Olives and olive oil in cancer prevention

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Epidemiologic studies conducted in the latter part of the twentieth century demonstrate fairly conclusively that the people of the Mediterranean basin enjoy a healthy lifestyle with decreased incidence of degenerative diseases. The data show that populations within Europe that consume the so-called 'Mediterranean diet' have lower incidences of major illnesses such as cancer and cardiovascular disease. Studies have suggested that the health-conferring benefits of the Mediterranean diet are due mainly to a high consumption of fibre, fish, fruits and vegetables. More recent research has focused on other important factors such as olives and olive oil. Obviously fibre (especially wholegrain-derived products), fruits and vegetables supply an important source of dietary antioxidants. What is the contribution from olives and olive oil? Apparently the potential is extremely high but epidemiologic studies rarely investigate consumption of these very important products in-depth, perhaps due to a lack of exact information on the types and amounts of antioxidants present. Recent studies have shown that olives and olive oil contain antioxidants in abundance. Olives (especially those that have not been subjected to the Spanish brining process) contain up to 16 g/kg typified by acteosides, hydroxytyrosol, tyrosol and phenyl propionic acids. Olive oil, especially extra virgin, contains smaller amounts of hydroxytyrosol and tyrosol, but also contains secoiridoids and lignans in abundance. Both olives and olive oil contain substantial amounts of other compounds deemed to be anticancer agents (e.g. squalene and terpenoids) as well as the peroxidation-resistant lipid oleic acid. It seems probable that olive and olive oil consumption in southern Europe represents an important contribution to the beneficial effects on health of the Mediterranean diet. *European Journal of Cancer Prevention* 13:319–326 © 2004 Lippincott Williams & Wilkins.

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Introduction

High intakes of saturated animal and polyunsaturated plant fats are implicated in the aetiology of a number of diseases. In particular, positive associations between elevated intakes of dietary fat and cancer of the colon (Armstrong and Doll, 1975), breast (La Vecchia *et al.*, 1998), prostate (Chan *et al.*, 1998) and ovary (Risch *et al.*, 1994) have been shown. However, the epidemiologic data linking breast (Holmes *et al.*, 1999) and colon cancer (Giovannucci *et al.*, 1994), with total fat intake is equivocal. Recent evidence suggests that it is not only the amount but also the type of dietary fat that is important in the aetiology of some cancers (Bartsch *et al.*, 1999, 2002).

Within this concept epidemiologic studies conducted in the latter part of the twentieth century show fairly conclusively that populations within the Mediterranean basin enjoy a healthy lifestyle with decreased incidence of degenerative diseases. The data suggest that populations within Europe that consume the so-called typical Mediterranean diet have lower incidences of major illnesses such as cancer and cardiovascular disease, despite a high intake of fat. The implication is that the health-conferring benefits of the Mediterranean diet are due mainly to a higher consumption of fibre, fish, fruits and vegetables, but more recent research has focused on other important factors such as olives and olive oil.

Although the antioxidant content of olives (Ryan *et al.*, 1999) and olive oil is well researched (Montedoro *et al.*, 1992a,b, 1993), it was not until recently that more precise profiles (Owen *et al.*, 2000a–d, 2003) became available which may well explain their chemoprotective effects (Martin-Moreno *et al.*, 1994; La Vecchia *et al.*, 1995; Trichopoulou *et al.*, 1995; Braga *et al.*, 1998).

Preamble

Olive oil is produced either by centrifugation or hydraulic pressing of malaxed olive drupes (pomace) harvested from the olive groves of Europe and although there are many publications related to the identification of individual compounds in olives and olive oil, comprehensive analyses on the solvent-extractable phenolic fractions are limited. Major contributions in this area come from the data of Ryan *et al.* (1999), Montedoro *et al.* (1992a,b),

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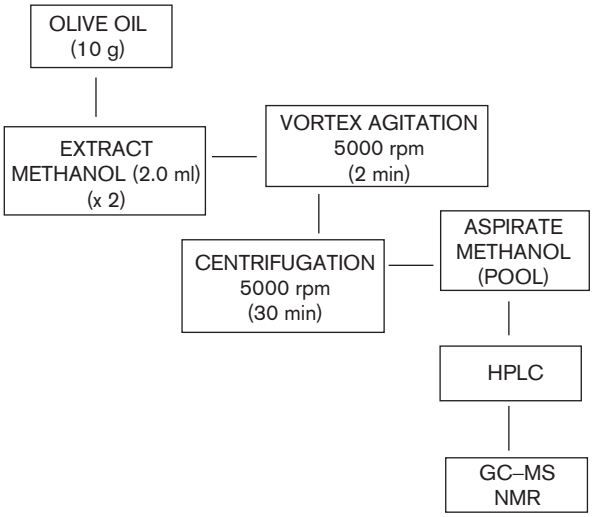
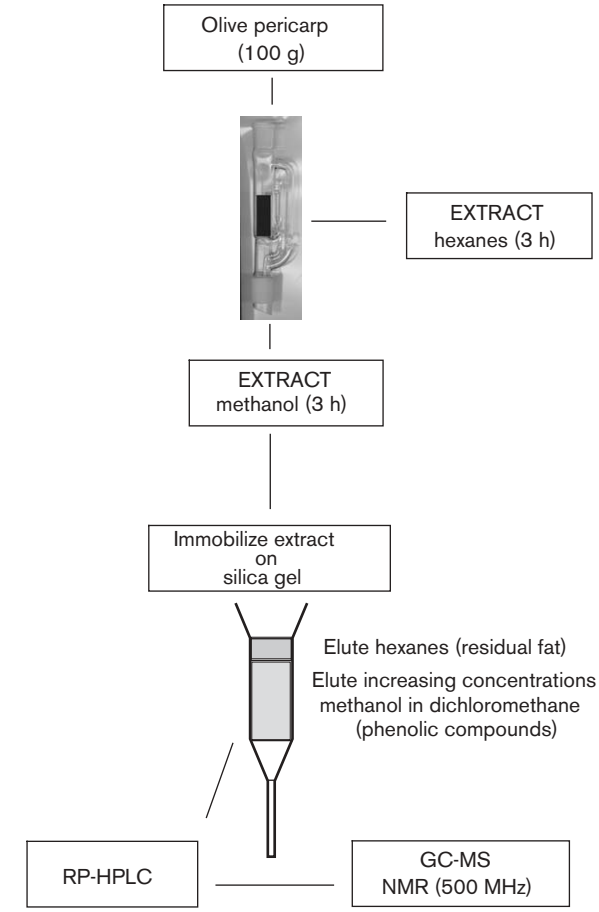
Fig. 1



Protocol for the extraction of phenolic compounds from olive oil. HPLC, high-performance liquid chromatography; GC–MS, gas chromatography–mass spectrometry; NMR, nuclear magnetic resonance.

Fig. 2



Protocol for the extraction of phenolic compounds from olives. HPLC, high-performance liquid chromatography; GC–MS, gas chromatography–mass spectrometry; NMR, nuclear magnetic resonance.

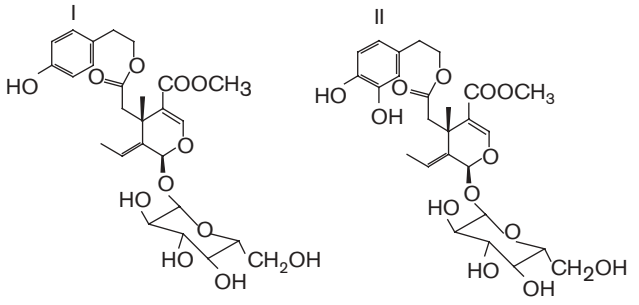
Angerosa *et al.* (1995) and more recently Owen *et al.* (2000a–c). The structures of the major individual phenolic compounds isolated from olives and olive oil by semi-preparative high-performance liquid chromatography (HPLC) were confirmed by liquid chromatography–mass spectrometry (LC–MS) and nuclear magnetic resonance (NMR) (Ryan *et al.*, 1999), NMR (Montedoro *et al.*, 1992a,b, 1993), gas chromatography–mass spectrometry (GC–MS) (Angerosa *et al.*, 1995) and by electrospray ionization mass spectrometry (ESI–MS), GC–MS and NMR (Owen *et al.*, 2000a–d, 2003). The methods used by Owen and colleagues to purify the phenolic compounds in olive oil and olives are depicted in Figures 1 and 2.

Phenolic antioxidant content of olive oils

The major phenolic compounds in olive oil comprise simple phenols, polyphenols, secoiridoids (SID) and lignans. Other than lignans, the remaining compounds are formed from ligstroside (I), oleuropein glycoside (II; Fig. 3) and acteosides (XII and XIII; see Fig. 8). Examples of the structures are shown in Figures 4 and 5. A typical analytical HPLC chromatogram of a methanolic extract of extra virgin olive oil (Fig. 6) displays seven major identifiable peaks of which 1–4 and 6–7 correspond to hydroxytyrosol (VIII), tyrosol (VII), the dialdehydic form of oleuropein (SID-1) lacking a carboxymethyl group (VI), the dialdehydic form of ligstroside (SID-2) lacking a carboxymethyl group (V), the aglycone (SID-3) of oleuropein glycoside (IV) and the aglycone (SID-4) of ligstroside (III). Peak 5 in the chromatogram represents the lignans (+)-1-acetoxypinoresinol (IX) and (+)-pinoresinol (X) which co-elute in this system. Small amounts of (+)-hydroxypinoresinol (XI) can also be detected in extra virgin olive oils.

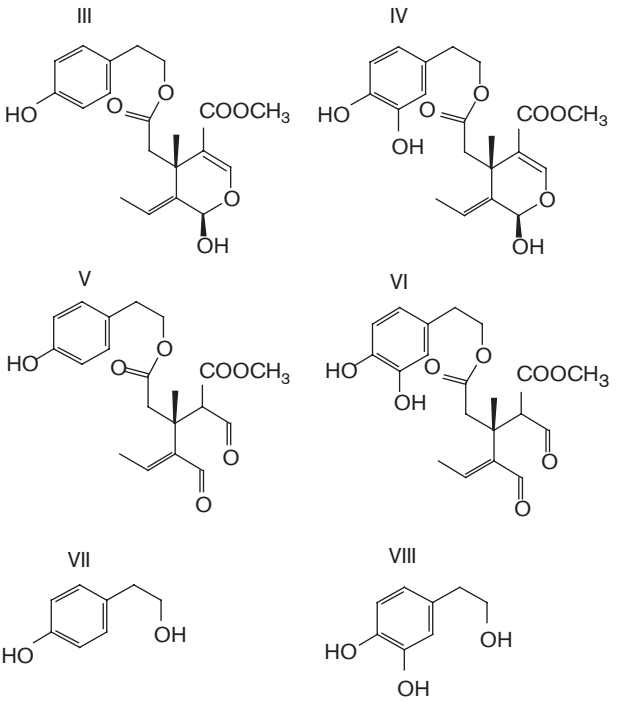
Montedoro *et al.* (1992a,b, 1993) reported total phenolic content (using Folin–Ciocalteau reagent) of olive oils over 500 mg/kg which is contrary to the data of Owen *et al.* (2000a–d, 2003) who showed that, on average, olive oils (Table 1) contained 196 ± 19 mg/kg total phenolics as judged by HPLC analysis. However the value for extra virgin (232 ± 15 mg/kg) was significantly higher than that of refined olive oils (62 ± 12 mg/kg; $P < 0.0001$). A comprehensive evaluation of the individual phenolics in olive oils was conducted by Owen *et al.* (2000c), who showed that the difference in total phenolics between extra virgin and refined olive oils was also reflected in the concentration (Table 1) of the major individual components. Appreciable quantities of hydroxytyrosol and tyrosol were detected (Table 1) in olive oils as judged by HPLC with an average of 11.66 ± 2.60 (SEM) and

Fig. 3



Structure of precursor secoiridoid glucosides detected in immature olives.

Fig. 4

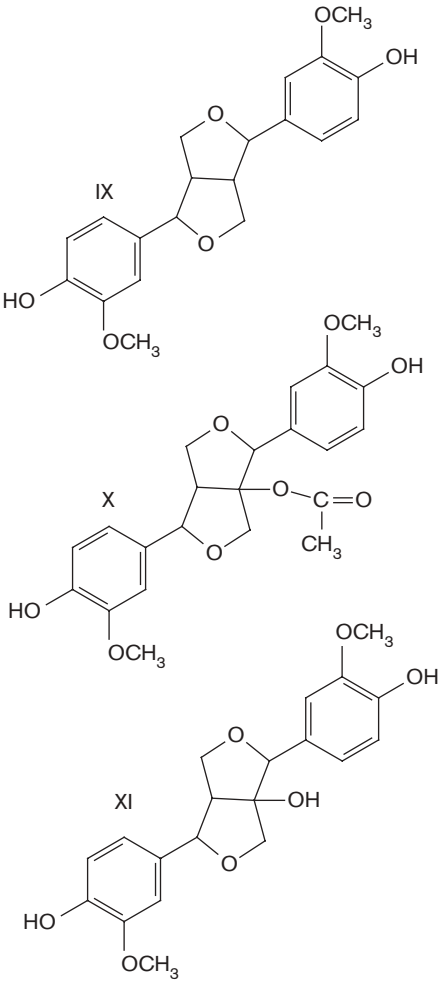


Structures of the phenolic compounds detected in olive oil. III. Aglycone of ligstroside. IV. Aglycone of oleuropein glucoside. V. Dialdehydic form of ligstroside aglycone lacking a carboxymethyl group. VI. Dialdehydic form of oleuropein glucoside aglycone lacking a carboxymethyl group. VII. Tyrosol. VIII. Hydroxytyrosol.

22.13 ± 3.82 mg/kg respectively. Again, there was a significant difference in the concentration of these phenolics in extra virgin (hydroxytyrosol, 14.42 ± 3.01; tyrosol, 27.45 ± 4.05 mg/kg) and refined olive oils (hydroxytyrosol, 1.74 ± 0,84; tyrosol, 2.98 ± 1.33 mg/kg: $P < 0.05$ and $P < 0.01$, respectively).

The concentration of SID (Table 1) in olive oils was variable with mean values of 7.97 ± 2.57 mg/kg (SID-1) and 15.75 ± 3.54 mg/kg (SID-2) and were higher in extra virgin olive oils (SID-1, 9.62 ± 3.18; SID-2, 18.09 ± 4.31) compared with refined olive oils (SID-1, 2.00 ± 0.87; SID-2, 7.30 ± 3.01) but these differences were not significant. On the other hand despite appreciable inter-oil variation the concentration (Table 1) of lignans in extra virgin (41.53 ± 3.93 mg/kg) was significantly higher ($P < 0.001$) than in refined olive oils (7.29 ± 2.56 mg/kg).

Fig. 5



Structures of the lignans detected in olive oil. IX. (+)-Pinoresinol. X. (+)-1-Acetoxypinoresinol. XI. (+)-1-Hydroxypinoresinol.

The aglycones of oleuropein glucoside and ligstroside were also evident in considerable quantities in the HPLC (Fig. 6) and GC–MS chromatograms but the non-homogeneity of the peaks in many of the oils prevented definitive quantitation. This is now ascribed to the presence of several enantiomers of each compound and in current studies they can now be safely quantitated.

Table 1 Content of phenolic compounds in olive oils

	All ($n=23$)	VOQ ($n=18$)	RVO ($n=5$)	P-value*
Total	196 ± 19	232 ± 15	62 ± 12	<0.0001
Hydroxytyrosol	11.66 ± 2.60	14.42 ± 3.01	1.74 ± 0.84	<0.05
Tyrosol	22.13 ± 3.82	27.45 ± 4.05	2.98 ± 1.33	<0.01
Total simple phenols	33.79 ± 4.48	41.87 ± 6.17	4.72 ± 2.15	<0.01
Secoiridoid-1	7.97 ± 2.57	9.62 ± 3.18	2.00 ± 0.87	ns
Secoiridod-2	15.75 ± 3.54	18.09 ± 4.31	7.30 ± 3.01	ns
Total secoiridoids	23.71 ± 5.61	27.72 ± 6.84	9.30 ± 3.81	ns
Lignans	34.09 ± 4.42	41.53 ± 3.93	7.29 ± 2.56	<0.001
Total individual phenolics	91.59 ± 10.57	111.12 ± 9.99	21.31 ± 8.03	<0.001

Data expressed in mg/kg ± SEM; VOQ, extra virgin olive oil; RVO, refined virgin oil.
*VOQ versus RVO; ns, not significant. Reprinted from Owen RW *et al.* Phenolic compounds and squalene in olive oils: the concentration and antioxidant potential of total phenols, simple phenols, secoiridoids, lignans and squalene. *Food Chem Toxicol* 2000 Aug; **38**:649–59 with permission from Elsevier.

Table 2 Content of phenolic compounds in olive pericarp and brine[a]

Compound	Black olive pericarp	Black olive brine	Green olive pericarp	Green olive brine
Hydroxytyrosol	5.78 ± 0.19	0.60 ± 0.01	4.48 ± 0.09	1.361 ± 0.001
Tyrosol	0.40 ± 0.04	0.062 ± 0.001	trace	trace
Phloretic acid	1.08 ± 0.04	0.051 ± 0.002	trace	trace
DHCA	1.79 ± 0.03	0.183 ± 0.001	trace	trace
Acteoside-1	4.83 ± 0.09	0.032 ± 0.001	trace	trace
Acteoside-2	1.95 ± 0.18	0.011 ± 0.001	trace	trace
Luteolin	0.35 ± 0.02	Trace	trace	trace
Apigenin	0.13 ± 0.01	Trace	trace	trace
Total	16.40 ± 0.15	0.939 ± 0.002	4.48 ± 0.09	1.361 ± 0.001

[a]Mean ± SEM from duplicate analyses; in g/kg pericarp dry wt or g/l brine. DHCA, dihydrocaffeic acid. Reprinted from Owen R *et al.* Isolation, structure elucidation and antioxidant potential of the major phenolic and flavonoid compounds in brined olive drupes. *Food Chem Toxicol* 2003; **41**:703–17 with permission from Elsevier.

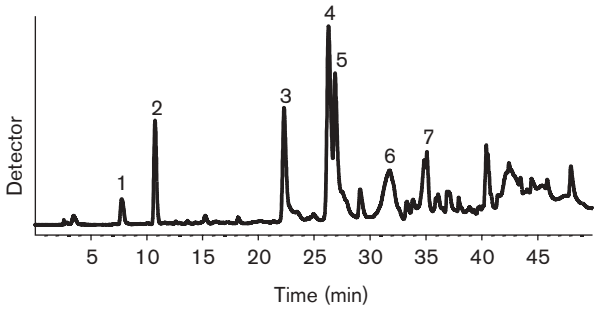
Phenolic antioxidants content in olives and associated brines

The phenolic compounds in olive pericarp comprise simple phenols, polyphenols, acyl glycosides and flavonoids. Typical analytical HPLC chromatograms of a black olive extract and its associated brine are presented in Figure 7a and 7b. Phenolic compounds present in a methanolic extract of black olive pericarp assayed by analytical HPLC (peaks 1–8, Fig. 7a) were the following: hydroxytyrosol (VIII), dihydrocaffeic acid (DHCA, XV), tyrosol (VII), phloretic acid (dihydro-*p*-coumaric acid, XIV), the acyl glycosides acteoside-1 (XII) and the isomeric form acteoside-2 (XIII), and the flavonoids luteolin (XVI) and apigenin (XVII) (Fig. 8).

Black olive brine (Fig. 7b) contains predominantly hydroxytyrosol (VIII), DHCA (XV), and tyrosol (VII) in essentially the same ratios as in the pericarp, plus a small amount of phloretic acid (XIV). In contrast methanolic extracts of green olive pericarp and corresponding brines contain primarily hydroxytyrosol with only trace amounts of other phenolic substances.

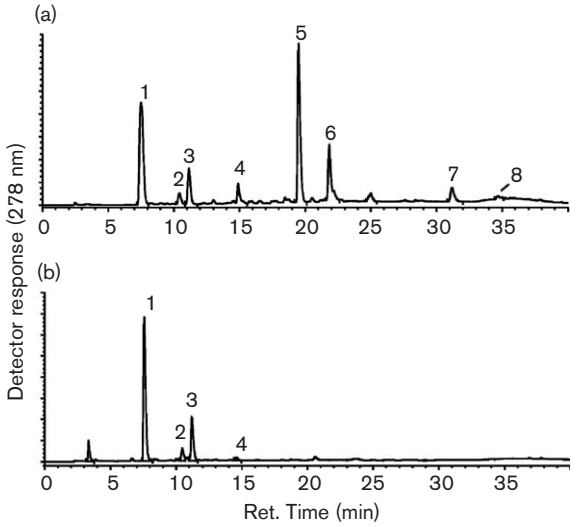
For the two types of olives and their associated brines the amounts of phenolic substances were determined by HPLC analysis, as summarized in Table 2. Black olive pericarp contained 16.40 g/kg total phenolics represented by simple phenols (55%), glycosides (42%) and flavonoids (3%). The simple phenols hydroxytyrosol, tyrosol, dihydrocaffeic acid and phloretic acid represented 35, 3, 11 and 7% of total phenols, respectively. The contributing glycosides were acteoside-1 (30%) and acteoside-2 (12%) while the flavonoids were luteolin (2%) and apigenin (0.8%). The pericarp of green olives contained only 4.48 g/kg total phenolics, almost entirely represented by hydroxytyrosol with only traces of other components. The profiles of the brines mirrored those of the respective pericarps. Black olive brine contained 0.93 g/l total phenolics (representing 5.4% of the total phenolics in pericarp + brine); green olive brine contained 1.36 g/l (23% of the total).

Fig. 6



Analytical high-performance liquid chromatogram of a methanolic extract of an extra virgin olive oil. (1) Hydroxytyrosol, (2) tyrosol, (3) dialdehydic aglycone of oleuropein glucoside lacking a carboxymethyl group, (4) dialdehydic aglycone of ligstroside lacking a carboxymethyl group, (5a) (+)-1-acetoxypinoresinol, (5b) (+)-pinoresinol, (6) aglycone of oleuropein and (7) aglycone of ligstroside.

Fig. 7



Analytical HPLC chromatograms of a methanolic extract of black olive pericarp (a) and its associated brine (b). (1) Hydroxytyrosol, (2) tyrosol, (3) dehydrocaffeic acid, (4) phloretic acid, (5) acteoside, (6) acteoside isomer, (7) luteolin, (8) apigenin. Reprinted from Owen R *et al.* Isolation, structure elucidation and antioxidant potential of the major phenolic and flavonoid compounds in brined olive drupes. *Food Chem Toxicol* 2003; **41**:703–17 with permission from Elsevier.

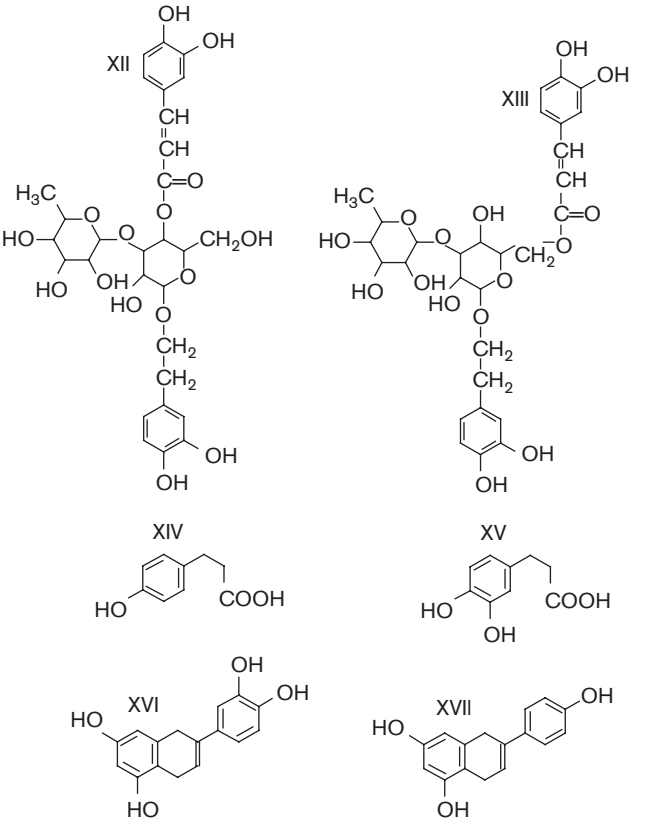
Squalene content of olive oils

Kiritsakis (1990) reported that olive oil contained the highest amounts of squalene among a range of seasoning oils. Olive oil was shown to contain between 136 and 708 mg/100 g whereas among a large number of other seasoning oils only rice bran oil contained significant quantities (332 mg/100 g). These data are in good agreement with those of Owen *et al.* (2000c) who compared the squalene content of extra virgin olive oils, refined olive oils and a selection of seed oils. A mean of 290 ± 38 mg/100 g was detected. However, while there was only a weak significant difference between extra virgin (424 ± 21 mg/kg) and refined (340 ± 31 mg/100 g; $P < 0.05$) olive oils, highly significant differences were evident between olive oils ($P < 0.0001$) and seed oils (24 ± 5 mg/100 g).

Antioxidant capacity of phenolic compounds in olive oil

The antioxidant potential of phenolic compounds in olive oil has also been the subject of considerable interest. This not only has relevance to a chemoprotective effect in humans but is also a major factor in the high stability (shelf life) of olive oils. The relatively high content (over 70%) of the mono-unsaturated fatty acid oleic acid is also of importance here because it is far less susceptible to oxidation than the polyunsaturated fatty acid linoleic acid which predominates, for example, in sunflower oil (Owen *et al.*, 2000a).

Fig. 8



Structures of the phenolic compounds detected in olives and their associated brines: the structures of tyrosol (VII) and hydroxytyrosol (VIII) are shown in Figure 4. XII. Acteoside. XIII. Acteoside isomer. XIV. Phloretic acid. XV. Dehydrocaffeic acid. XVI. Luteolin. XVII. Apigenin.

Much of the data have already been reviewed (Owen *et al.*, 2000d and references therein). Using the hypoxanthine/xanthine oxidase model (Owen *et al.*, 2000c) for the generation of reactive oxygen species, Owen *et al.* (2000c) studied the antioxidative capacity of methanolic extracts of a range of extra virgin ($n = 18$) and refined olive oils ($n = 5$) in comparison to seed oils ($n = 7$). All extracts were shown to exhibit antioxidant properties to a greater or lesser extent. On average, scavenging of the hydroxyl radical ($HO^{\cdot}$) was significantly higher by extracts of extra virgin olive oil. In fact, extracts of the seed oils exhibited minimum antioxidant activity and the potency of the extra virgin olive oil extracts was significantly greater than that of seed oils ($P < 0.0001$) and refined olive oil ($P < 0.05$).

In addition to their direct antioxidant capacity, extracts of olive oil were also potent inhibitors of xanthine oxidase activity as judged by HPLC analysis against a standard curve of uric acid. On average, while seed oils had little effect (inhibition, 6%), xanthine oxidase activity was inhibited to an extent of 48% by extracts of refined and

73% by extracts of extra virgin olive oils ($P < 0.05$ and $P < 0.0001$ in comparison with seed oils, respectively).

A comparison was also made between the antioxidant capacity of a concentration range of methanol extracts of each of the three oil types. While extracts of a seed oil (sunflower) and a refined olive oil had minimal effects on the hydroxylation of salicylic acid by $HO^{\cdot}$ and on xanthine oxidase activity, an extract of extra virgin olive oil had significant dose-dependent effects on both the hydroxylation of salicylic acid by $HO^{\cdot}$ and on xanthine oxidase activity.

Also the phenolic substances isolated and purified from olive oil were potent antioxidants in comparison with the classical *in vivo* and *in vitro* free radical scavengers vitamin E (Trolox) and dimethylsulphoxide respectively. Of the three classes of phenolic substances detected in significant quantities in olive oil tyrosol (simple phenol), SID-1 (secoiridoid) and (+)-1-acetoxypinoresinol (lignan) gave stronger responses than the classical antioxidant Trolox.

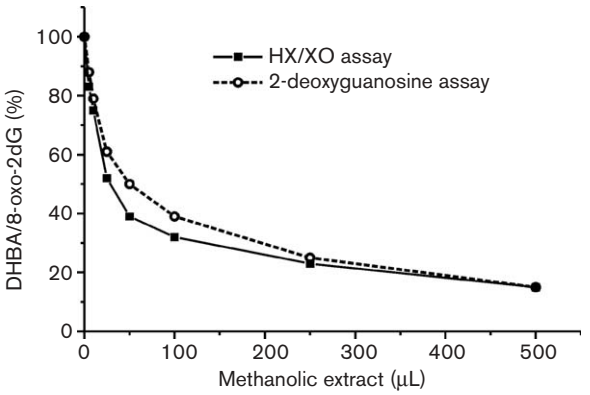
Owen *et al.* (2000e) have shown that the faecal matrix is capable of generating reactive oxygen species in abundance and furthermore established the potential of phenolic compounds isolated from olive oil to scavenge reactive oxygen species generated in the stool (Owen *et al.*, 2000a). The data showed that all three classes of phenolic antioxidants significantly attenuated the signals obtained in their absence. The IC_{50} (inhibitory concentration, 50%) values obtained were of the same order as in the standard assay.

Antioxidant capacity of phenolic compounds in olives

The antioxidant capacity of olive pericarp extracts was determined by not only the hypoxanthine/xanthine oxidase assay but also the production of 8-oxo-2dG in the 2-deoxyguanosine assay (Owen *et al.*, 2003). The reduction of total oxidation products as a function of the volume of extract added to the assay is shown in Figure 9.

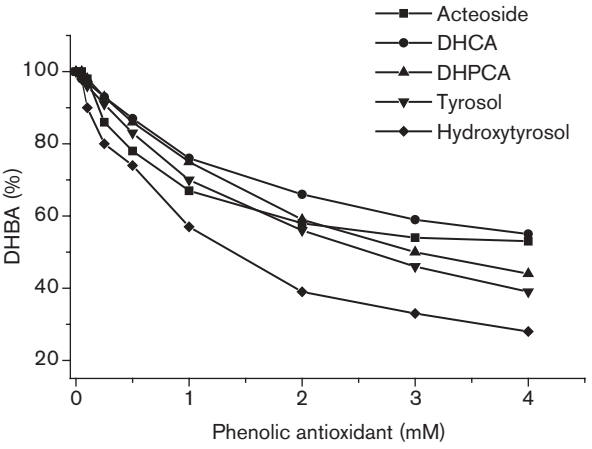
Black olive extract with its greater concentration of phenolic compounds exhibited a higher antioxidant capacity than green olive extract. The black and green olive extracts had IC_{50} values of 30 and 130 µl, respectively, for the hypoxanthine/xanthine oxidase assay and 50 and 90 µl for the 2-deoxyguanosine assay. The higher efficiency in the hypoxanthine/xanthine oxidase assay for black olive extract is probably due to the observed significant inhibition of xanthine oxidase activity ($IC_{50} = 200$ µl). The brines for the two types of olives also inhibited the attack of reactive oxygen species in a similar dose-dependent manner but with higher IC_{50} values.

Fig. 9



Effect of black olive extracts on the detection of reactive oxygen species in the hypoxanthine/xanthine oxidase (HX / XO) and 2-deoxyguanosine assays. Reprinted from Owen R *et al.* Isolation, structure elucidation and antioxidant potential of the major phenolic and flavonoid compounds in brined olive drupes. *Food Chem Toxicol* 2003; **41**:703–17 with permission from Elsevier.

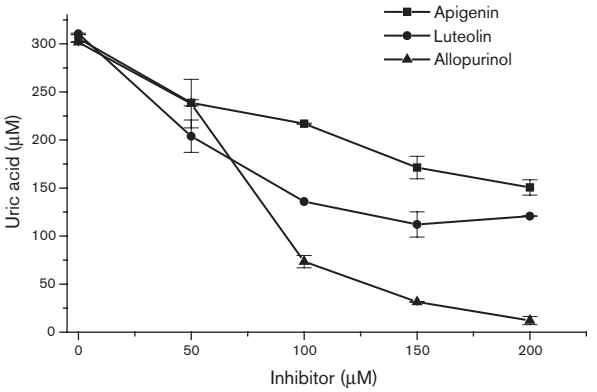
Fig. 10



Effect of purified phenolics from black olives in the hypoxanthine/xanthine oxidase assay. IC_{50} values are: hydroxytyrosol (1.34 mM), tyrosol (2.51 mM), phloretic acid (DHPCA, 3.0 mM), dihydrocaffeic acid (DHCA, 6.35 mM), acteoside (10.8) mM. Reprinted from Owen R *et al.* Isolation, structure elucidation and antioxidant potential of the major phenolic and flavonoid compounds in brined olive drupes. *Food Chem Toxicol* 2003; **41**:703–17 with permission from Elsevier.

Hydroxytyrosol (VIII), dihydrocaffeic acid (XV), tyrosol (VII), phloretic acid (XIV) and acteoside-1 (XII) were tested individually in the hypoxanthine/xanthine oxidase assay. Each compound inhibited the hydroxylation of salicylic acid by reactive oxygen species in a dose-dependent manner as shown in Figure 10 with the IC_{50} value listed in the figure legend. There was no evidence of direct xanthine oxidase inhibition in the HPLC chromatograms.

Fig. 11



Effect of purified phenolics from black olives on the inhibition of xanthine oxidase activity. Reprinted from Owen R *et al.* Isolation, structure elucidation and antioxidant potential of the major phenolic and flavonoid compounds in brined olive drupes. *Food Chem Toxicol* 2003; **41**:703–17 with permission from Elsevier.

The flavonoids luteolin and apigenin are poorly soluble in aqueous media. Therefore, they were dissolved in dimethylsulphoxide to study their possible effects on xanthine oxidase activity. Because dimethylsulphoxide itself is a potent inhibitor of hydroxyl radical attack on salicylic acid, it was not possible to perform the inhibition studies described for the other phenols. However, in a test for xanthine oxidase activity (uric acid production), both luteolin (XVI; $IC_{50} = 77\,\mu mol/l$) and apigenin (XVII; $IC_{50} = 186\,\mu mol/l$) proved to be relatively potent inhibitors of xanthine oxidase, comparable with allopurinol ($IC_{50} = 74\,\mu mol/l$), as shown in Figure 11.

Discussion

Olives and extra virgin olive oils contain high concentrations of phenolic antioxidants and squalene. Therefore in an area such as the Mediterranean basin where olives are liberally consumed and olive oil is the cooking and garnishing fat of choice, intake of phenolic antioxidants and squalene in the diet is likely to be considerably higher than in other areas of Europe. Probably this is a major factor which determines the far lower incidence of cancer in the region.

Habitual high intakes of olives and extra virgin olive oil will provide a continuous supply of antioxidants, which may mediate their effects by reducing oxidative stress via inhibition of lipid peroxidation, thereby inhibiting formation of DNA adducts (Bartsch *et al.*, 1999, 2002), factors that are currently linked to a host of diseases including cancer. The compounds described here are fat soluble and therefore a considerable proportion are likely to be absorbed and thereby should have chemopreventive effects against, among others, breast cancer. The non-absorbed remainder will reach the large bowel where they can exert their chemopreventive effects against colorectal cancer.

The identification of lignans as major antioxidant components of the phenolic fraction in extra virgin olive oil especially has considerable impact, because they have been studied in far greater depth than the simple phenols and secoiridoids. The transformation of (+)-pinoresinol into the so-called mammalian lignans enterodiol and enterolactone has also been demonstrated recently (Owen *et al.*, 2001) indicating that it is not only secoisolariciresinol diglucoside (Thompson *et al.*, 1996) that is the precursor of these lignans. Animal, cellular and metabolic studies have shown they possess important biological effects that may contribute to their potential as chemopreventive agents (Adlercreutz *et al.*, 1992).

Olive oils also contain high concentrations of squalene and, because squalene is to a large extent transferred to the skin, its major protective effect is thought to be against skin cancer, and this is supported by studies showing inhibition of this neoplasm in rodents (Newmark, 1997) and low incidence within populations of the Mediterranean basin. The mechanism is probably by scavenging singlet oxygen generated by UV light.

These observations have ramifications for the chemopreventive effect of the Mediterranean diet of which olives and olive oil are essential components. The differences highlighted in this review between extra virgin and refined olive oils and black and green olives in antioxidant content indicates that, in future epidemiologic and case–control studies, both olive type and the nature and source of olive oil consumed should be differentiated in ascertaining cancer risk. The study of the inter-relation between reactive oxygen species and dietary antioxidants in olives and olive oil is an area of real promise for elucidating mechanisms of breast and colorectal carcinogenesis and possible future chemopreventive strategies.

Acknowledgements

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