In: Handbook of Olive Oil
Editor: Jozef Miloš
ISBN: 978-1-53612-356-2

Chapter 7

OLEOCANTHAL: THE NEW PROMISING COMPOUND OF EXTRA VIRGIN OLIVE OILS

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ABSTRACT

Traditionally, the healthy properties of extra virgin olive oil have been attributed to its monounsaturated fatty acid high content. However, increasing evidence points out the participation of different minor antioxidant components, such as phenolic compounds. For the last decade, increasing research efforts have been made to explore the beneficial effects of these phenolic compounds on several physiological and physiopathological processes. Depending on the grade of bioavailability of each phenolic compound, they seem to carry antioxidant, anti-inflammatory and antimicrobial properties. One of the newest phenolic compounds discovered in the extra virgin olive oil food matrix is oleocanthal. The discovery of this molecule opens new perspectives on the biomedical applications of this natural compound with similar properties to those of so-called nonsteroidal anti-inflammatory drugs (NSAIDs). Oleocanthal has also exhibited antitumor properties on several tumor cell lines via different molecular mechanisms. Moreover, it has been proposed as an effective agent for the treatment of Alzheimer´s disease. Therefore, it is necessary to increase research efforts about oleocanthal and its promising applications as a preventive and/or therapeutic agent for several diseases.

Keywords: oleocanthal, olive oil, phenolic compounds, antioxidant, anti-inflammatory, anticancer agent

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OLIVE OIL: FROM ANCIENT TIMES TO TODAY

Olive oil (OO), also known as *green gold*, is one of the most relevant dietary components of the Mediterranean diet (MD), providing nutritional value and palatability to food [1]. It is obtained from olives, which come from the olive tree (*Olea europaea*), whose cultivation is thought to begin in some geographical point of the Eastern Mediterranean area between 4000-3000 B.C. Since then, the economy and culture of several civilizations has been ruled by OO, being used for culinary and medicinal purposes, among others [2].

Oil is derived from the Arabian term "Az-Zaite" (olive juice). There are references about it in a variety of ancient manuscripts, including Mycenaean tablets, the Bible and the legend of the Foundation of Athens. These texts not only gather knowledge about the types, ways of transport and culinary, religious, and industrial uses, but also perfectly illustrate how important OO was for these people: it served as a symbol of peace and glory, as fuel, medicine and a healthy ingredient. In fact, the olive tree was considered a sacred tree. This divine meaning inspired Egyptians to use olive tree leaves to make pharaohs crowns. It is noteworthy that Egyptian kings were instructed in OO uses, mainly those related to offering and nutrition. In Athens, winners of the Panathenaic games were rewarded with a full load of OO from Atica's plantation [3]. From a medicinal point of view, ancient Greeks appreciated OO so much that they used it in the treatment of about 60 health conditions (mainly wounds and burns), as Hippocrates mentions in his writings [4]. Actually, it is well established that in Mediterranean countries such as Spain, olive growing was introduced by Phoenicians or Greeks; later, this growing rose and fell with the Roman Empire, until Visigoths and Arabians recovered its glory [3].

The olive tree can have a millenarian life. In terms of cultivating conditions, it is not a very demanding tree, preferring chalky, sandy and well-drained soils also withstanding extreme temperatures [3]. OO is extracted from olives through a multi-step and mechanical-physical processing; as a result, different products with nutritional and gastronomical decreasing values can be obtained: extra virgin olive oil (EVOO), virgin olive oil (VOO), OO and olive-pomace oil. In general, OO is composed by one saponifiable fraction and another unsaponifiable fraction; the former (98-99%) comprises the triacylglycerides and free fatty acids responsible for oil acidity, such as oleic acids (60-80%), palmitic and estearic acids (13%) and linoleic and α-linolenic acids [5]. This saponifiable fraction depends on latitude, weather conditions, tree variety and olive maturity grade. According to Boskou et al. (2007) [6], OOs can be classified into two types: low linoleic-palmitic acids and high oleic acid content (for example, OOs from Spain, Italy and Greece), and those with the inverse proportion (such as Tunisians oils) [3]. On the other hand, the unsaponifiable fraction is composed by minor components (1-2%) of great biological interest, which contribute to oil color, taste and flavor. These minor components can be divided into fatty acid derivatives (mono- and diglycerides,

phosphatides, waxes and sterol esters) and other non-related fatty acid components such as hydrocarbons, aliphatic alcohols, free sterols, tocopherols (vitamin E), chlorophylls, carotenoids and polar phenolic compounds (PCs).

Traditionally, EVOO healthy properties have been attributed to its monounsaturated fatty acid high content; however, increasing evidence points to minor antioxidant components, absent in other types of seed oils, as responsible for this phenomenon. So far, up to 36 PCs have been identified in EVOO, both lipophilic and hydrophilic, which can be grouped into phenolic acids (vanillic, caffeic and ferulic acids), phenolic alcohols (tyrosol, hydroxytyrosol and oleocanthal (OC)), secoiridoids (oleuropein), hydroxy-isocromans (1-phenyl-6,7-dihydroxy-isochroman and 1-(3´-methoxy-4-hydroxy) phenyl-6,7-dihydroxy-isochroman), flavonoids (apigenin and luteolin), and lignans (pinoresinol) (Table) [3, 5, 7-9]. PCs composition and concentration (200 to 1000 mg/kg) also depend on agronomic factors, including olive tree variety and geographical location, cultivation techniques, and olive harvest maturity and processing. In this sense, the formation of some components (i.e., lignans) during the EVOO extraction process has been observed; after that, phenolic composition would be stable over the 12-18 months of EVOO maximum storage [8, 10]. In this way, PCs are suspected to be responsible for slowing rancidity, extending EVOO lifetime [10]. The proportion between oleic acid/linoleic acid/tocopherols/polyphenols significantly conditions oil stability at room and frying temperatures. In addition, most of these PCs provide bitter and astringent flavors to OO. Interestingly enough, consumers demand VOOs and EVOOs with a softer flavor; thus, supermarkets sell OOs with low content of minor components, that is, with decreased organoleptic and nutritional qualities [3].

Table. Main phenolic compounds (PCs) in extra virgin olive oil (EVOO)

Extra virgin olive oil (EVOO) phenolics	Key family member
Phenolic acids	Vanillic acid Caffeic acid Ferulic acid
Phenolic alcohols	Tyrosol Hydroxytyrosol Oleocanthal
Secoiridoids	Oleuropein
Flavonoids	Apigenin Luteolin
Lignans	Pinoresinol
Hydroxy-isocromans	1-phenyl-6,7-dihydroxy-isochroman 1-(3´-methoxy-4-hydroxy) phenyl-6,7-dihydroxy-isochroman.

OO is the main source of dietary fat in MD, with a traditional consumption of 25-50 mL in salad dressings and cooked foods [4]. The moderate consumption of OO meets the recommended intakes of monounsaturated fatty acids and linoleic acid to prevent deficiencies; its balance between omega-3 and omega-6 fatty acids is better than other vegetable oils [3]. The OO food matrix is suspected to prevent the breakdown of the PCs prior to absorption in the gastrointestinal tract. In general, humans absorb most of the PCs with varying rates of metabolism. Poorly absorbed OO phenolics seem to exert antioxidant properties at local level, as supported by research about the free radical scavenging capacity of these compounds in both the fecal matrix and intestinal epithelial cells. Furthermore, unabsorbed PCs may act as antimicrobial agents in the gastrointestinal tract. Hence, assuming that PCs absorption rate is in the range of 40%–95%, Mediterranean populations might be consuming between 4–9 mg/day of the PCs present in OO [9].

Finally, it should be taken into account that OO phenolics also have hypolipemiant, anti-atherogenic and anti-inflammatory properties. Thus, the whole OO food matrix may act in the organism at several levels, playing an essential role in the prevention of diseases related to oxidative stress, including cardiovascular and neurodegenerative diseases and cancer [11-13].

HEALTHY EFFECTS OF EXTRA VIRGIN OLIVE OIL PHENOLIC COMPOUNDS

Over the last decade, increasing biomedical research has been performed to study EVOO PCs on several physiological and physiopathological conditions. Globally, they seem to exert healthy effects thanks to its antioxidant, anti-inflammatory and antimicrobial properties; although this is going to depend on the bioavailability of each compound in our organism. In terms of PCs metabolism, it has been observed that the most present metabolites in urine are produced from hydrotyroxol, oleuropein, aglycone and OC versus those deriving from other PCs such as tyrosol, luteolin, apigenin, pinoresinol and acetoxypinoresinol. As commented previously, antioxidant and antimicrobial properties seem to depend on the degree of absorption at gastrointestinal level [14].

Concerning antimicrobial properties of EVOO PCs, *in vitro* studies show a synergistic effect against the pathogenic bacteria *E.coli*, *H. pylori* y *L. monocytogene*, among others; however, antimicrobial effects have also been registered against beneficial bacteria such as *L.acidophilus* y *B. bifidum* [14]. EVOO PCs might maintain balance of normal intestinal microbiota, which metabolizes these compounds in the lower gastrointestinal tract. Likewise, it should be mentioned that antimicrobial activity could

be related to anti-inflammatory effects of EVOO CFs, as derived from studies about the connection between inflammatory response and gut pathogens in obesity and atherosclerosis [15].

On the other hand, the antioxidant effect of EVOO PCs may be responsible for the improvement of cardiovascular health, among other examples. Some of the proposed mechanisms underlying this healthy effect are: protection against lipid peroxidation of low density lipoproteins (LDL), increased cholesterol levels of high density lipoproteins (HDL), and decreased arterial pressure or inhibition of platelet aggregation [10]. Consequently, the European Food Safety Authority [16] claims that the daily intake of 5 mg/kg of PCs decreases the risk of cardiovascular disease. Other antioxidant mechanisms of EVOO PCs have been observed as well, such as increased total antioxidant capacity in serum, increased erythrocyte glutathione peroxidase activity and regulation of balance between glutathione and reduced glutathione [14].

With regard to anti-inflammatory properties, EVOO consumption might also attenuate systemic inflammatory response decreasing the risk of chronic inflammatory diseases. *In vitro* studies about oleuropein aglycone show how this polyphenol seems to down-regulate expression of tumor necrosis factor alpha (TNF-α) in monocytes [17]. Moreover, Rosignoli et al. (2013) [18] have demonstrated that other EVOO PCs are able to influence inflammatory response by modulating monocyte function, such as production of superoxide anion (O_2^-), prostaglandin E2 (PGE2), TNF-α and expression of cyclooxygenase 2 (COX2). Recently, one of the most promising EVOO PCs with similar anti-inflammatory properties to those from nonsteroidal anti-inflammatory drugs (NSAIDs) is OC, which is going to be discussed with more detail in the next section.

Overall, previously commented properties may translate to the higher longevity observed in Mediterranean populations. In fact, research of Cañuelo et al. (2012) [7] showed how tyrosol administration in *Caenorhabditis elegans* induced the rise in life expectancy of the nematode. As authors point out, this anti-ageing effect would be mediated by resistance to high temperatures and oxidative stress, as it can be concluded from results about compounds related to heat shock (HSF-1) and insulin pathways (DAF-2 and DAF-16).

Healthy properties of EVOO and its components are therefore supported by a large body of scientific evidence. It can also be assumed that these healthy effects could be achieved by EVOO quantities normally consumed as part of the MD [14].

Oleocanthal: A Multiterapeutic Agent

A dialdehydic form of (−) deacetoxy-ligstroside aglycone (Figure) was first identified in EVOO by Montedoro et al. (1993) [19]. A decade later, Andrewes et al. (2003) [20]

determined that this compound was responsible for the burning pungent sensation caused by the consumption of certain EVOOs.

In a brief communication published in Nature, Beauchamp et al. (2005) [21] linked this perception with those caused by solutions of the NSAID ibuprofen and thus, with a possible shared pharmacological activity. This naturally occurring phenolic secoiridoid was named OC (*oleo-* for olive, *-canth-* for sting, and *-al* for aldehyde) and its chemical structure was related to the secoiridoid glycosides ligstroside and oleuropein. As suspected by authors, *de novo* synthetics enantiomers of OC dissolved in non-irritating corn oil, mimicked irritation observed in EVOO, confirming that sole OC, and not a mixture of compounds, was responsible for this sensation; it was also found to cause a dose-dependent inhibition of COX-1 and COX-2 activities but had no effect on lipoxygenase *in vitro*. Therefore, despite structural dissimilarity, it was demonstrated that both OC and ibuprofen act through prostaglandin biosynthesis pathway. Authors pointed out that OC could be used in the treatment of some diseases; an effective dose of up to 9 mg of OC/day (assuming a consumption of 50 g of EVOO containing up to 200 µg/ml OC) would correspond to about 10% of the ibuprofen dosage currently recommended for adult pain relief. Two research groups subsequently described the first total synthesis of (−) enantiomer of OC (patent CA2607977 A1) [22, 23] and patented a method to enrich an extract containing this interesting PC [24].

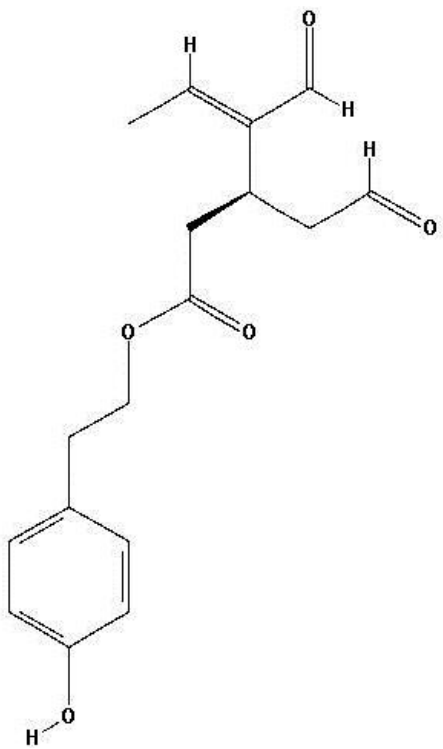

Figure. Chemical structure of oleocanthal.

OC represents about 10% of total PCs in EVOO; it would be present in those oils (sometimes referred as “one cough” or “two cough oils”) which are too bitter and pungent for most consumers [25, 26]. It must not be forgotten that other PCs such as vanilloid compounds may be also responsible for the pungency sensation elicited by EVOO [27]. The sensorial experience caused by OC is exceptionally located in the oropharyngeal region of the oral cavity; it seems to be positively correlated with OC concentration and its biological activity. According to Peyrot de Gachons et al. (2011) [26], OC triggers this pungency sensation through the activation of the thermo-transient receptor potential cation channel, subfamily A, member 1 (TRPA1), which is differently expressed in afferent trigeminal fibers. This activation may occur via a different mechanism from most electrophilic agonists of TRPA1. Yet, some interindividual differences in this perception have been observed [21, 27, 28]. Following the hypothesis of Fischer et al. (1965) [29] about proportionality between perception intensity, and pharmacological properties, Parkinson et al., (2014) [4] propose that these differences among individuals may be due to variation in local expression of TRPA1s, leading also to different inflammatory responses in tissues such as muscle.

Although OC is not so affected by heat than other EVOO PCs (hydroxytyrosol, oleuropein and oleuropein aglycon), a significant decrease in its biological activity has been observed after extended heating time (up to 31%). Concerning to the degradation process, the authors of this study suggest that OC’s chemical structure (number of hydroxyl groups bonded to the aromatic ring) and consequent antioxidant activity may be partially responsible for the differences observed between PCs [30]. As commented previously, it has been reported that after a high intake of EVOO, one of the most abundant metabolites found in human urine are derived from OC, among other EVOO PCs [31].

The MD which includes a regular consumption of EVOO, has been associated with a lower risk of suffering from several pathological conditions including cardiovascular and cerebrovascular diseases, diabetes mellitus, metabolic syndrome, cancer and neurodegenerative diseases [8]. The following provides current evidence about the effect of OC on high prevalent diseases, such as neurodegenerative diseases, cancer and inflammatory diseases.

Alzheimer’s Disease

Regarding the effect of MD on brain health, several studies have indicated that OO may delay age-related cognitive decline [32]. Furthermore, research in Mediterranean and non-Mediterranean countries showed a lower incidence of Alzheimer’s disease (AD) in populations with higher adherence to MD [33-35]. Apart from unsaturated fatty acids, it is likely that OC and other PCs contained in EVOO exert a neuroprotective action that

has not been completely elucidated yet. So far, a few *in vitro* studies have been performed to clarify the effects of OC on the two major types of hallmark lesions of AD: neurofibrillary tangles and β-amyloid senile plaques.

Firstly, Li et al. (2009) [32] demonstrated that OC is able to inhibit in a potent and selective manner tau fibrillization by forming an adduct with lysine residues via initial Schiff base formation. It is noteworthy that, for this inhibitory effect, both aldehyde groups of OC seem to be essential. Following this observation, Monti et al. (2011) [36] provided new insights about mechanism of inhibition of tau fibrillization mediated by OC.

On the other hand, Pitt et al. (2009) [37] reported that OC interacts with forming or pre-formed amyloid β-derived diffusible ligands (ADDLs) altering their structure. This interaction might be responsible for an increasing immunoreactivity and the subsequent clearance by oligomer-specific antibodies from synapses, thus avoiding synaptic toxicity. Therefore, these results indicate the potential of OC to be used in diagnosis and immunotherapy of AD. Related to β-amiloide senile plaques, a recent *in vitro* and *in vivo* study conducted by Abuznait et al. (2013) [38] showed that OC treatment up-regulated expression and activity of P-glycoprotein (P-gp) and LDL lipoprotein receptor related protein-1 (LRP1) in cultured mice's brain endothelial cells, two major transport proteins responsible for removal of β-amyloid peptide across the blood-brain barrier. Moreover, the authors performed the first *in vivo* assay with OC, which was intraperitoneally administered (10 mg/kg/day, twice daily over 2 weeks) to C57BL/6 wild-type mice. Results not only confirmed *in vitro* observations about enhanced clearance, but also showed up-regulation of β-amyloid peptide degrading enzymes, such as insulin degrading enzyme (IDE) and neprilysin (NEP) due to OC treatment. Most recently, Qosa et al. (2015) [39] investigated for the first time the effect of oleocanthal in TgSwDI mice, an animal model of AD. OC administration (5 mg/kg/day, daily over 4 weeks) significantly decreased amyloid load in the hippocampal parenchyma and microvessels of mice. OC enhanced cerebral clearance of Aβ, not only up-regulating expression of P-glycoprotein and LRPI at the blood-brain barrier but also activating the ApoE-dependent amyloid clearance pathway in the mice's brains. On the other hand, it was also observed that OC treatment reduced astrocytes activation and IL-1β levels. It should be taken into account that mTOR inhibition may also be involved in the neuroprotective effect of OC [40].

Therefore, evidence points out that OC is a promising therapeutic agent for the treatment of AD, acting on both tau fibrillization and β-amyloid aggregation. This broad effect seems not to be shared by any other NSAIDS, including aspirin, ibuprofen and naproxen, which have been extensively investigated for their beneficial effects on AD [32].

Cancer

Once again, epidemiology data from Mediterranean populations highlight that there exists a lower incidence of many types of cancer (breast, prostate, lung and gastrointestinal cancer) compared to non-Mediterranean populations. It has been established that EVOO PCs exert antitumor effect, as derived from *in vitro* studies in several tumor cell lines [4, 41, 42].

In particular, OC has shown to exert antitumor effects interfering with multiple pathways and stages (proliferation, migration, and invasion) of cancer. The first results about OC anti-cancer properties were obtained in mouse epidermal JB6 Cl41 cells; it was observed that OC treatment suppressed the proliferation and malignant transformation of this tumor cell line by inhibiting the extracellular signal-regulated kinase (ERK) pathway [43]. The same year, Elnagar et al. (2011) [44] confirmed that OC had significant anti-proliferative, anti-migratory and anti-invasive effects in both human breast (MDA-MB-231) and prostate (PC3) cancer lines, likely due to its ability to inhibit c-Met phosphorylation. Later, it has been observed that OC acts as a potent inhibitor of heat shock protein 90 (Hsp90), a chaperone involved in different cancer hallmarks. In fact, Margarucci et al. (2013) [45] reported a statistically significant reduction of two Hsp90 client proteins Akt and Cdk4 levels as consequence of Hsp90 inhibition by OC treatment in human macrophage cell line U937.

Recently, it has been confirmed that OC potentially disrupts the pathogenesis of c-Met kinase related malignancies, a proto-oncogene receptor which is well known to have a significant oncogenic role in many tumors. Hence, in the study conducted by Akl et al. (2014) [46], OC treatment caused a dose- and time-dependent suppression of the growth of three different human breast cancer cell lines (MDA-MB-231, MCF-7 and BT-474); on the contrary, no remarkable effects were recorded on the viability and growth of non-tumorigenic human MCF10A mammary epithelial cells. Data suggest that this inhibitory effect might be associated with the blockage of the activation of c-Met receptor tyrosine kinase by its natural ligand hepatocyte growth factor (HGF). As expected, the subsequent activation of downstream effectors of HGF/c-Met axis was also affected, including Akt, mitogen-activated protein kinase (MAPK), cyclin D1, cyclin-dependent kinase 6 (CDK6) and the cyclin-dependent kinase inhibitor (CKI) proteins p21 and p27. Moreover, in the highly metastatic MDA-MB-231 line, OC induced G1 cell cycle arrest and dose-dependent inhibition on HGF-induced migration and invasion signals. It was also observed that *in vitro* cell cycle arrest by OC triggers apoptosis through caspase-8-dependent pathway, which results in activation of caspase-8, cleavage of death domain kinase (RIP) and caspase-3, leading to the proteolytic cleavage of Poly (ADP-ribose) polymerase (PARP) and activation of programmed cell death; in addition, this pro-apoptotic effect was associated with a marked reduction of the total c-Met protein expression. On the other hand, antimigratory and anti-invasive activities of OC were

associated with suppression of Brk/paxillin/Rac1 signaling pathway. Furthermore, related to the effect of OC treatment on epithelial-to-mesenchymal transition (EMT) of the above-mentioned breast cancer cell lines, marked increases in the expression of the epithelial markers E-cadherin and zonula occludens 1 (Zo-1) were observed, whereas decreased levels of the mesenchymal marker vimentin in MDA-MB-231 cells were recorded. Globally, it can be concluded that OC seems to stabilize the epithelial phenotype reducing mesenchymal phenotype in breast cancer cells; moreover, reduction in mammary cancer cell scattering, motility, and invasion due to OC treatment point out a potential role of this PC in stabilizing cell to cell adhesion. Authors also performed *in vivo* confirmatory testing of OC antitumor potential using a MDA-MB-231 xenograft growth in female athymic nude mice. Results showed that OC administration resulted in the suppression of tumor growth, decreasing cancer cell proliferation as indicated by reduction of Ki-67 and CD31 levels. Most recently, Khanfar et al. (2015) [40] has reported that OC is also able to suppress mammalian Target of Rapamycin (mTOR) phosphorylation without affecting its total levels in metastatic breast cancer cell line MDA-MB-231. Thus, this inhibitory mechanism might partially explain not only antitumor properties but also neuroprotective effect of OC. To further explore the mechanism by which OC induces cell death in cancer cells, Legendre et al., (2015) [42] conducted a study on PC3 (prostate), MDA-MB-231 (breast), and BxPC3 (pancreatic) tumor cell lines. Authors reported that OC induced loss of viability in cancer cells in a dose-dependent manner within 30 minutes after OC treatment; it was also observed that upon different *in vitro* conditions (presence/absence of serum) OC promoted primary necrosis or a combination of apoptosis and secondary necrosis (via lysosomal membrane permeabilization (LMP)), respectively. This phenomenon seems to be mediated by inhibition of acid sphingomyelinase (ASM) activity. Interestingly, in the non-cancerous BJ human fibroblasts OC treatment induced G1 cell cycle arrest which is resumed after 72h without this affecting their viability. It has also been found that OC treatment inhibit proliferation, migration, invasion, and metastasis of human hepatocellular carcinoma *in vitro* and *in vivo*, without affecting viability of normal liver LO2 cells [47]. Results obtained in this study confirm previous evidence about OC inhibitory effect is mediated by inducing cell cycle arrest and apoptosis, up-regulating cleavage of PARP and caspase-3. It was also observed that phosphorylated signal transducer and activator of transcription 3 (STAT3) levels were decreased after OC exposure, which leads to decreased levels of the cell cycle protein cyclin D1, the anti-apoptotic proteins Bcl-2 and survivin, and the invasion-related protein matrix metalloproteinase-2 (MMP-2). It can be deduced from experimental evidence that OC suppresses STAT3 activation by IL-6 and modulates expression of positive (p-JAK1 and p-JAK2) and negative (SHP-1) STAT3 regulators. Besides the above-mentioned molecular mechanisms, related to EMT this study describes for the first time that OC reduced Twist protein levels and mRNA expression due to a reduced binding of STAT3 to the Twist gene promoter.

Finally, it should be mentioned that other potential mechanisms different from direct suppression of the HGF/c-Met signaling pathway may also be involved. It has been reported that OC exerts its anti-inflammatory activity through inhibition of 5-lipoxygenase [48], macrophage inflammatory protein 1- alpha (MIP-1 a) and interleukin-6 (IL-6) [49, 50], as discussed in the next subsection.

Inflammatory Diseases

Nowadays, it is accepted that inflammation contributes to the onset and progression of joint and bones diseases, including osteoarthritis and rheumatoid arthritis. Scientific evidence shows that synovial fibroblasts, synovial macrophages and chondrocytes produce pro-inflammatory mediators that lead to the up-regulation of cartilage-degrading factors. Among others, this degenerative process is mediated by the overexpression of the inducible nitric oxide synthase (iNOS) in chondrocytes and the subsequent nitric oxide (NO) overproduction [49]. Recent statistical data from the World Health Organization (2010) [51] showed that Mediterranean populations have the lowest risk of chronic inflammatory disease in the world. It has been hypothesized that a low and chronic consumption of PCs with anti-inflammatory properties such as OC may contribute to this phenomenon [4].

It has been observed that OC and synthesized derivatives induce a dose-dependent decrease in lipopolysaccharide-induced iNOS expression in murine chondrocytes. In this way, NO and its stable end product nitrite (NO_2^-) levels were also reduced by OC treatment, with a low cytotoxic effect. It has to be emphasized that NO is thought to have a pivotal role in the cartilage degenerative process; NO_2^- has been found to be elevated in the synovial fluid and serum of patients with rheumatoid arthritis and osteoarthritis [52]. As previously commented, OC has been also demonstrated to downregulate mRNA levels of the inflammatory mediators (MIP-1α) and IL-6 in murine cultured macrophages and chondrocytes, as well as synthesis of IL-1β, TNF-α and granulocyte-macrophage colony-stimulating factor (GM-CSF). Consequently, decreased levels of proinflammatory mediators also reduce the overproduction of NO by iNOS, whose enzymatic activity depends on several stimuli including inflammatory signals [49]. Since OC acts inhibiting COX enzymes and the subsequent synthesis of prostaglandin 2 (PG2), it may also attenuate arthritic pain via this molecular pathway [4].

So far, standard pharmacological treatment based on the use of NSAIDs has failed to treat this multifactorial process and significant adverse effects have been described. Therefore, phytochemicals such as OC have to be considered as a safe and effective therapeutical option for the management of degenerative joint and bones diseases [49].

FUTURE CONSIDERATIONS

Currently, a large body of scientific evidence has highlighted that EVOO PC oleocanthal as a new promising therapeutic agent for the treatment of several health conditions, such as cancer and neurodegenerative and inflammatory diseases. In addition, oleocanthal-based compounds may mean an opportunity to increase antitumor efficacy in c-Met kinase related malignancies, as shown by Mohyieldin et al. (2016) [53]. However, as with other potential drugs, it should be taken with caution. Experts agree that i*n vitro* findings must not be totally extrapolated to *in vivo* conditions [4, 54]. It should be mentioned that rigorous toxicological and pharmacokinetic testing of OC must be performed to start with safety preclinical and clinical trials to test oleocanthal treatment efficacy in human diseases in the upcoming years. Moreover, *in vitro* results show that oleocanthal up-regulates the activity and expression of a multidrug efflux transporter such as P-glycoprotein [55], which can affect chemotherapeutic treatment in different types of patients.

This precaution needs to be even stronger when the effects of a compound removed from its natural food matrix [4, 25] are reported. Over the last decades, new nutritional knowledge has produced changes about the established paradigm related to the use of isolated compounds as drugs; currently, the application of the whole food matrix seems to be more proper due to relationship between matrix compounds. In this way, EVOO phenolics are suspected to act in a synergistic manner to achieve antioxidant, anti-inflammatory and antimicrobial activities which have been observed both *in vitro* and *in vivo*. Nevertheless, the way in which compounds interact is not yet well understood. Therefore, further research about this and other aspects of healthy effects of natural products is needed.

Last but not least, socio-economic strategies are needed to support research about OO. It must not be forgotten that the main producing countries, including Spain, Portugal, Italy and Greece, must play a central role to preserve and promote high quality EVOOs, as cultural heritage. In this way, R&D coordinated investment has to be implemented not only to protect native and eco-sustainable olive tree species but also to widen scientific knowledge about OO compounds for preventive and/or therapeutic future application. Hence, along with OO industry, public institutions (i.e., universities and technology center) become essential partners to face this challenge.

REFERENCES

[1] Mataix Verdú, J; Muñoz Alférez, MJ. Sentidos químicos y alimentación. En: Nutrición y alimentación humana. II. Situaciones fisiológicas y patológicas. Editor:

MataixVerdú J. Ergón, Majadahonda (Madrid, 2009), 877-887. [Chemical senses and diet. In: Nutrition and Human Food. II. Physiological and pathological conditions. *Editor: MataixVerdú J. Ergón*, *Majadahonda* (Madrid, 2009), 877-887.]

[2] Rhizopouhu, Sofía; Olea europaea, L. A Botanical Contribution to Culture. *American-Eurasian J. Agrie. & Environ. Sci*, (2007), 2, 382–387.

[3] Sánchez, FJ. Aceite de oliva, clave de vida en la Cuenca Mediterránea. *An. R. Acad. Nac. Farm.*, 2007, 73, 653-692 [Olive oil, key component of the Mediterranean Basin life. *An. R. Acad. Nac. Farm.*, 2007, 73, 653-692.]

[4] Parkinson, L; Keast, R. Oleocanthal, a Phenolic Derived from Virgin Olive Oil: A Review of the Beneficial Effects on Inflammatory Disease. *Int. J. Mol. Sci.*, 2014, 15, 12323-12334.

[5] López, H; Ruiz, MD; Cabrera, C. Grasas y aceites. En: Tratado de Nutrición. Tomo II: Composición y Calidad Nutritiva de los Alimentos. Editor: Gil A, *Editorial Médica Panamericana* (Madrid, 2010), 258-265 [Fats and oils. In: Treatise on Nutrition. Volume II: Composition and Nutritional Quality of Food. Editor: Gil A, *Editorial Médica Panamericana* (Madrid, 2010), 258-265.]

[6] Boskou, D. Olive oil. *World. Rev. Nutr. Diet.*, 2007, 97, 180-210.

[7] Cañuelo, A; Gilbert-López, B; Pacheco-Liñán, P; Martínez-Lara, E; Siles, E; Miranda-Vizuete, A. Tyrosol, a main phenol present in extra virgin olive oil, increases lifespan and stress resistance in *Caenorhabditis elegans*. *Mech. Ageing Dev.*, 2012, 133, 563–574.

[8] Rodríguez-Morató, J; Xicota, L; Fitó, M; Farré, M; Dierssen, M; de la Torre, R. Potential role of olive oil phenolic compounds in the prevention of neurodegenerative diseases. *Molecules.*, 2015, 20, 4655-4680.

[9] Parkinson, L; Cicerale, S. The Health Benefiting Mechanisms of Virgin Olive Oil Phenolic Compounds. *Molecules.*, 2016, 21, E1734.

[10] Servili, M; Sordini, B; Esposto, S; Urbani, S; Veneziani, G; Di Maio, I; Selvaggini, R; Taticchi, A. Biological Activities of Phenolic Compounds of Extra Virgin Olive Oil. *Antioxidants (Basel)*, 2014, 3, 1-23.

[11] Pinto, JA; Martínez, JR. El aceite de oliva y la dieta mediterránea. Servicio de Promoción de la Salud. Instituto de la Salud Pública. Dirección General de Salud Pública y Alimentación. *Consejería de Sanidad y Consumo de la Comunidad de Madrid*. Madrid, 2005 [Olive oil and Mediterranean Diet. Health Promotion. Public Health Institute. Directorate General of Public Health and Food. Department of Health and Consumption of the Regional Government of Madrid. Madrid, 2005.]

[12] Bendini, A; Cerretani, L; Carrasco-Pancorbo, A; Gómez-Caravaca, AM; Segura-Carretero, A; Fernández-Gutiérrez, A; Lercker, G. Phenolic molecules in virgin olive oils: a survey of their sensory properties, health effects, antioxidant activity and analytical methods. An overview of the last decade. *Molecules*, 2007, 12, 1679-1719.

[13] Fitó, M; de la Torre, R; Farré-Albaladejo, M; Khymenetz, O; Marrugat, J; Covas, MI. Bioavailability and antioxidant effects of olive oil phenolic compounds in humans: a review. *Ann. Ist. Super. Sanita.*, 2007, 43, 375-381.

[14] Cicerale, S; Lucas, LJ; Keast, RSJ. Antimicrobial, antioxidant and anti-inflammatory phenolic activities in extra virgin olive oil. *Curr. Opin. in Biotechnol.*, 2012, 23, 129–135.

[15] Caesar, R; Fak, F; Backhed, F. Effects of gut microbiota on obesity and atherosclerosis via modulation of inflammation and lipid metabolism. *J. Intern. Med.*, 2010, 268, 320–328.

[16] EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) Scientific Opinion on the substantiation of health claims related to polyphenols in olive and protection of LDL particles from oxidative damage (ID 1333, 1638, 1639, 1696, 2865) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. *EFSA J.*, 2011, 9, 1–25.

[17] Dell'Agli, M; Fagnani, R; Galli, GV; Maschi, O; Gilardi, F; Bellosta, S; Crestani, M; Bosisio, E; De Fabiani, E; Caruso, D. Olive oil phenols modulate the expression of metalloproteinase 9 in THP-1 cells by acting on nuclear factor-kB signaling. *J. Agric. Food. Chem.*, 2010, 58, 2246-2252.

[18] Rosignoli, P; Fuccelli, R; Fabiani, R; Servili, M; Morozzi, G. Effect of olive oil phenols on the production of inflammatory mediators in freshly isolated human monocytes. *J. Nutr. Biochem.*, 2013, 24, 1513-1519.

[19] Montedoro, G; Servili, M. Simple and hydrolyzable compounds in virgin olive oil. Spectroscopic characterizations of the secoiridoid derivatives. *J. Agric. Food Chem.*, 1993, 41, 2228–2234.

[20] Andrewes, P; Busch, JL; de Joode, T; Groenewegen, A; Alexandre, H. Sensory properties of virgin olive oil polyphenols: identification of deacetoxy-ligstroside aglycon as a key contributor to pungency. *J. Agric. Food. Chem.*, 2003, 51, 1415-1420.

[21] Beauchamp, GK; Keast, RS; Morel, D; Lin, J; Pika, J; Han, Q; Lee, CH; Smith, AB; Breslin, PA. Phytochemistry: ibuprofen-like activity in extra-virgin olive oil. *Nature.*, 2005, 437, 45-46.

[22] Smith, AB; 3rd, Han, Q; Breslin, PA; Beauchamp, GK. Synthesis and assignment of absolute configuration of (-)-oleocanthal: a potent, naturally occurring non-steroidal anti-inflammatory and anti-oxidant agent derived from extra virgin olive oils. *Org. Lett.*, 2005, 7, 5075–5078.

[23] Smith, AB; 3rd, Sperry, JB; Han, Q. Syntheses of (−)-oleocanthal, a natural NSAID found in extra virgin olive oil, the (−)-deacetoxy-oleuropein aglycone, and related analogues. *J. Org. Chem.*, 2007, 72, 6891–6900.

[24] Estanove, C; Pruvost, F; Saldmann, F. *Procede d'enrichissement d'un extrait contenant de l'oleocanthal*, (WO2008107557 A1, 2008) [Enrichment method for an extract containing oleocanthal (WO2008107557 A1, 2008).

[25] Fogliano, V; Sacchi, R. Oleocanthal in olive oil: between myth and reality. *Mol. Nutr. Food. Res.*, 2006, 50, 5-6.

[26] Peyrot des Gachons, C; Uchida, K; Bryant, B; Shima, A; Sperry, JB; Dankulich-Nagrudny, L; Tominaga, M; Smith, AB; 3rd, Beauchamp, GK; Breslin, PA. Unusual pungency from extra-virgin olive oil is attributable to restricted spatial expression of the receptor of oleocanthal. *J. Neurosci.*, 2011, 31, 999-1009.

[27] Bennett, SM; Hayes, JE. Differences in the chemesthetic subqualities of capsaicin, ibuprofen, and olive oil. *Chem Senses*., 2012, 37, 471-478.

[28] Cicerale, S; Breslin, PA; Beauchamp, GK; Keast, RS. Sensory characterization of the irritant properties of oleocanthal, a natural anti-inflammatory agent in extra virgin olive oils. *Chem. Senses.*, 2009, 34, 333-339.

[29] Fischer, R; Griffin, F; Archer, RC; Zinsmeister, SC; Jastram, PS. Weber ratio in gustatory chemoreception; an indicator of systemic (drug) reactivity. *Nature.*, 1965, 207, 1049–1053.

[30] Cicerale, S; Conlan, XA; Barnett, NW; Sinclair, AJ; Keast, RS. Influence of heat on biological activity and concentration of oleocanthal--a natural anti-inflammatory agent in virgin olive oil. *J. Agric. Food. Chem.*, 2009b, 57, 1326-1330.

[31] García-Villalba, R; Carrasco-Pancorbo, A; Nevedomskaya, E; Mayboroda, O; Deelder, A; Segura-Carretero, A; Fernández-Gutiérrez, A. Exploratory analysis of human urine by LC-ESI-TOF MS after high intake of olive oil: Understanding the metabolism of polyphenols. *Anal. Bioanal. Chem.*, 2010, 398, 463–475.

[32] Li, W; Sperry, JB; Crowe, A; Trojanowski, JQ; Smith, AB; 3rd, Lee, VMY. Inhibition of tau fibrillization by oleocanthal via reaction with the amino groups of tau. *Neurochem.*, 2009, 110, 1339–1351.

[33] Scarmeas, N; Stern, Y; Tang, MX; Mayeux, R; Luchsinger, JA. Mediterranean diet and risk for Alzheimer's disease. *Ann. Neurol.*, 2006, 59, 912–921.

[34] Feart, C; Samieri, C; Rondeau, V; Amieva, H; Portet, F; Dartigues, JF; Scarmeas, N; Barberger-Gateau, P. Adherence to a Mediterranean diet, cognitive decline, and risk of dementia. *JAMA*, 2009, 302, 638–648.

[35] Gardener, S; Gu, Y; Rainey-Smith, SR; Keogh, JB; Clifton, PM; Mathieson, SL; Taddei, K; Mondal, A; Ward, VK; Scarmeas, N; Barnes, M; Ellis, KA; Head, R; Masters, CL; Ames, D; Macaulay, SL; Rowe, CC; Szoeke, C; Martins, RN. AIBL Research Group. Adherence to a Mediterranean diet and Alzheimer's disease risk in an Australian population. *Transl. Psychiatry*, 2012, 2, e164.

[36] Monti, MC; Margarucci, L; Tosco, A; Riccio, R; Casapullo, A. New insights on the interaction mechanism between tau protein and oleocanthal, an extra-virgin olive-oil bioactive component. *Food Funct.*, 2011, 2, 423−428.

[37] Pitt, J; Roth, W; Lacor, P; Blankenship, M; Velasco, P; De Felice, F; Breslin, P; Klein, WL. Alzheimer's-associated Aβ oligomers show altered structure, immunoreactivity and synaptotoxicity with low doses of oleocanthal. *Toxicol. Appl. Pharmacol.*, 2009, 240, 189–197.

[38] Abuznait, AH; Qosa, H; Busnena, BA; El Sayed, KA; Kaddoumi, A. Olive-oil-derived oleocanthal enhances β-amyloid clearance as a potential neuroprotective mechanism against Alzheimer's disease: *in vitro* and *in vivo* studies. *ACS Chem. Neurosci.*, 2013, 4, 973-982.

[39] Qosa, H; Batarseh, YS; Mohyeldin, MM; El Sayed, KA; Keller, JN; Kaddoumi, A. Oleocanthal enhances amyloid-β clearance from the brains of TgSwDI mice and *in vitro* across a human blood-brain barrier model. *ACS Chem. Neurosci.*, 2015, 6, 1849-1859.

[40] Khanfar, MA; Taha, MO. Elaborate ligand-based modeling coupled with multiple linear regression and k nearest neighbor QSAR analyses unveiled new nanomolar mTOR inhibitors. *J. Chem. Inf. Model*., 2013, 53, 2587–2612.

[41] Menéndez, JA; Vazquez-Martin, A; Garcia-Villalba, R; Carrasco-Pancorbo, A; Oliveras-Ferraros, C; Fernandez-Gutierrez, A; Segura-Carretero, A. tabAnti-HER2 (erbB-2) oncogene effects of phenolic compounds directly isolated from commercial Extra-Virgin Olive Oil (EVOO). *BMC Cancer.*, 2008, 8, 377.

[42] LeGendre, M; Preslin, PA; Foster, DA. (-)-Oleocanthal rapidly and selectively induces cancer cell death via lysosomal membrane permeabilization. *Mol Cell Oncol.*, 2015, 2, e1006077.

[43] Khanal, P; Oh, WK; Yun, HJ; et al. p-HPEA-EDA, a phenolic compound of virgin olive oil, activates AMP-activated protein kinase to inhibit carcinogenesis. *Carcinogenesis*., 2011, 32, 545–553.

[44] Elnagar, AY; Sylvester, PW; el Sayed, KA. (−)-Oleocanthal as a c-Met inhibitor for the control of metastatic breast and prostate cancers. *Planta. Med.*, 2011, 77, 1013–1019.

[45] Margarucci, L; Monti, MC; Cassiano, C; Mozzicafreddo, M; Angeletti, M; Riccio, R; Tosco, A; Casapullo, A. Chemical proteomics-driven discovery of oleocanthal as an Hsp90 inhibitor. *Chem. Commun. (Camb).*, 2013, 49, 5844–5846.

[46] Akl, MR; Ayoub, NM; Mohyeldin, MM; Busnena, BA; Foudah, AI; Liu, YY; Sayed, KA. Olive phenolics as c-Met inhibitors: (-)-Oleocanthal attenuates cell proliferation, invasiveness, and tumor growth in breast cancer models. *PLoS One.*, 2014, 9, e97622.

[47] Pei, T; Meng, Q; Han, J; Sun, H; Li, L; Song, R; Sun, B; Pan, S; Liang, D; Liu, L. (-)-Oleocanthal inhibits growth and metastasis by blocking activation of STAT3 in human hepatocellular carcinoma. *Oncotarget.*, 2016, 7, 43475-43491.

[48] Vougogiannopoulou, K; Lemus, C; Halabalaki, M; Pergola, C; Werz, O; Smith, AB; 3rd, Michel, S; Skaltsounis, L; Deguin, B. One-Step Semisynthesis of Oleacein and the Determination as a 5-Lipoxygenase Inhibitor. *J. Nat. Prod.*, 2014, 77, 441-445.

[49] Scotece, M; Gómez, R; Conde, J; Lopez, V; Gómez-Reino, JJ; Lago, F; Smith, AB; 3rd, Gualillo, O. Further evidence for the anti-inflammatory activity of oleocanthal: inhibition of MIP-1α and IL-6 in J774 macrophages and in ATDC5 chondrocytes. *Life Sci.*, 2012, 10, 91, 1229-1235.

[50] Scotece, M; Gómez, R; Conde, J; Lopez, V; Gómez-Reino, J; Lago, F; Smith, I; Gualillo, O. Oleocanthal inhibits proliferation and MIP-1 expression in human multiple myeloma cells. *Curr. Med. Chem*., 2013, 20, 2467–2475.

[51] WHO. World Health Statistics 2010; World Health Organization: Geneva, Switzerland, 2010.

[52] Iacono, A; Gómez, R; Sperry, J; Conde, J; Bianco, G; Meli, R; Gómez-Reino, JJ; Smith, AB; 3rd, Gualillo, O. Effect of oleocanthal and its derivatives on inflammatory response induced by lipopolysaccharide in a murine chondrocyte cell line. *Arthritis Rheum*., 2010, 62, 1675-1682.

[53] Mohyeldin, MM; Akl, MR; Ebrahim, HY; Dragoi, AM; Dykes, S; Cardelli, JA; El Sayed, KA. The oleocanthal-based homovanillyl sinapate as a novel c-Met inhibitor. *Oncotarget.*, 2016, 7, 32247-32273.

[54] Scotece, M; Conde, J; Abella, V; Lopez, V; Pino, J; Lago, F; Smith, AB; 3rd, Gómez-Reino, JJ; Gualillo, O. New drugs from ancient natural foods. Oleocanthal, the natural occurring spicy compound of olive oil: a brief history. *Drug. Discov. Today.*, 2015, 20, 406-410.

[55] Abuznait, AH; Qosa, H; O'Connell, ND; Akbarian-Tefaghi, J; Sylvester, PW; El Sayed, KA; Kaddoumi, A. Induction of expression and functional activity of P-glycoprotein efflux transporter by bioactive plant natural products. *Food. Chem. Toxicol.*, 2011, 49, 2765-2772.