease in cardiovascular risk by reducing SFA intake and concluded that replacing dietary energy from SFA with that from polyunsaturated fatty acids (PUFA) seems to be a useful coronary heart disease preventive strategy, more useful than the replacement with carbohydrates. However, the effects of replacement with MUFA were unclear due to the inclusion of only one small trial. Joris and Mensink (2016) analyzed recent studies carried out during the last 3 years on the relationship between MUFA and cardiovascular risk markers or coronary heart diseases. In summary, the new studies were in agreement with the earlier findings pointing to MUFA having a favorable effect on serum lipoprotein profile compared with a mixture of SFA, being the effects similar to those of linoleic acid and α-linolenic acid. Besides, replacement of SFA or high-glycemic index foods with MUFA lowered coronary heart disease risk. Recently, the association between cis MUFA intake, from both plant (MUFA-P) and animal (MUFA-A) sources, and coronary heart diseases risk, has been separately studied in 63,442 women from the Nurses' Health Study (1990–2012) and 29,942 men from the Health Professionals Follow-Up Study (1990–2012). It was concluded that plant-based foods are the main source of MUFAs with coronary heart disease prevention properties (Zong et al. 2018), which is favorable for oils from olive trees (EVOO, VOO, OO and olive pomace oil).

One of the studies analyzed by Joris and Mensink (2016) was the prospective cohort study PREDIMED (Primary Prevention of Cardiovascular Disease with a Mediterranean Diet), which showed that the intake of MUFA was associated with a lower risk of cardiovascular disease and death, reducing 30% the incidence of cardiovascular events (the study was originally published in 2013 but has been retracted and republished after data revision obtaining similar results, Estruch et al. 2018), in addition to other positive effects such as diabetes prevention (Salas-Salvadó et al. 2018). The PREDIMED study, revealed that the volunteers following the Mediterranean diet, with extra virgin olive oil as the main source of dietary fat, showed that MUFA represented 22% of the daily dietary energy intake (equivalent to 50 mL/day of virgin olive oil) and 40% of the total fat consumption. This higher amount of fat compared to the maxima of 35% recommended by international bodies (FAO-WHO 2008; EFSA 2010a), pointed out that the quality of dietary fat is much more important than the quantity. Additionally, it is important to note that although MUFA are the major components of EVOO, it also contains minor bioactive compounds, *inter alia* polyphenols, which may have contributed to the results observed in the PREDIMED Study since olive oil phenolic compounds have shown anti-inflammatory effects (Fitó et al. 2008) and are recognized for their capacity reducing plasma low-density lipoprotein (LDL) levels, as supported by a positive scientific opinion from the European Food Safety Authority (EFSA 2011).

Most of the studies performed on the health beneficial effects of olive oil's MUFA have been carried out with EVOO and VOO. To the best of our knowledge, there is no clinical trial specifically assessing the effects of olive pomace oil on cardiovascular health. However, based on its' fatty acids composition, comparable to that of EVOO and VOO, the benefits derived from MUFA consumption could also be expected from olive pomace oil, turning into an interesting vegetable source of oleic acid. In addition, olive pomace oil also contains a wide range of minor compounds that may have a positive impact on health.

Biological activity of olive pomace oil minor components

Olive pomace oil contains other minor constituents with biological activity, some of them are present at very low concentration in EVOO, VOO and OO and, therefore, are exclusive to this type of oil. In this section, the bioactivity of triterpenic dialcohols (erythrodiol and uvaol), triterpenic acids (oleanolic, ursolic and maslinic acids), squalene, and fatty alcohols will be reviewed. Although the content of phenolic compounds in olive pomace oil is low (Table 1), they will be also discussed due to their relevant bioactivity. Literature on the health effects of tocopherols and phytosterols is very abundant, yet to the best of our knowledge specific studies on the contribution of these minor components of olive oils, including olive pomace oil, are lacking; their biological effects and their potential contribution to cardiovascular health will be briefly reviewed. The chemical structures of these bioactive compounds are shown in

Figure 1. As mentioned before, most studies published on the biological activity and potential health effects of olive pomace oil's minor components are in vitro or in vivo studies in animal models, with a clear need for randomized clinical trials using this oil as a source of edible fat in the diet.

Table 1. Amounts of the main minor components in olive-extracted oils.

mg/Kg	EVOO/VOO	ROO	ROPO
Tocopherols	110–300	150–300	185–300
Sterols	800–2400	1000–2000	1800–3000
Squalene	900–10000	500–6000	500–6000
Triterpenic acids	90–200	<100	<200
Triterpenic dialcohols	<200	<200	200–1000
Aliphatic fatty alcohols	<350	<350	1000–3000
Phenolic compounds	100–800	<100	<100

Alba-Mendoza et al. 1996; Pérez-Camino and Cert, 1999; Bockisch 1998; Graciani 2006; García-González et al. 2013; Boskou 2015; Sánchez-Gutierrez et al. 2017.
EVOO/VOO: extra virgin olive oil/ virgin olive oil.
ROO: refined olive oil.
ROPO: refined olive pomace oil.

Biological activity of pentacyclic triterpenes (oleanolic and maslinic acids, erythrodiol and uvaol) in olive pomace oil

Pentacyclic triterpenes constitute a characteristic group in olive pomace oil. Crude olive pomace oils are very rich in triterpenic acids (2000–10000 mg/Kg) (Pérez-Camino and Cert, 1999; García et al. 2008; Ruiz-Méndez et al. 2013), particularly oleanolic and maslinic acids. However, most of these triterpenic acids are lost during refining resulting in final contents in the edible oil of < 200 mg/Kg (García et al. 2006; Sánchez-Gutierrez et al. 2017). Likewise, triterpenic alcohols (erythrodiol and uvaol) are also partly lost during refining, although about 500 mg/Kg of triterpenic alcohols remain in refined olive pomace oil (Pérez-Camino and Cert, 1999). Despite this, these components are likely to induce beneficial health effects. In vitro studies on the antioxidant activity of pentacyclic triterpenes have focused on their ability to protect LDL against oxidations, considering the importance of oxidized LDL (ox-LDL) in the development

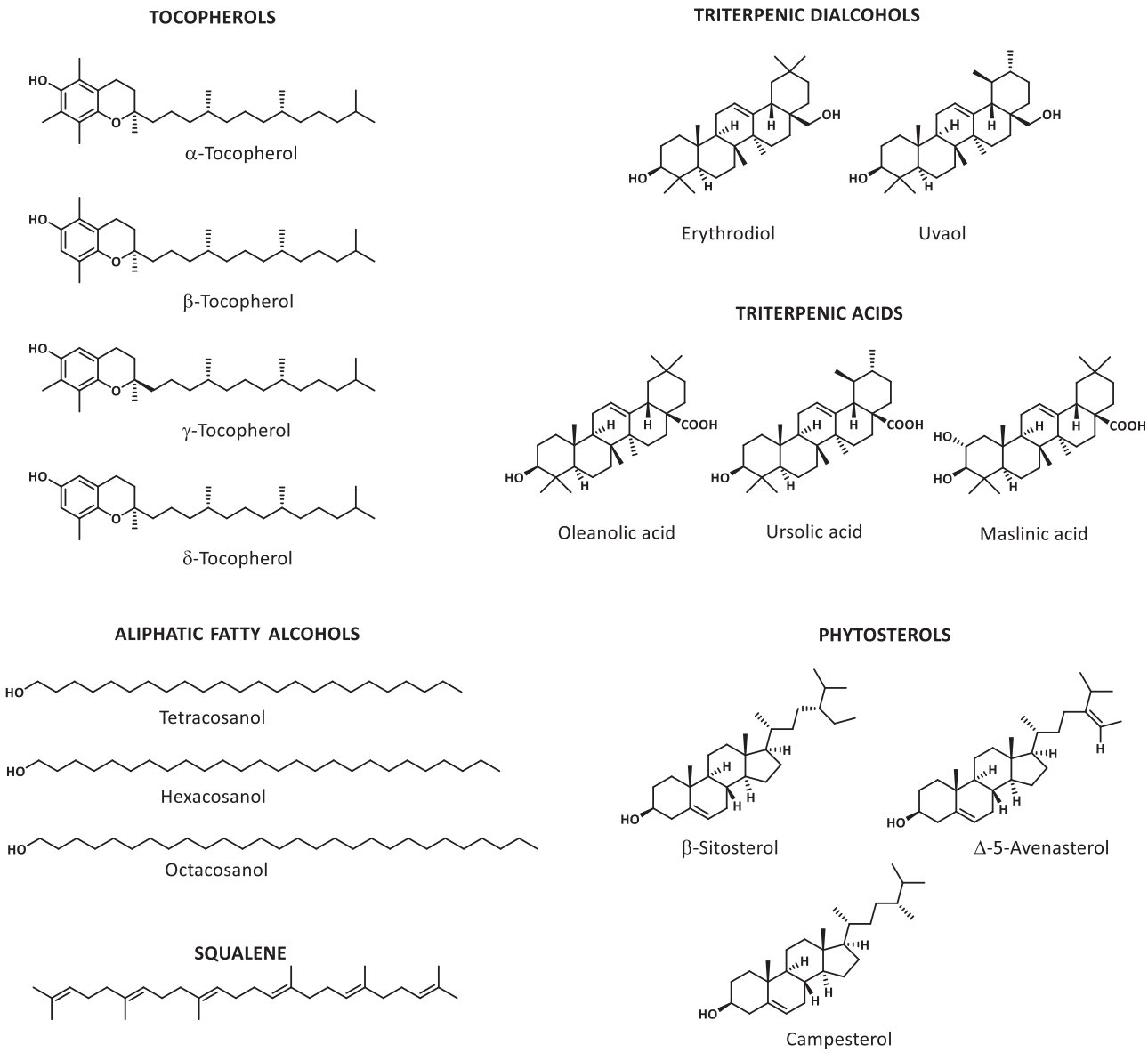

Figure 1. Chemical structure of the minor components in olive pomace oil.

of atherosclerosis. Andrikopoulos et al. (2002) described that oleanolic acid (OA) and its isomer ursolic acid (UA) may protect LDL against oxidation (estimated according to in vitro copper ion-induced LDL oxidation), both triterpenic acids showing similar capacity to chiosmastic gum, a strong human LDL oxidation protector (Andrikopoulos et al. 2003). Likewise, Allouche et al. (2010) proposed that maslinic acid (MA), uvaol and erythrodiol might exert cardiovascular benefits by different mechanisms of action using in vitro assays. While MA showed strong antioxidant activity, uvaol and erythrodiol exhibited both antioxidant and antithrombotic properties related to LDL particles, according to in vitro copper ion-induced LDL oxidation and LDL-supported thrombin generation, respectively.

Hyperlipemia is an established risk factor for cardiovascular diseases (CVDs). Triterpenes may positively modulate blood lipids, as seen in an animal study where oral administration of MA and OA (100 mg/Kg) to rats fed a high-cholesterol diet for two weeks resulted in a hypolipidemic effect, reducing serum triglycerides, total cholesterol and LDL-cholesterol over 70% (Liu et al. 2007). These triterpenes also restored the levels of the hepatic enzymes lactate dehydrogenase (LDH), alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT), increased in high-cholesterol fed control rats, in addition to restoring glycogen contents and morphological alterations observed in hepatocytes in treated animals compared to controls. Furthermore, gene expression of the enzyme acyl-CoA cholesterol acyltransferase (ACAT) was partially suppressed compared with the model control group fed the cholesterol-rich diet without triterpenes. No difference was observed between the therapeutic effects of both triterpenic acids (MA and OA). These authors concluded that the possible mechanism involved in the hypolipidemic action of triterpenes would be related to their effect lowering ACAT gene expression, and inhibiting intestinal absorption, synthesis or storage of cholesterol (Liu et al. 2007).

The cardioprotective effect of MA has also been tested in a rat model of isoproterenol (ISO)-induced myocardial infarction (Hussain Shaik et al. 2012). Pretreatment with MA (15 mg/Kg) for 7 days protected against ISO-induced cardiotoxicity, decreasing the activity of the cardiac marker enzymes creatine kinase (CK), ALT, AST and γ-glutamyl transferase (GGT). Also, serum lipid profile was improved in MA-pretreated rats (decreased cholesterol, triglycerides, LDL and very low density lipoprotein (VLDL)-cholesterol and increased high density lipoprotein (HDL)-cholesterol), with lower malondialdehyde (MDA) levels (biomarker of lipid peroxidation) and increased paraoxonase (PON) activity (atheroprotective enzyme found in HDL particles).

Diol triterpenes, erythrodiol and uvaol, have demonstrated in mice the capacity to reduce cardiac hypertrophy and left ventricle remodeling induced by angiotensin II by diminishing fibrosis and modulating growth and survival of cardiac myofibroblasts. In addition, inhibition of the angiotensin II-induced proliferation in a peroxisome proliferator-activated receptor gamma (PPARγ)-dependent manner has been observed in myofibroblasts, while at high doses activation of pathways of programed cell death, that are dependent on c-Jun N-terminal kinase (JNK) and PPAR-γ, have also been described, protecting from cardiac hypertrophy (Martín et al. 2012).

It is well known that hypertension is a strong independent risk factor for stroke, coronary heart disease and congestive heart failure and thus its improvement contributes to the prevention of CVDs. In Dahl salt-sensitive hypertensive rats, OA and UA also modulated lipid profile and blood glucose levels favorably after the intraperitoneal administration of 60 mg/Kg/day for 6 weeks (Somova et al. 2003). Moreover, these triterpenes decreased heart rate and prevented the development of severe hypertension in these rats (Somova et al. 2003). Other authors have also revealed the anti-hypertensive effects of pentacyclic triterpenes. Thus, OA inhibited the progression of fibrosis and lowered portal pressure in rats with CCl_4-induced cirrhosis after daily administration of 30 and 60 mg/Kg of OA (Liu et al. 2012). The portal anti-hypertensive effect might be related to the observed increased expression of endothelial nitric oxide synthase (eNOS) and enhanced nitric oxide (NO) levels in the liver. Likewise, OA prevented dexamethasone-induced hypertension in rats after administration for five days (60 mg/kg), the observed effects attributed to its antioxidant character and NO-releasing action (Bachhav et al. 2011).

NO is synthesized from L-arginine by means of eNOS, which plays a key role on the maintenance of endothelial homeostasis (Heiss, Rodríguez-Mateos, and Kelm 2015). Alterations in the vascular tone result in changes in blood pressure, thus linking hypertension and endothelial dysfunction (Silva, Pernomian, and Bendhack 2012). Accordingly, vasodilatory effects of OA and erythrodiol in rats' thoracic aorta have been demonstrated, mainly mediated by endothelial production of NO (Rodríguez-Rodríguez et al. 2004). Furthermore, dose-dependent vasorelaxation induced by OA, MA, erythrodiol and uvaol was observed in aortas isolated from spontaneously hypertensive rats, again with involvement of NO (Rodríguez-Rodríguez et al. 2006). Recently, Luna-Vázquez et al. (2016) have demonstrated the involvement of NO, in addition to hydrogen sulfide, in the vasodilator effect of UA and uvaol, possibly by means of activation of eNOS and cystathionine gamma-lyase (CSE), respectively.

Triterpenes have also shown anti-inflammatory activity, which is common to many pathological processes. The preventive antioxidative and anti-inflammatory effects of MA have been evaluated in macrophages isolated from murine peritoneum, which plays a role in the defensive system of these animals. Interestingly, MA (50–100 μM) was able to reduce the generation of hydrogen peroxide and the release pro-inflammatory cytokines interleukin (IL)-6 and tumor necrosis factor (TNF)-α from lipopolysaccharide (LPS)-stimulated murine macrophages (Márquez-Martín et al. 2006). The anti-inflammatory activity of MA has also been shown in primary cortical astrocytes (Huang et al. 2011). These cells were treated with triterpenes at 0.1, 1 and 10 μM for 24 h before being exposed to LPS. MA treatment exerted potent anti-inflammatory action by inhibiting the

production of NO and TNF-α. Moreover, MA attenuated LPS-induced translocation of the transcription factor NF-κB (p65 subunit) to the nucleus and prevented IκBα phosphorylation in a dose-dependent manner. MA also suppressed the expression of cyclooxygenase 2 (COX-2) and inducible nitric oxide synthase (iNOS) at protein and mRNA levels. These results suggested that MA has the potential to reduce neuroinflammation by inhibiting nuclear factor kappa B (NF-κB) signal transduction pathway in cultured cortical astrocytes (Huang et al. 2011).

Likewise, an in vivo study with apolipoprotein E (Apo-E) knockout mice showed that OA had antiatherogenic effects, which were lipid-independent, since no changes were observed in cholesterol and triglycerides in mice after consuming 100 mg/kg/day for 8 weeks (Buus et al. 2011). In this study, OA reduced atherosclerotic lesion in Apo-E-deficient mice by decreasing iNOS expression, pointing again to its anti-inflammatory activity.

Recently, the effects of MA on the expression of genes and proteins involved in inflammation in LPS-stimulated RAW 264.7 cells (murine blood macrophage cell line) were investigated (Fukumitsu et al. 2016). MA inhibited TNF-α production and reduced the transcription of genes involved in inflammation (IL-1β, IL-6, iNOS and cyclooxygenase (COX)-2) and also inhibited NF-κB translocation to the nucleus and phosphorylation of IκBα. In this same study, the effect of MA on carrageenan-induced paw edema and collagen antibody-induced arthritis in mice was tested (200 mg/Kg MA in the edema model or 100 mg/Kg in the arthritis model) showing anti-inflammatory and anti-arthritis effects as revealed by the suppression of paw edema, arthritis score and inflammatory cells.

Similarly, the anti-inflammatory and antioxidant properties of uvaol were evaluated in a mouse model of allergy (Agra et al. 2016). The results revealed that uvaol can be a good candidate for the treatment of allergic inflammation by inhibiting eosinophil influx and IL-5 production in ovalbumin-induced allergy. Recently, Kashyap et al. (2016) reviewed the promising anti-inflammatory activities of OA and UA, concluding that the major anti-inflammatory effects of these molecules are mediated via inactivation of NF-κB, STAT3/6, and Akt/mTOR pathways.

Diabetes is another important risk factor in the development of CVDs. Triterpenes have also shown potential effects in the treatment of type 2 diabetes. There is evidence that triterpenes reduce glucose absorption, decrease endogenous glucose production and increase insulin sensitivity as demonstrated in the following studies. OA and MA (80 mg/Kg) ameliorated postprandial hyperglycemia in streptozotocin (STZ)-induced diabetic rats after co-administration of the triterpenes and carbohydrate compared with non-triterpene treated diabetic rats (Khathi et al. 2013). OA and MA not only down-regulated the expression of sodium-dependent glucose co-transporter-1 (SGLT-1) and glucose transporter-2 (GLUT-2) in the small intestine of STZ-induced diabetic rats, but also inhibited α-amylase, sucrase and α-glucosidase activity. The inhibition of enzymes involved in the hydrolysis of carbohydrates together with the down-regulation of glucose transporters delay glucose absorption in the small intestine, and consequently prevent postprandial plasma glucose increase (Khathi et al. 2013).

In type 2 diabetes, endogenous glucose production is increased and synthesis of glycogen is reduced by increasing gluconeogenesis and glycogenolysis, respectively. Wen et al. (2005) demonstrated that MA may inhibit the activated form of glycogen phosphorylases (GP) isolated from rat liver (enzyme involved in glycogenolysis reaction), the inhibitory effect of this triterpenoid being 6-times stronger than that of caffeine, a well-known GP inhibitor. In the same study, in vivo hypoglycemic activity was evaluated in adrenaline-induced diabetic mice, which is known to indirectly stimulate glycogenolysis and, thus, increase blood glucose concentration. After oral administration of MA (100 mg/kg) for 7 days, fasting plasma glucose was 46% lower compared to control animals. These results show that MA reduces glucose availability by limiting endogenous glucose production through GP inhibition. In line with this mechanism, in vivo treatment of $Lep^{db/db}$ obese diabetic mice with OA (20 mg/kg/day) for 2 weeks resulted in lower gluconeogenesis, lower fasting blood glucose levels, and improved glucose and insulin tolerance (Wang et al. 2013).

Insulin resistance, the hallmark of type 2 diabetes, is closely related to lipid metabolism disorders. Accordingly, OA improved hepatic insulin resistance through the inhibition of mitochondrial reactive oxygen species (ROS), and hypolipidemic and anti-inflammatory effects in the $Lep^{db/db}$ obese animal model (Wang et al. 2013). Likewise, MA showed hypoglycemic effects by reducing insulin resistance in KK-A(y) mice, a model of genetic type 2 diabetes (Liu et al. 2007), after daily intake of 10 and 30 mg/kg for 2 weeks. MA treatment in mice fed with a high-fat diet also reduced model-associated adiposity and insulin resistance, and increased the accumulated hepatic glycogen content. Complementary in vitro assays in human hepatoma HepG2 cells showed that MA exerted anti-diabetic effects by increasing glycogen content and inhibiting glycogen phosphorylase activity (Liu et al. 2014).

The benefits described on cardiovascular health and type 2 diabetes are closely related with obesity, which prevalence is drastically increasing in Western countries. Accumulating evidence suggests that triterpenes act as anti-obesity agents, reducing lipid absorption, improving lipid homeostasis (Sung et al. 2010; Liu et al. 2013), and promoting food intake and body weight regulation (De Melo et al. 2010). In a recent review by Silva, Oliveira, and Duarte (2016), the hypoglycemic and anti-obesity mechanisms of triterpenes have been reported. However, most in vivo studies that evaluate type 2 diabetes preventive activity of triterpene have been developed in animals, lacking human studies, excepting the recently multicentre randomized double-blind controlled trial PREDIABOLE (Santos-Lozano et al. 2019). The effect of olive oil enriched in oleanolic acid (600 mg/Kg) on the incidence of type 2 diabetes in 176 patients with prediabetes (impaired glucose tolerance and impaired fasting glucose) compared to a control group that consumed the same amount of commercial non-enriched olive oi was

evaluated. The results showed that the intake of oleanolic acid enriched olive oil reduced the risk of developing diabetes in prediabetic patients.

On the other hand, triterpenes present remarkable antimicrobial activity. These compounds act against important human pathogens such as mycobacteria, human immunodeficiency virus (HIV), and different protozoal species (Jesus et al. 2015). Special interest has focused on HIV. MA showed potent inhibitory activity against HIV-1 protease (Xu et al. 1996; García-Granados, 1999), also observed for OA and its derivatives (Nakamura, 2004). The inhibition of this enzyme produces immature and noninfectious virions and molecules, consequently blocking the life cycle of HIV (Filho et al. 2010). These effects could ultimately improve HIV patient's quality of life. However, the antiretroviral azidothymidine (AZT) resulted to be better compared to MA and OA and new studies searching for new compounds, acylated triterpenes from olive oil industry wastes, among others, with antiviral (as inhibitors of the HIV-1-protease) effects have been developed (Parra et al. 2014). This study also evaluated the anti-proliferative effect of these new synthetic compounds against the b16f10 murine melanoma cancer cells showing promising results.

In this sense, pentacyclic triterpenoids obtained from natural plant materials have shown to inhibit tumor cell proliferation, induce apoptosis, increase the life span of tumor-bearing mice compared to control animals, as well as prevent angiogenesis, invasion and metastasis of tumor cells to distant organ sites in preclinical models of cancer (Zhang, Men, and Lei 2014). As an example, erythrodiol, uvaol and OA showed significant cytotoxic effect and inhibited proliferation in a dose- and time-dependent manner in human MCF7 breast cancer cell line. Erythrodiol growth inhibition occurred through apoptosis, also causing a relevant decrease of ROS production and DNA damage, whereas uvaol and OA showed cell cycle arrest (Allouche et al. 2011).

Taken all together, triterpenes may play a key role in the prevention of cardiovascular diseases and their risk factors by modulating lipid profile, improving endothelial function, inducing hypotensive effects, lowering inflammation and improving biomarkers related to the prevention of diabetes and obesity. These compounds have also been described as potent anti-microbial and anti-carcinogenic agents. Therefore, triterpenes, as bioactive compounds present in olive pomace oil, may contribute to the beneficial effects ascribed to the Mediterranean diet on the prevention of cardiovascular diseases (Ros et al. 2014) and other associated pathologies like diabetes and obesity.

Squalene and its biological properties

Another interesting bioactive compound is squalene, a triterpenic polyunsaturated hydrocarbon present in high concentration in olive pomace oil. Although squalene is partially lost during the process of refining the olive pomace, its content in the edible oil ranges from 500 to 6000 mg/Kg (Table 1) (Nergiz and Çelikkale 2011), providing a daily dietary intake of 25–300 mg/day per 50 mL of olive pomace oil. Squalene is an interesting bioactive substance with recognized anti-carcinogenic, antioxidant, hypocholesterolemic, detoxifying and emollient activities, in addition to hydrating the skin and to transporting drug, among other properties (Ramírez-Torres et al. 2010; Spanova and Daum, 2011).

Squalene is one of the major components of skin surface lipids and a key factor in the maintenance of skin health. The capacity of squalene for blocking photo-induced lipid peroxidation in cellular skin components has been demonstrated by quenching singlet oxygen (Kostyuk et al. 2012). This is due to the antioxidant activity associated to squalene. Cho et al. (2009) reported that the intake of 13.5 g/day of squalene in healthy volunteers significantly decreased wrinkles in aged human skin, increased type I procollagen and decreased UV-induced deoxyribonucleic acid (DNA) damage. Squalene also seems to play an important role in the retina health, reducing oxidative damage in rod photoreceptor cells (Fliesler and Keller 1997) or alcohol damage in the chick embryo retina (Aguilera et al. 2005).

Plasmatic levels of squalene in humans are originated from endogenous synthesis and from dietary sources, being the latter especially important in populations consuming large amounts of olive oil. Although squalene is an important intermediate in the endogenous synthesis of cholesterol, its intake does not increase in serum cholesterol concentration. Strandberg, Tilvis, and Miettinen (1990) reported that a daily dietary intake of 900 mg of squalene for a period of 7-10 days in humans produced a 17-fold increase in serum squalene, but serum triglyceride and cholesterol levels were not changed. More interestingly, Chan et al. (1996) described that the combined therapy of pravastatin and squalene, administered to patients with hypercholesterolemia, significantly reduced total cholesterol and LDL-cholesterol, in addition to increasing HDL-cholesterol. These effects were much more significant than treatment with the statin alone, without squalene. The advantages of squalene may be due to increased fecal elimination of cholesterol and bile acids (Strandberg, Tilvis, and Miettinen 1990; Strandberg, Tilvis, and Miettinen 1989). Recently, circulating squalene levels in addition to other cholesterol precursors have been proposed as disease biomarkers by providing deeper insight into the disease process according to their relative abundance (Brown et al. 2014).

Guillén et al. (2008) showed that squalene feeding (1 g/kg/day) during 10 weeks reduced atherosclerotic lesion size in Apo-E deficient male mice, together with a lower hepatic fat content compared to control animals not receiving squalene. The same research group demonstrated in HepG2 cells (a liver cell model) that squalene administration was able to counteract nonalcoholic hepatic steatosis (Hoang et al. 2016). Another interesting article described that squalene ameliorates atherosclerotic lesions through the inhibition of oxidized LDL uptake by macrophages by reducing CD36 scavenger receptor expression (Granados-Principal et al. 2012). Likewise, Bullon et al. (2009) showed that squalene (2.5 mg/Kg/day) administration for 50 days also reduced endothelial activation and fibrosis in gingival mucosa of atherosclerotic rabbits. Moreover, a cardio-

protective action of squalene has been demonstrated in isoproterenol-induced myocardial infarction in male albino rats after administration of a diet containing 2% squalene for 45 days (Sabeena Farvin et al. 2004). Motawi, Sadik, and Refaat (2010) also reported that squalene acts as a cytoprotectant capable of attenuating cyclophosphamide-induced alterations in rat myocardium. Taken all together, these findings suggest that squalene is a promising agent in the prevention and management of cardiovascular diseases.

In a recent intervention study, Casado-Diaz et al. (2017) supplemented the diet of postmenopausal women with a by-product from olive pomace rich in squalene (providing approximately 530 mg squalene/day). These authors found reduced lipid peroxidation and oxidized LDL (oxLDL) levels, and enhanced vitamin E and coenzyme Q10 levels in serum after two months. Furthermore, when using serum from these women to supplement culture medium (10%), they observed stimulated osteoblastogenesis and inhibited adipogenesis in human mesenchymal stem-cells from bone marrow, demonstrating the squalene ability to prevent and treat degenerative pathologies associated with aging, such as oxidation and osteoporosis (Casado-Diaz et al. 2017). In addition, an interesting recent study evaluated the effects of dietary squalene supplementation (0.02–0.1%) for 4 weeks on an acute colitis model induced by dextran sulfate sodium (DSS) in C57BL/6 mice (Sánchez-Fidalgo et al. 2015). In this study, squalene was able to reduce the oxidative stress status and return expression of pro-inflammatory proteins to basal levels, acting through p38 mitogen-activated protein kinase (MAPK) and NF-κB signaling pathways. Furthermore, squalene is widely used as co-adjuvant vaccine since it increases Th1 monocyte responses by potentiating toll-like receptor (TLR)4 adjuvant agonists through caspase and IL-18 dependent mechanisms (Desbien et al. 2015).

Oxidative and inflammatory activities are closely related with carcinogenic processes, where it has been demonstrated that squalene has positive effects (Ghanbari et al. 2012; Gaforio et al. 2015). Of particular interest is the possible role of squalene in the prevention of breast cancer. In this sense, in vitro cellular studies suggested that squalene may be partially responsible for the lower incidence of breast cancer in populations that consume the Mediterranean diet, due to its protective activity against oxidative DNA damage in normal mammary cells (Warleta et al. 2010).

Therefore, diets with an adequate intake of oils containing squalene, such as olive pomace oil, might be sufficient to achieve the protective benefits related to skin, eyes, and cardiovascular health as well as osteoporosis and cancer prevention.

Aliphatic fatty alcohols and their biological activity

Aliphatic fatty alcohols constitute an important group present in olive pomace oil (1000-3000 mg/kg) (Fernández-Arche et al. 2009). The main linear aliphatic alcohols characterized are tetracosanol (C24), hexacosanol (C26), octacosanol (C28) and docosanol (C22) and are found in significantly larger amounts in olive pomace oil than in the rest of categories of olive oil (Giacometti, 2001) (Table 1). A review of clinical studies reported that the intake of mixtures of long chain fatty alcohols decreases the concentration of LDL-cholesterol and increases HDL-cholesterol in humans after a period of dietary intervention (Hargrove et al., 2004). In particular, reports suggest that consumption of 5-20 mg per day of mixed C24-C34 alcohols, including octacosanol (C28) and triacontanol (C30) decreases LDL-cholesterol levels by 21-29% and raises HDL-cholesterol levels between 8–15% (Hargrove et al. 2004). Although the above-mentioned revision reflects that many studies have been conducted on fatty alcohols, little has been done with the fatty alcohols specific to olive pomace oil. An important study has been undertaken by Fernández-Arche et al. (2009) who evaluated the potential anti-inflammatory activity of long-chain fatty alcohols in olive pomace oil. These authors reported a total concentration of fatty alcohols of 1853 mg/Kg in this oil, constituted mainly by tetracosanol, hexacosanol and octacosanol. In addition, they confirmed that fatty alcohols isolated from olive pomace oil can decrease the production by immune cells of various inflammatory mediators (eicosanoids, cytokines and NO) by different mechanisms, including interfering at different stages of their metabolic pathways, inhibiting the activity of phospholipase A2 (PLA2) and the expression of inducible NOS (iNOS). These results suggested that fatty alcohols may confer a protective function to olive pomace oil against inflammatory damage, which is inherent in many pathological processes.

As far as the authors know, there are no human intervention studies to assess the activity of fatty alcohols contained in olive pomace oil on cardiovascular function. All the same, considering the content of these compounds in olive pomace oil (as high as 1853 mg/Kg) (Fernández-Arche et al. 2009) and that 50 mL is a recommendable amount of monounsaturated oil to consume daily, this implies a daily intake of 93 mg of long-chain fatty alcohols. Moreover, the size of the main fatty alcohols (C24 - tetracosanol, C26 - hexacosanol and C28 - octacosanol) are within the range of C24-C34, which are recognized to improve the lipid profile in humans after a consumption of 5-20 mg long-chain fatty alcohols. Therefore, olive pomace oil is an important dietary source of fatty alcohols and it would be of great interest to confirm this benefit on cardiovascular function in addition to other complementary biological activities through the development of long-term intervention studies in humans.

Phytosterols, tocopherols and their biological properties

Two important components of olive pomace oil are phytosterols and tocopherols. Actually, olive pomace oil shows the highest level of phytosterols (1800 and 3000 mg/Kg) compared with the rest of olive oils (extra virgin olive oil, virgin olive oil and olive oil, with contents of phytosterols ranging between 800 and 2400 mg/Kg) (Antonopoulos et al. 2006) (Table 1). β-sitosterol is by far the most abundant (>93%), followed by campesterol, Δ7-stigmastenol, stigmasterol and brassicasterol.

On the other hand, tocopherols or vitamin E are also abundant in olive pomace oil (Table 1). The term vitamin E comprise eight compounds found in nature (α-, β-, γ-, and δ-tocopherol, and α-, β-, γ, and δ-tocotrienol). Of the eight naturally occurring forms of vitamin E, α-tocopherol is the most abundant in foods, including olive pomace oil, being also the main form retained in human plasma (Péter et al. 2013). Although during the refining process part of the tocopherol content is lost, olive pomace oil contains about 200 mg/Kg, which is increased in the final commercial olive pomace oil product due to the contribution of tocopherol contained in the virgin olive oil added to the refined olive pomace oil. Therefore, addition of up to 200 mg/kg of α-tocopherol (E307) is permitted (CEE 2011) in olive pomace oil, since this category of olive-extracted oil likely contains more tocopherols than the commercialized olive oil or virgin olive oil (up to 300 mg/Kg) (Table 1).

Numerous studies have shown that dietary supplementation with phytosterols reduces cholesterol levels by either inhibiting cholesterol absorption (Quilez et al. 2003) or by decreasing production of Apolipoprotein B (apoB) in both liver and intestine (Ho and Pal, 2005). Specifically, EFSA has admitted that a daily intake of sterols-stanols of 1.5–3 g for 2–3 weeks can reduce LDL-cholesterol by 11% and, therefore, reduce the risk of coronary heart disease (EFSA 2012a). It is considered a harmless ingredient with no observed signs of toxicity at the doses recommended by EFSA or even higher (Hepburn et al. 1999). Considering the average content of sterols present in olive pomace oil (1800–3000 mg/kg) and the reference intake of daily consuming 50 mL of olive oil, also extended to olive pomace oil, a daily intake of around 0.2 g of phytosterols may be estimated. This amount is still far from the value recommended by EFSA (1.5–3 g/day), but even so it would help control or reduce blood cholesterol levels in combination and complementing to the activity of MUFA and other minor components of olive pomace oil.

Animal studies suggest that phytosterols reduce atherosclerosis (John, Sorokin, and Thompson 2007), but this association is less clear in humans (Hansel et al. 2011). Thus, with the exception of subjects with sitosterolemia (a disease caused by mutations in the intestinal transporters that alter normal intestinal absorption of sterols), recent studies on a large number of volunteers showed no relationship between intake of phytosterols and a lower incidence of cardiovascular disease, or the said association was even negative, suggesting the need to undertake new long-term studies in humans to determine the bioactivity of phytosterols, other than reducing the level of blood cholesterol (Hansel et al. 2011).

Data on the effect of these compounds on vascular function are scarce, outstanding the double-blind crossover study developed by De Jongh et al. (2003). These authors administered daily, during 4 weeks, 2.3 g of a mixture of phytosterols (β-sitosterol, campesterol, stigmasterol and others) to 41 children (5–12 years) with a family history of hypercholesterolemia. The results showed a significant decrease in total cholesterol (11%) and LDL-cholesterol (14%) compared with the placebo group, although no effect on endothelial function was observed.

Another noteworthy in vitro study on the anti-inflammatory activity of phytosterols was conducted by De la Puerta, Martinez-Dominguez, and Ruiz-Gutierrez (2000). These authors observed anti-inflammatory effects of β-sitosterol reducing ear edema in mice more efficiently than other compounds such as oleuropein or hydroxytyrosol. Moreno (2003) evaluated the effect of β-sitosterol on macrophages and observed a reduction in the production of oxygen radicals and the release of arachidonic acid, reducing inflammation. However, many of the results observed in in vitro studies are not extrapolated to in vivo studies; therefore, long-term placebo-controlled studies in humans are still needed to clarify this and other aspects of the bioactivity of phytosterols (Derdemezis et al. 2010).

As for tocopherols, it is well-known that vitamin E is a powerful antioxidant that prevents propagation of free radicals in membranes and plasma lipoproteins. Therefore, α-tocopherol prevents lipid peroxidation and preserves cellular membrane integrity. Vitamin E deficiency can be due to fat malabsorption disorders or genetic abnormalities that affect vitamin E transport. Severe deficiency symptoms include ataxia, peripheral neuropathy, muscle weakness and damage to eye retina. This essential role of vitamin E as an antioxidant in the human body has recently been underlined by EFSA (EFSA 2010b).

Vitamin E intake recommendations are based on estimates for the minimum levels needed to avoid deficiency symptoms. In the US, the Recommended Daily Allowance (RDA) is 15 mg α-tocopherol in adults. In Europe, EFSA set the adequate intake of vitamin E for adults at 13 mg/day for men and 11 mg/day for women (EFSA 2015). However, most reported dietary intake values worldwide are below RDA (Péter et al. 2015). Possible strategies to increase vitamin E intake are encouragement of consumption of vitamin E-rich food sources (e. g. vegetables, dairy products, eggs), adequate fortification of food products (e. g. vegetable oils), and supplementation. In this sense, the daily consumption of 50 mL of olive pomace oil, containing up to 185-200 mg/Kg of tocopherol, would represent an intake of 10 mg of vitamin E. Therefore, olive pomace oil is an interesting source of vitamin E.

However, it is important to be aware of the adverse effects of tocopherols on prothrombin times and factors related to blood clotting (Booth et al. 2004). Although these effects have only been observed at high doses (IOM. Institute of Medicine 2000), individuals with already compromised blood clotting capacity or with low vitamin K status are the most susceptible to vitamin E (Corrigan and Ulfers, 1981; Diplock, 1995). Apart from this consideration, clinical trials have not provided evidence that regular intake of vitamin E supplements prevents cardiovascular disease or reduces morbidity and mortality by cardiovascular events. However, participants in these studies have been largely middle-aged or elderly individuals with established heart disease or risk factors for heart disease. Therefore, understanding the effectiveness of vitamin E in preventing

coronary heart diseases might require longer studies in younger participants.

In short, olive pomace oil is an important dietary source of vitamin E which covers the recommended daily intake, leaving aside the effects of high doses of vitamin E on health as results are controversial.

Phenolic compounds and their biological activity

The content of phenolic compounds in olive pomace oil (50-150 mg/Kg) is small compared to that of virgin or extra virgin olive oils (200-800 mg/Kg) (Mateos et al. 2001; García et al. 2003; Brenes et al. 2004) (Table 1). The most abundant phenolic compounds found in olive oils are secoiridoid derivatives (the dialdehydic form of decarboxymethyl ligstroside aglycone, the dialdehydic form of decarboxymethyl oleuropein aglycone, the aldehydic form of oleuropein aglycone, and the aldehydic form of ligstroside aglycone) and lignans (pinoresinol and 1-acetoxypinoresinol), together with phenylethyl alcohols (hydroxytyrosol and tyrosol) formed by hydrolysis of their corresponding secoiridoid derivatives, phenolic acids (hydroxybenzoic and hydroxycinnamic acid derivatives), and flavonoids (apigenin and luteolin) (Mateos et al. 2001; Bendini et al. 2007). In the case of crude olive pomace oil, the most relevant phenolic compounds are hydroxytyrosol, hydroxytyrosol acetate, tyrosol, catechol, 4-ethylphenol, vanillin, and the lignans pinoresinol and 1-acetoxypinoresinol. However, lower amounts are present in olive pomace oils extracted with hexane, being hydroxytyrosol, its acetate and lignans the most representative phenolic compounds in refined olive pomace oil (Brenes et al. 2004).

Olive oil polyphenols have widely demonstrated to have potent health benefits (Visioli and Bernardini 2011; Martín-Peláez et al. 2013; Hu et al 2014, among others). Although the following studies refer to virgin or extra virgin olive oil, the bioactivity of phenols can be extrapolated to olive pomace oil, bearing in mind that the concentration of phenols and the presence of other minor components are different. A brief summary of the most relevant studies will be described.

One of the most accepted health properties of polyphenols in virgin or extra virgin olive oil is their effect on cardiovascular disease. These properties are associated with their antioxidant, hypocholesterolemic, antithrombotic, antiatherogenic and hypotensive properties, in addition to the ability to improve endothelial function and vascular response (Martín-Peláez et al. 2013). Despite the numerous studies undertaken that demonstrate these and other beneficial properties (Visioli and Bernardini 2011; Hu et al, 2014), the EFSA has only approved to date a health claim on the capacity conferred to the intake of 5 mg/day of olive oil phenols to prevent oxidation of LDL (EFSA 2011). Other effects such as increasing the concentration of HDL have not been clearly substantiated and a cause-effect relationship has not been admitted (EFSA 2012b).

Other benefits not recognized by EFSA for lack of sufficient studies (EFSA 2011) are related to the hypotensive and anti-inflammatory activities of polyphenols, although new results that support both properties have recently been published. Thus, an improvement in blood pressure and endothelial function in pre- and hypertensive volunteers with early signs of atherosclerosis has been associated with the intake of olive oil rich in phenolic compounds (Moreno-Luna et al. 2012; Valls et al. 2015; Widmer et al. 2013). Improved endothelial function is related to decreased levels of thromboxane B2 and leukotriene B4 described in healthy (Bogani et al., 2007) or hyperlipidemic volunteers (Visioli et al. 2005) as well as decreased plasminogen activator inhibitor-1 (PAI-1) and activated factor VII (FVIIa) in hypercholesterolemic patients (Ruano et al. 2007). There is controversy on the effects of phenolic compounds on vascular cell adhesion molecules (sICAM-1 and sVCAM-1), with the observation of a postprandial decrease after virgin olive oil intake (Pacheco et al. 2007), while the sustained consumption in patients with stable coronary disease showed no changes in their plasmatic concentration, although levels of pro-inflammatory cytokines and C-reactive protein (CRP) significantly improved (Fitó et al. 2008). More recently, the ability of virgin oil olive oil to modulate the expression of pro-atherogenic genes related to inflammation (IFNy, ARHGAP15 and IL7R) and oxidative stress (ADRB2) in mononuclear cells of peripheral blood has been confirmed (Konstantinidou et al., 2010).

The consumption of virgin and extra virgin olive oil rich in polyphenols is also beginning to be associated with a decreased risk of type 2 diabetes by reducing fasting glucose in healthy volunteers (Oliveras-López et al. 2012), as well as preventing cytotoxic amyloid aggregation of human amylin (Rigacci et al. 2010). The benefits described on cardiovascular health and type 2 diabetes have relevance in the so-called metabolic syndrome, which is increasingly prevalent in Western countries. The intake of virgin or extra virgin olive oil may be a strategy to combat this syndrome, with recent observation of a decrease in the oxidative damage to lipids and DNA in individuals with metabolic syndrome adhering to the Mediterranean diet, based on extra virgin olive oil as a source of fat (Mitjavila et al. 2013). In addition, the ability of phenols of extra virgin olive oil to positively modulate the expression of various genes involved in inflammatory pathways in the postprandial period in patients with metabolic syndrome has also been demonstrated (Camargo et al. 2010).

Scientific evidence from preclinical and clinical studies on the health effects of olive pomace oil related to health

So far, this review has focused on the effects of pure olive pomace oil's minor compounds or fractions enriched in these bioactive components, since much of the literature on oil pomace oil is focused on these components. However, this section will focus on clinical and preclinical studies developed with olive pomace oil, although such studies are scarce and have been developed mostly in animal experiments.

A study by Perona et al. (2005) in rats fed for three weeks with monounsaturated oil diets (high oleic sunflower

oil, olive oil and olive pomace oil) showed that consumption of olive pomace oil protects the liver against oxidation, proving to be slightly more potent than other oils, possibly due to its content of minor components such as triterpenes. A later study of this group, conducted in mice fed for 8 weeks with fat diets (fish oil, refined olive oil and olive pomace oil) confirmed the antioxidant character of olive pomace oil, partially preventing the formation of reactive oxygen species in stimulated murine macrophages (De la Puerta et al. 2009). Both studies demonstrated the activity of olive pomace oil preventing oxidative stress damage.

In a study developed in mice lacking ApoE, the influence of olive pomace oil showed to prevent atherosclerosis. Thus, three groups of mice were tested, one consuming a standard diet (control group), another a standard diet enriched with virgin olive oil and the third group fed on a standard diet enriched with olive pomace oil. The results showed that mice in the olive pomace oil enriched-diet had lower indexes of atherosclerotic lesions compared to the other diets, accompanied by an improvement in the lipid profile and a lower level of oxidative stress. Although both vegetable oils had the same level of MUFA and squalene, they differed in their content of phytosterols, tocopherols and triterpenes, among others, which were higher in olive pomace oil. Therefore, the authors concluded that the minor components of olive pomace oil go beyond the effect of the phenolic compounds of virgin olive oil, and were responsible for reducing the development of atherosclerosis in apoE knockout mice (Acín et al. 2007).

Moreover, it has been shown that the consumption of diets rich in olive pomace oil enhances the functionality of blood vessels in hypertensive rats and in animals with normal blood pressure (Rodriguez-Rodriguez et al. 2007). The daily intake for 12 weeks of high-fat diets containing 15% refined olive oil, olive pomace oil containing 200 mg/Kg of OA or olive pomace oil supplemented with 800 mg/Kg OA, showed an improvement in dilation of the arteries, particularly in those animals that had taken the fortified oil. The mechanisms associated with this improvement were enhanced expression of the endothelial nitric oxide synthase enzyme (eNOS), although there was no lowering of blood pressure. Later, these authors demonstrated in the same animal model (spontaneously hypertensive rats fed high-fat diets containing olive pomace oil) that, despite the absence of changes in blood pressure, olive pomace oil consumption improved the function of endothelial mesenteric arteries by increasing the endothelium-derived hyperpolarizing factor (EDHF), very altered in endothelial dysfunction of rats with hypertension (Rodriguez-Rodriguez et al. 2009).

In contrast, Valero-Muñoz et al. (2014) evaluated the effect of administering OA-enriched olive pomace oil to hypertensive rats for 8 weeks. These authors found that this oil attenuated the high blood pressure, associated with an improvement in the endothelium-dependent relaxation and increased vascular eNOS expression. Also, they observed a decrease of vascular inflammation associated with decreased expression of TNFα in the aorta in combination with a vascular antifibrotic effect, as suggested by the decreased expression of transforming growth factor beta (TGF-β) and collagen I. In addition, this oil improved cardiac hemodynamics and induced a decrease in kidney and lung weights. Therefore, this study supports, in part, the results previously mentioned and confirms the cardiovascular benefit of olive pomace oil enriched in triterpenic acids.

Moreover, olive pomace oil affects the composition of triglyceride-rich lipoproteins (TRL) in the postprandial period (the period after eating). A short-term, crossover, randomized, blind study was developed to evaluate the effect of two oils (refined olive and olive pomace oils). The fat composition was the same in both oils, but olive pomace oil contained a series of minor components (phytosterols, tocopherols, triterpenic acids and alcohols, and fatty alcohols) that were lacking in refined olive oil. After eating meals containing 70 g of each oil, fewer TRL particles but with higher triglyceride levels were observed after ingestion of olive pomace oil (Cabello-Moruno et al., 2007). In addition, these triglycerides showed a faster rinse speed than those derived from the intake of refined olive oil, associated with an increased hydrolysis by lipoprotein lipase rather than hepatic accumulation (Cabello-Moruno et al., 2014). This could present a major impact in the atherogenic processes.

Using the same postprandial study protocol as above but comparing virgin olive oil with olive pomace oil and an olive pomace oil enriched with OA (575 mg/kg), the isolated TRL particles obtained at 2 and 4 h after ingestion of each oil were put in contact with macrophages. The results showed that all the studied oils, and particularly that enriched in OA, induced changes in TRL composition, which reduced the production of inflammatory markers like monocyte chemoattractant protein-1 (MCP-1), IL-6, IL-1β, TNFα, and the expression and levels of COX-2, suggesting a beneficial effect of oleanolic acid and olive pomace oil in inflammation (Graham et al. 2012).

At the last, Cabello-Moruno et al. (2015) deepened into the activity of minor components of olive pomace oil on the expression of the VLDL receptor, also using macrophages treated with TRLs particles obtained after the intake of 70 g of olive pomace oil compared with refined olive oil without minor components. Again, the results showed the influence of olive pomace oil in the composition of the TRL particles, which increased the expression of the VLDL receptor in macrophages, although the changes did not prevent the uptake of TRL by macrophages and the formation of foam cells, a hallmark in the atherosclerotic process.

Dietary recommendations of monounsaturated fatty acids (MUFA) consumption by international bodies

A review on fatty acid intake in the adult population of 40 countries around the world and its comparison with the World Health Organization (WHO) recommendations (Harika et al. 2013) highlights the excess of fat consumption. In more than half the countries analyzed, the intake of SFA surpassed the upper limit of 10% energy intake recommended as the by different international bodies (FAO-WHO 2008; EFSA 2010a). This could be a consequence of the higher palatability of foods rich in SFA compared to that with non-

saturated fat, in addition to generally being cheaper than lipids with a healthier profile. However, most of the guidelines emphasize replacing the excess of SFA with polyunsaturated fatty acids (PUFA) to prevent cardiovascular diseases (CVD) (FAO-WHO 2008; Perk et al. 2012; Harris et al. 2009). In this sense, it is important to consider the amount of omega-6 and omega-3 in the diet, since a moderate intake of seed oils rich in linoleic acid (PUFA, omega-6) and low consumption of fish rich in omega-3 produces an imbalance in the omega-3/omega-6 ratio in the diet with the consequent risk of suffering a range of chronic diseases common in western society (Simopoulos 2008). However, in agreement with the American Heart Association (AHA) report (Harris et al. 2009) and others experts (Harris, 2006), although consumption of high amounts of omega-3 PUFA in cellular membranes is associated to a reduction of CVD risk, this does not mean that a reduction of omega-6 PUFA intake to decrease omega-6/omega-3 ratio arrives at the same result. All the same, the consumption of seed oils, such as sunflower, corn or soybean, among others, is the main source of dietary omega-6 PUFA. The replacement of seed oils by olive oil, in addition to bringing us closer to the Mediterranean dietary pattern, would result in a benefit to the omega-3/omega-6 ratio, although it is still necessary to eat more oily fish rich in omega-3. Most dietary guidelines do not establish a dietary value of reference for MUFA intake, except for the Spanish Society of Community Nutrition (SENC) (Aranceta and Serra Majem 2011), which recommends a value of 20% of dietary energy intake, and the report issued by FAO/WHO, which indicates determining a range of MUFA by subtracting trans fatty acids, SFA and PUFA from the total fat, leading to a value between 16 and 19% of the total dietary calorie intake (FAO-WHO 2008). In this sense, the average intake of MUFA in the European adult population oscillates between 11 and 18% of the total dietary calorie intake, equivalent to 24–40 g/day. Greece (22-23%), followed by Spain and Portugal, shows the highest consumption levels for its higher olive oil production and Mediterranean dietary habits (EFSA 2010a).

Therefore, the changes in the eating habits experienced in recent decades point to the need to implement strategies which promote a healthy diet to recover the traditional Mediterranean dietary habits. The content and quality of fat is one of the most controversial aspects in the field of Nutrition and Dietetics. Although all national and international recommendations and guidelines advise a total fat intake of between 20 and 35% of the total daily energy intake for the general population (FAO-WHO 2008; EFSA 2010b), the PREDIMED study (Estruch et al. 2018) opens the door for greater fat intake, as high as 40% of the total energy, as long as virgin olive oil constitutes the main source of fat in the diet (20-25%, 45-55 g/day) and this situation represents an interesting opportunity for olive pomace oil.

Concluding remarks

Olive pomace oil is a natural source of healthy compounds. The oil shares compositional parameters with other olive oil categories, which are widely recognized as health standards and one of the pillars of the Mediterranean diet. Considering the high MUFA content in olive pomace oil, with demonstrated health benefits, this type of olive oil represents an interesting alternative to reach the recommendation (FAO) of consuming 20% of total dietary energy in the form of monounsaturated fatty acids, with the total fat intake representing up to 35% of total caloric value of the diet. In this sense, nutritional guidelines recommend the consumption of olive oil instead of seed oils, yet olive pomace oil is barely mentioned. Hence scientific studies are required to strengthen the health potential of olive pomace oil, and thus improve its acceptance and recognition by consumers and health organizations.

Olive pomace oil is an important source of minor, bioactive compounds with relevant health potential. The major components identified with health implications are pentacyclic triterpenes, aliphatic fatty alcohols, sterols, tocopherols, squalene and phenolic compounds. The refining process of crude pomace oil, which cannot be avoided for commercial purposes, significantly reduces the content of phenolic compounds, whereas other minor components remain at concentrations that can induce positive health effects. In general, studies conducted with olive pomace oil are scarce and most have been carried out in animals. In fact, there few clinical studies which are acute, postprandial studies. Nonetheless, numerous in vitro and preclinical studies, developed with pure compounds or extracts isolated from plant sources have evidenced the potential of each of the aforementioned bioactive compounds in olive pomace oil, particularly preventing cardiovascular disease risk factors. Therefore, olive pomace oil, based on the quality of its monounsaturated fat and the content in minor components, can play an important role in disease prevention. In consequence, it is relevant to carry out clinical studies to evaluate the health benefits derived from sustained consumption of olive pomace oil. The results that these studies may yield should favor the acceptance by consumers who nowadays are skeptical about olive pomace oil but are increasingly conscious of the role dietary fat plays in health.

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