# Health effects of the minor components of olive oil

## *Part I Minor components and their effects for health and the stability of olive oil*

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## 1 Introduction

Olive oil is characterized by its delicate and unique flavour. This unique flavour and aroma is due to a variety of constituents that are present in very low concentrations. While the major part (>95%) of the oil consists of fatty acids bound to glycerol, the so-called triglycerides, there is a large number of constituents, which are present only in small amounts. Nevertheless, these so-called minor components are of great importance; some of them have been reported to be beneficial to human health, others improve the stability of the oil and, not least, some are responsible for the unique flavour of the oil.

The minor constituents of olive oil can be subdivided into tocopherols, phenols, flavour compounds, hydrocarbons, and sterols. In this paper the most important compounds of the first three classes will be reviewed with respect to their implications for human health and their contribution to the stability and taste of the oil. A separate fact sheet "Health effects of minor components of olive oil (II)" will deal with the hydrocarbons and sterols.

## 2 Minor components of olive oil

### 2.1 Tocopherols

Olive oil contains α–tocopherol, the tocopherol with the highest vitamin E activity, in quantities varying from 1.2 to 43 mg/100g (1-3). On average, the amount present in the oil is about 12 to 25 mg/100g, as reported by one group (3). Others found even higher values of 24 to 43 mg/ 100g (2). Obviously, the amount present in the oil depends on various factors. Although scientific material dealing with this question is relatively sparse it appears that the cultivar, the ripeness of the fruit as well as the conditions and the duration of storage should be of particular importance. Other tocopherols (β and γ) are only present in trace amounts (1;3).

### 2.2 Phenolic compounds

The olive pulp contains phenolic compounds, which are mainly water-soluble. However, small quantities are also found in the oil. The class of phenols comprises a variety of different substances. It includes simple phenolic compounds such as vanillic acid, gallic acid, coumaric acid, caffeic acid, tyrosol, or hydroxytyrosol. On average, these simple phenols account for 4.2 mg/ 100g in extra virgin olive oil and for 0.47 mg/ 100g in refined olive oil. Furthermore, olive oil contains secoiridoids such as oleuropein and ligstroside (2.8 mg/100g in extra virgin and 0.93 mg/100g in refined olive oil, respectively), or more complex molecules such as lignans (4.15 mg/100g in extra virgin and 0.73 mg/ 100g in refined olive oil, respectively) and flavonoids, e.g. apigenin or luteolin (data from (4)). The content of phenolic compounds in the oil depends on the cultivar and the ripeness of the olives at the time of harvest, e.g. the concentration of hydroxytyrosol, tyrosol, and luteolin increases with increasing maturity of the fruits (5), whereas the total amount of phenolic compounds and α–tocopherol decreases with increasing ripeness (2). Until now, there are only few investigations of the bioavailability of these substances. Visioli and colleagues found that tyrosol and hydroxytyrosol are dose-dependently absorbed in a range of 60 to 80% of the amount ingested (6).

### 2.3 Flavour compounds

More than 70 compounds are thought to contribute to the unique flavour and taste of olives and olive oil. Among these are products of oxidative degradation of unsaturated fatty acids such as aldehydes, e.g. hexanal, nonanal, 1-hexanol, or 2,4-decadienal. Furthermore, aliphatic and aromatic hydrocarbons, alcohols, ketones, ethers, esters as well as furan and thioterpene derivatives add appreciably to the odour and palatability of the oil (1).

## 3 Impact of the minor constituents on human health

### 3.1 Tocopherols

Oxidative injury is assumed to play a crucial role in the development of several diseases, e.g. coronary heart disease (CHD) and cancer, and the probability that antioxidants may protect against oxidative injury and low-density lipoprotein (LDL) oxidation has gained growing evidence in the past years.

Since the 1980s several epidemiological studies have been carried out to evaluate the relationship between the intake of vitamin E and cardiovascular disease. These studies have used high doses of vitamin E provided as supplements rather than vitamin E-rich food. It could be observed that high-dose vitamin E supplements (>67 mg α–tocopherol/d) for at least two years significantly lowered the CHD risk (risk reduction 31-65%) (reviewed in (7)). On the other hand, short-term as well as low-dose supplementations (< 67 mg/d) had no significant effects on CHD (8).

In contrast to these results from observational studies, the intervention trials completed so far have not yielded unequivocal results. In the Cambridge Heart Antioxidant Study (CHAOS) the application of 268 or 536 mg α–tocopherol per day has led to a substantial reduction in non-fatal myocardial infarction, while death from coronary heart disease and overall mortality was not reduced (9). In a secondary prevention study conducted by a group of Italian scientists the administration of 300 mg α–tocopherol per day for 3.5 years also did not reduce the risk of death or myocardial infarction (10). Last year another study was finished showing that treatment with 268 mg α–tocopherol daily for 4.5 years had no apparent effect on cardiovascular outcomes in patients at high risk for cardiovascular disease (11). Taken together, the studies conducted so far do not provide convincing evidence that vitamin E supplementation should be recommended as a general healthcare measure.

However, there are many data on beneficial effects of vitamin E on metabolic processes relevant to various diseases. Boscoboinik and colleagues showed that α–tocopherol in physiologically relevant concentrations inhibited the proliferation of vascular smooth muscle cells, a process known to be of importance in the formation of the so-called intermediate atherosclerotic lesion (12). Another group observed a reduction in the release of reactive oxygen, lipid peroxidation, interleukin-1β-secretion, and adhesion to endothelial cells in the monocytes of healthy humans after an 8-week supplementation with 800 mg/d (13). Also, platelet aggregation was found to be inhibited by the uptake of vitamin E in the range of 268 to 804 mg α–tocopherol/d (14). These effects are not related to the antioxidant property of vitamin E, as they are not shared by other lipid-soluble antioxidants. Rather, α–tocopherol appears to exert direct effects on the expression of genes such as adhesion molecules (15) or on the activity of enzymes such as 5-lipoxygenase (16) or protein kinase C (14).

These results indicate that vitamin E may exert beneficial effects with regard to cardiovascular disease by various mechanisms. However, as these studies were conducted with high-dose vitamin E supplements, it remains to be investigated whether these effects can be obtained by taking up vitamin E in the amounts naturally present in foods such as olive oil. One of the reasons why the intervention trials mentioned above have not shown convincing protective effects even of high-dose supplementation of vitamin E might be that atherogenesis is a long-lasting process and the oxidative modification of lipoproteins is thought to be an initial process of atherosclerotic lesion formation. Therefore, the true value of dietary vitamin E might not be unsheathed until long-lasting primary prevention studies have been conducted (17).

Such primary prevention studies have already been conducted in animal models of atherosclerosis. Pratico and colleagues were able to show that oxidative stress is of functional importance in the development of atherosclerosis in an animal model and that this oxidative stress and also the formation of atherosclerotic lesions in the aorta can be suppressed by oral administration of vitamin E (18). In addition, a study published last year by Terasawa et al. reported that artificially induced vitamin E deficiency increased the severity of atherosclerosis in the same mouse model (19).

In addition to its anticipated beneficial effects with regard to cardiovascular diseases vitamin E is an effective weapon against cancer. In numerous animal models, vitamin E has been found to be protective against cancer of various locations (reviewed in (20)). Furthermore, studies in humans have shown that low vitamin E levels in serum or plasma are associated with an increased risk of cancer of the lung, the uterine cervix and the prostate. The intervention trials conducted in humans until now have also yielded promising first results. Heinonen and colleagues found that a long-term supplementation (between 5 and 8 years) of 50 mg α–tocopherol per day substantially reduced prostate cancer incidence (-32%) and mortality from prostate cancer (-41%) in male smokers (21). In a study on the effect of vitamin E on premalignant lesions of the upper aerodigestive tract, it was observed that administration of high doses of α–tocopherol (268 mg/d) resulted in beneficial clinical and histologic responses (22). In the Chinese rural area of Linxian, which is known for its high cancer rates, supplementation of 30 mg α–tocopherol per day in combination with selenium (50 µg/d) and β-carotene (15 mg/d) reduced total mortality by 9%. This reduction was mainly attributable to lower cancer rates, especially stomach cancer, and the reduced risk began to arise one to two years after the start of supplementation (23).

In conclusion, the numerous studies on the health effects of vitamin E conducted so far show, that this micronutrient may be salubrious in various regards. Possibly, some of the effects will only be obtained when vitamin E is administered as a supplement in large doses. Nevertheless, vitamin E in the amounts present in olive oil is still likely to be beneficial for human health. Additionally, it is most likely, and some of the studies presented in this fact sheet (see also chapter 3.2) support this assumption, that due to synergistic effects the combination of vitamin E and the other minor components present in extra virgin olive oil will be more salubrious than the sum of the single components.

### 3.2 Phenolic compounds

Phenolic compounds have repeatedly been reported to be potent antioxidants. Owen et al. have assessed the antioxidative potential of different phenolic compounds of olive oil and found that a wide range of these components show antioxidative properties, such as hydroxytyrosol, tyrosol, caffeic acid, vanillic acid, (+)-1-acetoxypinoresinol, and oleuropein (24). Interestingly, extracts of extra virgin olive oil, but not refined olive oil, containing a mixture of known and unknown phenolics were effective at far lower concentrations than the compounds tested individually, indicating that there are synergistic effects between the individual compounds increasing the antioxidative potential of the mixture. Moreover, extracts of extra virgin olive oil had a profound suppressive effect on xanthine oxidase activity. Xanthine oxidase is an enzyme that is implicated in carcinogenesis, and xanthine oxidase inhibitors have been shown to have a chemopreventive effect on cancer cells (24). Similar observations were made with regard to LDL susceptibility to oxidation. Oleuropein and tyrosol were reported to inhibit LDL in vitro oxidation, but a far more pronounced effect was achieved with a mixture of phenolic compounds from extra virgin olive oil in comparable concentrations (25;26). Furthermore, protocatechuic acid and 3,4-hydroxyphenylethanol (DHPE) were shown to be highly effective in protecting LDL from in vitro oxidation (27). In these studies LDL was isolated and the phenolics were added to the LDL preparations in vitro. Bonanome and colleagues, however, administered meals rich in extra virgin olive oil to healthy volunteers and reported that immediately after the meal phenolic compounds (in this case tysosol and hydroxytyrosol were measured) were present in all classes of plasma lipoproteins except very low-density lipoprotein, which was accompanied by an increase in their antioxidative capacity (28). Also, DHPE was found to counteract the cytotoxic effect of reactive oxygen metabolites on cells, therewith preventing cell damage (29). Deiana and colleagues observed that hydroxytyrosol inhibits DNA damage by peroxinitrite (30).

In addition to these antioxidative effects, phenolic compounds of extra virgin olive oil have a distinct anti-inflammatory effect. Petroni and colleagues reported that hydroxytyrosol inhibits the formation of a pro-inflammatory eicosanoid, leucotriene B4, in a dose-dependent manner (31). De la Puerta found that not only hydroxytyrosol, but also tyrosol, oleuropein, and caffeic acid inhibit leucotriene B4 formation by reducing the activity of the catalysing enzyme 5-lipoxygenase (32). This enzyme was also reported to be inhibited by olive fruit extract, and the substances found to be responsible for this effect were DHPE, oleuropein, and caffeic acid (33). Another interesting and possibly salubrious effect of olive oil phenols has been reported by Petroni and colleagues. Possibly also via an inhibitory effect on 5-lipoxygenase DHPE, and to a lesser extent also oleuropein, luteolin, apigenin, and quercitin, inhibit platelet aggregation and platelet eicosanoid formation in vitro (34).

### 3.3 Flavour compounds

The leaf and fruit of the olive tree is known to be naturally resistant to microbe and insect attack. One reason for this has been found by Kubo and colleagues, who observed antimicrobial activities of molecules which belong to the large group of flavour compounds (35). Among these were acyclic compounds such as hexanal, nonanal, 1-hexanol, 3-hexanal, 2-heptenal, or 2-nonenal and cyclic mono- and sesquiterpene hydrocarbons such as 3-carene or β-farnesene. Most of these compounds exerted antimicrobial activities against a range of different microorganisms, among these Staphylococcus aureus, Streptococcus mutans, Escherichia coli, Candida utilis, and Aspergillus niger (35). The implications of this finding are not clear today, but as some of these bacteria and fungi or toxins produced by them are harmful to humans, this antimicrobial effect is a further aspect that might contribute to the beneficial health effects of olive oil.

## 4 Impact of the minor constituents on the stability of olive oil

The minor components of olive oil referred to above do not only have beneficial effects on human health but are also important for the durability and stability of the oil. Several groups have independently reported that the amount of phenolic compounds in extra virgin olive oil highly correlates with its stability (2;36;37). There is less agreement, however, whether tocopherol also contributes to the stability of the oil. While Baldioli and colleagues did not observe any correlation between the oxidative stability of the oil and its α–tocopherol content (36), others found a small contribution of α–tocopherol (37), and a Spanish group even found a strong correlation between the oxidative stability of the oil and the α–tocopherol content (2).

## 5 Summary and conclusion

Olive oil, especially extra virgin olive oil, contains a large number of structurally heterogeneous components in very small concentrations. Among these so-called minor components are vitamins such as tocopherols (vitamin E), phenols, hydrocarbons, sterols, and flavour compounds. These substances are responsible for the unique taste and flavour of the oil, increase its stability and are beneficial to human health by preventing injurious or deleterious processes such as oxygen radical induced oxidation of lipids. Therefore, the presence of these compounds in the oil is – in addition to its favourable fatty acid composition - a further reason to recommend olive oil as a main source of fat in our daily diet .

# Part II Hydrocarbons (especially squalene) and sterols and their effects for human health

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## 1 Introduction

In this second fact sheet on minor components of olive oil findings on health beneficial effects of hydrocarbons, particularly squalene, and sterols present in olive oil will be reviewed.

## 2 Minor components of olive oil

### 2.1 Hydrocarbons

The major hydrocarbon of olive oil is squalene, a triterpene and intermediate of the cholesterol biosynthesis pathway. Extra virgin olive oil contains squalene in an amount of about 400-450 mg/100g, while refined olive oil contains about 25% less (1). Other authors found 200-700 mg/100g in extra virgin olive oil (reviewed in (2)). According to the latter author, the average intake of squalene is 30 mg per day in the USA. However, with a high consumption of extra virgin olive oil, the intake can reach 200-400 mg per day as observed in the Mediterranean countries (2). Several individuals might even consume up to 1 g of squalene per day with their diets, as suggested by Gylling and Miettinen (3).

In addition to squalene, other hydrocarbons are also present, e.g. the pro-vitamin A β-carotene, albeit in very small quantities (β-carotene: 0.03 - 0.36 mg/100g) (Kiritsakis and Markakis 1987).

### 2.2 Sterols

Sterols are an essential component of cell membranes, and both animals and plants produce them. The sterol ring is a common feature of all sterols; the differences are in the side chain. Cholesterol is exclusively an animal sterol. Over 40 phytosterols have been identified so far. The amount of total sterols in extra virgin olive oil found by different groups varies between 113-265 mg/100g oil (5;6). Two factors influencing this amount are the cultivar and the degree of ripeness of the olives (5). By far the major sterol in olive oil is β-sitosterol, mounting up to 90-95% of total sterols (5;6). Campesterol and stigmasterol make up for about 3% and 1%, respectively (5;6). Stanols are saturated sterols, which are virtually absent from typical diets (reviewed in (4)).

## 3 Impact of the minor constituents on human health

### 3.1 Hydrocarbons (squalene)

#### 3.1.1 Squalene and serum cholesterol concentrations

As already mentioned above, squalene is a metabolite of cholesterol synthesis. Thus, theoretically dietary squalene can be transformed into cholesterol in the body and might therefore increase serum cholesterol levels. The first prerequisite for this effect would be that considerable quantities are absorbed. Available evidence suggests that 60 to 80 percent of dietary squalene is absorbed from an oral dose (2;7). Furthermore, available evidence indicates that a substantial amount of dietary squalene is indeed converted to cholesterol in humans. However, this increase in cholesterol synthesis is not associated with consistent increases in serum cholesterol levels, possibly as a result of a concomitant increase in faecal elimination (8). Although Miettinen and Vanhanen observed an increase in serum total and LDL-cholesterol concentrations after a dietary supplementation with a very high daily dose of squalene (1g), the values could be normalized when the squalene dose was subsequently reduced to a lower level (0.5g per day) (9). Of particular interest is a study indicating that squalene, added to a protocol with low-dose pravastatin, even enhanced the efficacy of pravastatin as a cholesterol-lowering drug (10). Taken together, the concern that low doses of squalene contribute to high serum cholesterol levels appears to be misplaced. At reasonable dietary levels of 0.5g or less per day squalene appears to have no adverse effect on serum cholesterol concentrations.

#### 3.1.2 Squalene and cancer

Epidemiological studies suggest a cancer-protective effect of dietary olive oil. In Greece, women with high total fat intake, mainly as olive oil, have a breast cancer rate of only about one-third that of women in the United States (11). A case-control study in Spain showed a reduced risk for breast cancer in women with the highest olive oil consumption (12). In a large case-control study in Greece breast cancer risk was 25% lower in women consuming olive oil more than once a day (13). In another case-control study in Spain, women in the highest third of monounsaturated fatty acid (MUFA) consumption (largely from olive oil) had a strongly reduced risk of breast cancer (14). A recent case-control study performed in Italy indicated a decreased risk of breast cancer with increased intake of unsaturated fatty acids from edible oils. In Italy, about 80% of edible oil is olive oil, suggesting a protective effect of olive oil intake (15). Another recent case-control study in Italy reported a significant inverse trend of edible oil (mainly olive oil) intake and risk of pancreatic cancer (16). Two leading scientists in the field, Theresa J. Smith and Harold L. Newmark, suggested that this protective effect might be due to the large amount of squalene in extra virgin olive oil (2;11), an assumption supported by a good deal of experimental animal studies. The majority of these studies has investigated the effect of topically applied or systemic administered squalene on chemically-induced cancers of the skin, the colon, and the lung of mice. Taken together, these results clearly show that dietary squalene has distinct anti-carcinogenic effects (17-21).

#### 2.1.3 Other effects of dietary squalene

First studies indicate that the dietary intake of squalene might have beneficial effects beside its anti-cancer properties. Kohno and colleagues observed that squalene is a highly potent quencher of reactive singlet oxygen in human skin surface (22). In animal models, squalene also appears to play an important role in the health of the eye, with particular regard to rod photoreceptor cells of the retina (23). Furthermore, several groups have reported that animals fed squalene show an enhanced capacity to excrete toxins such as hexachlorbenzene or strychnine (24-26), although some of these effects required very high doses of squalene.

### 2.2 Sterols (β-sitosterol)

#### 2.2.1 Effect on serum cholesterol concentrations

Both, oral and parenteral administration of plant sterols and stanols results in reduced concentrations of plasma total and LDL-cholesterol (reviewed in (4;27)). It is likely, that most of this reduction is due to the inhibition of intestinal cholesterol absorption. Also, hepatic and intestinal cholesterol metabolism might be affected. It must be noted, however, that significant reductions in serum cholesterol levels have been achieved only in those studies, in which phytosterol supplements have been used. The doses given were in the range of 1-3 g per day, an amount which cannot be achieved with natural foods. Most of the studies used margarines fortified with sterols or stanols. In general, the decreases in total and LDL-cholesterol increased with increasing daily doses of sterols up to a dose of 2g per day, beyond which no further cholesterol-lowering effect could be observed (28). A recent meta-analysis of all randomised, double-blind trials concluded that at daily intakes of 2g plant sterols or stanols serum LDL-cholesterol concentrations were lowered by 9-14%, with no effects on HDL-cholesterol or triglycerides (27). Furthermore, the decrease in cholesterol concentrations is more distinct in hypercholesterolemic subjects and in subjects on a cholesterol-rich diet (reviewed in (4;27)). In one study, significant lipid-lowering effects were observed with a relatively low dose of 740 mg phytosterols per day in subjects consuming a cholesterol-rich diet (29). Therefore, it can not be excluded that the amounts of phytosterols taken up with a diet rich in extra virgin olive oil might be somewhat beneficial with regard to serum cholesterol concentrations, especially in hyperlipidemic patients consuming diets rich in cholesterol.

#### 3.2.2 Phytosterols and cancer

There are several reports on anti-tumor effects of phytosterols, especially β-sitosterol. Von Holtz and colleagues observed that compared with cholesterol-treated controls, treatment of human prostate cancer cells with β-sitosterol decreased their growth by 24% and induced apoptosis 4-fold (30). Apoptosis is the so-called programmed cell death, a prophylactic mechanism, by which cells commit suicide, e.g. when they have converted into cancer cells, in order to avert damage from the body. Furthermore, β-sitosterol appears to be effective in the treatment of benign prostatic hyperplasia (31-33). In addition to these findings on prostate cancer or prostate hyperplasia, there have been reports on salubrious effects of β-sitosterol on colon cancer cells and breast cancer cells in vitro (34-36). Furthermore, β-sitosterol was shown to nullify the effect of a carcinogen on the colon in rats (37). There are only few studies investigating the relationship between phytosterols and cancer in humans. In a study in Uruguay, De Stefani and colleagues found a strong inverse relationship between the total intake of phytosterols and stomach cancer (38). In an observational study, a research group from California, U.S.A., compared the sterol intakes of Seventh-Day-Adventists, a group known for its very low total cancer morbidity and mortality, with that of the general population. They found that the Seventh-Day-Adventists not only consumed less cholesterol, but also by far more phytosterols, and suggested that either the high total phytosterol intake or the high phytosterol-to-cholesterol-ratio of their diet contributed to the reduced occurrence of cancer (39).

The majority of the investigations referred to above are either in vitro studies using cell culture models of specific cancers or animal studies. Thus, the results on this topic must be handled with caution until there are more data available from studies in humans. Nevertheless, the findings are promising in that phytosterols, and particularly β-sitosterol, might exert distinct anti-cancerogenic effects with regard to cancers of prostate, colon, breast, and stomach.

## 4 Summary and conclusions

Among the minor components of olive oil there are hydrocarbons, particularly squalene, and phytosterols. In a huge amount of studies these substances have been shown to exert beneficial effects. Above all, anti-cancerogenic effects have been shown for both squalene and β-sitosterol. Therefore, the relatively high content of squalene and phytosterols is another valuable feature of olive oil, which contributes to the oil being so salubrious. Considering the possibility of additional synergistic effects between hydrocarbons, phytosterols, phenols, tocopherols, flavour compounds, and the favourable fatty acid composition this salubrity of the oil as a whole might even be higher than the sum of the single beneficial effects.