The Contribution of \( \alpha \)-Tocopherol and \( \gamma \)-Tocopherol to the Antioxidant Capacity of Several Edible Plant Oils

Garry G. Duthie, Peter T. Gardner, Philip C. Morrice, Donald B. McPhail

Natural Products Group, Rowett Institute of Nutrition and Health, University of Aberdeen, Aberdeen, UK
Email: G.Duthie@abdn.ac.uk

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Abstract

Many oils from plants are important components of our food chain and maintaining their oxidative stability (OS) is economically and nutritionally important. OS is dependent in part on antioxidant capacity (AC) arising from the electron donating ability of endogenous compounds in the oils. Attention has focused on the contribution to AC of phenolic compounds in oils as many have bioactivities \textit{in vitro}. However, the relevance of such phenolics to healthy nutrition remains unclear. In contrast, many plant-derived oils also contain tocopherol homologs, which as vitamin E are dietary-essential, lipid-soluble antioxidants. We have determined the AC of twelve “off the shelf” edible oils by assessing their ability to quench galvinoxyl, a stable free radical species. The stoichiometric reactivity of vitamin E with galvinoxyl indicates that the combined \( \alpha \)-tocopherol and \( \gamma \)-tocopherol homologs contribute between 20% (olive) and 85% (soya) to AC depending on type of oil. Tocopherols are important contributors to the AC of several vegetable oils. Breeding and photo mixotrophic programmes to enhance content in edible oil crops of this important vitamin may have a positive impact not only on oil stability during storage but also in contributing to provision of optimum dietary intakes for health.

Keywords

Vegetable Oils, Antioxidant Capacity, Vitamin E, Electron Spin Resonance, Diet and Health

1. Introduction

The demands of the processing, reformulation and culinary sectors of the food industry have contributed to a marked increase in the worldwide production of edible oils in the last 30 years [1]. For example, oils derived...
from plants are used in the formulation of cooking oils, ready meals, salad dressings, shortenings, spreads and bakery products. A desirable attribute of plant oils is good oxidative stability (OS). Oxidation is a major cause of deterioration of oils, leading to a decrease in product shelf life and increased economic losses [2]. The formation of oxidised products in the oils can also potentially adversely affect the health of the consumer [3]. OS is dependent in part on the presence of compounds with electron or hydrogen atom donating ability inhibiting the process of free radical-mediated lipid peroxidation [4].

Much attention has focused on the contribution made by a wide range of intrinsic phenolic compounds to the antioxidant capacity (AC) of edible oils as their conjugated π-electron systems allow ready donation of hydrogen atoms or electrons from their hydroxyl moieties to annihilate reactive free radical species [5] [6]. Numerous health benefits of these phenolic antioxidants have also been suggested although low bioavailability and rapid systemic clearance may limit in vivo efficacy [7]. However, many edible oils also contain vitamin E homologs, a specific class of phenolic compound, including \( \alpha \)-tocopherol which is readily bioavailable. The chromanol ring system of the vitamin E homologs is a highly effective hydrogen atom donor that, upon quenching free radical species, results in the formation of a stable tocopherol-tocopherylquinone redox system which is unable to participate in further lipid oxidising activity [8]. This antioxidant activity may underlie many biological effects of the tocopherols in addition to the recognized essentiality for preventing cell membrane damage by reactive oxygen species and conditions such as neuropathies and myopathies [9]. However, intakes of vitamin E have been recently reported as being below recommendations in significant parts of the populations of several Western countries [10].

In view of the role tocopherols may play in maintaining the OS of plant oils as well as their potential health benefits, we have measured the AC of ethanolic extracts of several commercially available edible oils and determined the contribution of the major vitamin E homologs, \( \alpha \)-tocopherol and \( \gamma \)-tocopherol, to the overall AC. The AC of the oils was measured by the extent to which they could quench galvinoxyl \([2,6-di-tert-butyl-\alpha-(3,5-di-tert-butyl-4-oxo-2,5-cyclohexadien-1-ylidene)-p-tolyloxy]\), a resonance stabilized, sterically-protected synthetic free radical. Electron spin resonance (ESR) spectroscopy, a technique that can detect and characterize free radical species, was used to quantify the reduction in galvinoxyl radical by the extracts [11]. ESR has several advantages over the more commonly used spectrophotometric methods such as FRAP, ABTS, DPPH and ORAC [12] in terms of sensitivity and that the radical species being monitored has a very well defined spectrum. There is also no interference from the non-radical (diamagnetic) components of the extract, therefore solution turbidity or the presence of other chromophores is not an issue [11].

2. Materials and Methods
2.1. Oils and Reagents

The following edible oils were sequentially purchased from commercial outlets: (1) Olive Oil Brand A (refined), (2) Rapeseed Oil Brand A, (3) Rapeseed Oil Brand B, (4) Walnut Oil, (5) Rapeseed Oil Brand C (6) Olive Oil Brand B (extra virgin), (7) Olive Oil C (extra virgin), (8) Soya Oil, (9) Sunflower Oil, (10) Sesame Oil, (11) Groundnut Oil, (12) Grapeseed Oil. All reagents were purchased from Sigma-Aldrich-Fluka (Gillingham, UK) and BDH Prolabo (Leicestershire, UK). Analytical grade reagents were used without further purification. Echinone was a gift from Hoffman la Roche, Basle, Switzerland.

2.2. Determination of Antioxidant Capacity of Oils

The procedures to estimate the antioxidant capacity of the oils were based on those previously described [11] using a Bruker ECS 106 spectrometer, operating at 9.5 GHz and equipped with a cylindrical (TM110 mode) cavity. The microwave power and modulation amplitude were 10 mW and 0.14 mT, respectively. Oils (2 ml) were added to ethanol (8 ml) and vortex mixed for 1 min. The solvent phase was recovered by centrifugation (5 min, 1500 \( \times g \)), then added to an equivalent amount of ethanol to remove residual haze. AC was estimated by addition of an ethanolic solution of galvinoxyl (3 ml, 0.5 mM) to the oil extract (3 ml). An ethanolic solution of quercetin (3 ml, 0.1 mM), a flavonoid with previously reported antioxidant capacity [11], was used as an internal standard. Addition of galvinoxyl solution to ethanol (3 ml) served as a control. The electron spin resonance (ESR) spectrum of the galvinoxyl radical was obtained 5 min after mixing by which time the reaction had gone to completion. The spectrum was double integrated to obtain the concentration of galvinoxyl radicals remaining/
ml oil extract. Data are calculated as the number of radical molecules reduced by the ethanolic extracts relating to 1 ml of oil. The coefficient of variation following extraction and ESR determination of 6 identical samples was 0.97%. All determinations were carried out in triplicate at 20°C and are expressed as mean ± SD. Reactivity to galvinoxyl of ethanolic solutions of $d\alpha$-tocopherol, $d\gamma$-tocopherol and linoleic acid were also determined.

### 2.3. Determination of Tocopherols in Oils

The concentrations of the vitamin E homologs, $d\alpha$-tocopherol and $d\gamma$-tocopherol, were determined by high pressure liquid chromatography (HPLC) using minor modifications of previously described procedures [13]. In brief, 0.025 g of oil were mixed with 2 ml ethanol (67% solution), added to hexane (700 ml) and shaken for 10 min. Following centrifugation (7500 × g), 600 ml of the hexane layer was removed, dried under nitrogen and dissolved in a mobile phase (20% dioxin, 20% ethanol, 60% acetonitrile) prior to application to the HPLC column (Beckman Ultrasphere ODS 5 µm 25 cm × 4.6 mm I.D in a column oven set at 29°C). Flow rate was 1.05 ml·min$^{-1}$ and injection volume was 150 µl. Homologs were detected using a Waters 470 Scanning Fluorescence Detector with emission/excitation set as follows: 0 - 5.1 min—330/470; 5.2 - 14.6 min—298/328; 14.7 - 30.0 min—349/480. Echinone was used as an internal standard.

### 2.4. Determination of Total Phenols

Total phenolic concentrations of the ethanolic extracts of the oils were determined spectrophotometrically at 765nm using Folin-Ciocalteu reagent [14] following the method described in [15]. Values are presented as gallic acid equivalents.

### 2.5. Determination of Fatty Acid Profiles in Oils

To obtain the total fatty acid composition of the oils, fatty acid methyl esters were formed and determined based on the Bligh and Dyer method (AOCS Method number: Ce 1j-07_p) [16]. Gas liquid chromatography operating conditions were: Column CP-SIL 88, 50 × 0.25 mm; carrier gas helium (flow rate of 1 ml·min$^{-1}$); column head pressure 170 - 190 pka; injector 290°C; temperature programme 165°C × 7 min, 5°C/min to 175°C, 10°C/min to 220°C, 220°C × 15 min; split injection (flow 70 ml·min$^{-1}$; injection volume 1 ml).

### 2.6. Statistical Analysis

Measurements were made in triplicate and presented as mean ± SD. Where appropriate, correlation coefficients were computed and tested for significance using the Microsoft Excel 2007 statistical function.

### 3. Results

Compared with the ethanolic control, all the oil extracts exhibited an ability to reduce the galvinoxyl radical. The activities ranged from $4.8 \times 10^{17}$ - $2.0 \times 10^{18}$ molecules of radical scavenged/ml oil. Soya oil was the most effective (83% galvinoxyl reduction) whereas the refined olive oil was least effective (20% reduction) (Figure 1). In comparison, 1 ml of a 50 µM solution of quercetin (internal standard) was able to reduce $1.8 \times 10^{18}$ molecules of galvinoxyl. There was no detectable decrease in radical signal in the presence of the ethanolic solutions of linoleic acid relative to the control ($98.8\% \pm 2.8\%$) suggesting that the polyunsaturated fatty acids in the oils were not significant oxidisable substrates for galvinoxyl.

The total tocopherol contents ($\alpha + \gamma$ homologs) of the edible oils varied markedly, soya and sesame being the richest sources (36 and 39 µg·ml$^{-1}$, respectively). In contrast, concentrations in the olive and sesame oils did not exceed 10 µg·ml$^{-1}$. The olive oils, sunflower oil and grapeseed oil were comprised almost exclusively of the $\alpha$-homolog, whereas the walnut, soya and sesame oils mainly contained the $\gamma$-form (Figure 2). Sesame oil also contained the greatest content of total phenols ($147 \mu g\cdot GAE\cdot ml^{-1}$) whereas soya contained the least (87 µg·GAE·ml$^{-1}$) (Figure 3).

Between 91% - 97% of the total fatty acid content could be accounted for in terms of 13 major constituents (Table 1) the remainder being ascribed to minor GLC determined peaks. The virgin olive oils were dominated (ca 70%) by the monounsaturated 18:1 cis fatty acid (oleic acid). This was also the major component of the rapeseed and groundnut oils. The 18:2 cis polyunsaturated fraction (linoleic acid) was in greatest abundance in the
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Figure 1. Antioxidant capacity of edible oils determined as the number of molecules of galvinoxyl radical reduced by ethanolic extracts derived from 1 ml of oil. Key: (1) Olive Oil Brand A (refined), (2) Rapeseed Oil Brand A, (3) Rapeseed Oil Brand B, (4) Walnut Oil, (5) Rapeseed oil Brand C (6) Olive Oil Brand B (extra virgin), (7) Olive Oil Brand C (extra virgin), (8) Soya Oil, (9) Sunflower Oil, (10) Sesame Oil, (11) Groundnut Oil, (12) Grapeseed Oil. (Q) quercetin control (50 µM). Data as mean ± SD of 3 determinations.

Figure 2. Vitamin E homologs in edible oils. dα-tocopherol indicated by shaded bars and dγ-tocopherol by open bars content of the oils. Key: (1) Olive Oil Brand A (refined), (2) Rapeseed Oil Brand A, (3) Rapeseed Oil Brand B, (4) Walnut Oil, (5) Rapeseed oil Brand C (6) Olive Oil Brand B (extra virgin), (7) Olive Oil Brand C (extra virgin), (8) Soya Oil, (9) Sunflower Oil, (10) Sesame Oil, (11) Groundnut Oil, (12) Grapeseed Oil. Data as mean ± SD of 3 determinations.

There was a highly significant correlation ($r = 0.802; p = 0.0017$) between the tocopherol content of the oil extracts and the radical reducing activity (Figure 4). For comparison, a standard curve for the reduction of galvinoxyl by dα-tocopherol in ethanolic solution is also shown. With the exception of the groundnut oil, the oils had antioxidant capacities greater than could be accounted for by their total tocopherol contents. This was most
Figure 3. Total phenols as gallic acid equivalents in edible oils. Key: (1) Olive Oil Brand A (refined), (2) Rapeseed Oil Brand A, (3) Rapeseed Oil Brand B, (4) Walnut Oil, (5) Rapeseed oil Brand C (6) Olive Oil Brand B (extra virgin), (7) Olive Oil Brand C (extra virgin), (8) Soya Oil, (9) Sunflower Oil, (10) Sesame Oil, (11) Groundnut Oil, (12) Grapeseed Oil. Data as mean ± SD of 3 determinations.

Figure 4. Extent of galvinoxyl radical reduction by oils relative to their total (α + γ) tocopherol content. The regressed line indicates the reduction attributable to total tocopherol. Key: (1) Olive Oil Brand A (refined), (2) Rapeseed Oil Brand A, (3) Rapeseed Oil Brand B, (4) Walnut Oil, (5) Rapeseed oil Brand C (6) Olive Oil Brand B (extra virgin), (7) Olive Oil Brand C (extra virgin), (8) Soya Oil, (9) Sunflower Oil, (10) Sesame Oil, (11) Groundnut Oil, (12) Grapeseed Oil.

Table 1. Percent fatty acid content of edible oils used in the present study.

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apparent in relation to the soya oil. There was no significant relationship between total phenols and galvinoxyl reduction \( (r = 0.272, \, p = 0.3928) \). Furthermore, no significant correlation was observed between the antioxidant capacities of the oils and the linoleic acid \((18:2 \, \text{cis})\) content \( (r = 0.369; \, p = 0.2381) \) which is consistent with the ESR data showing lack of reactivity between galvinoxyl and ethanolic solutions of linoleic acid and further supports as mentioned above that polyunsaturated fatty acids in the oils are not important confounding substrates for galvinoxyl.

4. Discussion

A diverse range of endogenous phenolic compounds are ubiquitously present in plant oils \[6\]. These range from simple phenolic acids to more complex structures such as flavones and lignans \[17\] \[18\]. Numerous reviews emphasize the important contribution of such phenolic compounds to the antioxidant capacity and oxidative stability of lipid rich foods including edible plant oils \[5\] \[19\] \[20\]. There is also growing interest in their exogenous addition to lipid-rich foods in order to decrease the use of synthetics antioxidants as consumers express a preference for natural alternatives \[21\]. In addition to conferring multiple reductive capacities, the potential health benefits of natural phenolics in edible oils is also under intense investigation as many demonstrate several bioactivities in mammalian cell cultures which are potentially disease preventative \[22\]. However, to date their nutritional relevance remains unclear as there is limited understanding in vivo of the bioavailability, metabolism and mechanism of activity of phenolic compounds from foods, including edible oils \[23\]-\[25\].

In contrast, the essentiality of dietary vitamin E as a lipophilic antioxidant in vivo has long been recognized \[26\]. Edible oils are an important dietary source of essential tocopherols \[27\] and the observed highly significant correlation between tocopherol content and galvinoxyl reduction also emphasizes their important contribution to maintaining oxidative stability of the bulk food product. The reaction stoichiometry between galvinoxyl and the \( \alpha \)- and \( \gamma \)-tocopherol homologs \[11\] is similar \((2.1 \, \text{and} \, 1.9 \, \text{molecules of radical reduced per molecule, respectively})\). Consequently, interpolating from the concentration curve in Figure 4, the total tocopherol content accounted for approximately \(45\% - 60\%\) of the antioxidant activity of grapeseed, walnut, sunflower and soya oils. The contribution of the tocopherols to antioxidant capacity was even more marked in the sesame and groundnut oils \((83\% \, \text{and} \, 100\%, \, \text{respectively})\). In contrast additional factors in the olive oils appear to be mainly responsible for reducing galvinoxyl, with tocopherols only accounting for \(20\% - 30\%\) of the overall antioxidant activity. Although the antioxidant activity of the oils unaccounted for by the tocopherols is likely to be due to the phenolic components, we cannot exclude the possibility of contributions from any \( \beta \)-and \( \delta \)-homologs or tocotrienols which were not determined in the present study due to lack of external standards to ensure definite peak identification.

Surprisingly we were unable to discern a significant correlation between galvinoxyl reduction and the concentration of total phenols in the oils. The reason for this is unclear but may reflect an acknowledged relative lack of specificity of the widely used Folin-Cioalteu method which may not fully reflect the total number of phenolic molecules present \[28\] as some phenols may react with the folin reagent and others do not. For example, a single ring phenol with a \(1.3\) \( \text{OH} \) configuration and lacking hydroxyls with extended conjugation onto an unsaturated chain may be under-represented compared with a \(1.2\) conformation. In contrast, the presence of more complex polyphenolic structures that have multiple oxidation potentials may be over-represented when reacting with phosphotungstic and phosphomolybdic acids present in the reagent \[29\].

Advances in understanding of the genetics of biosynthesis of phenolics in plants has stimulated research in manipulating the phenolic content of food crops through conventional plant breeding, agronomic practices and molecular manipulation \[30\]. In addition to phenolic modulation in relation to plant resistance to pathogenic and environmental stress and product shelf-life extension, there is also much interest in optimizing endogenous content to benefit human health. For example, many phenolics in edible oils are reported to reduce oxidative damage to biological molecules in mammalian systems either directly or by upregulating the cellular defensive response elements \[31\]. However, a relatively low bioavailability and/or rapid systemic clearance may indicate that dietary sources of many phenolics are insufficient to convey such potentially disease preventative activity \[32\]. In contrast, tocopherols are readily absorbed from the food matrix and their absorption, transport protein-mediated selection and distribution to peripheral tissues is well-described \[33\]. Moreover, inadequate dietary vitamin E intakes have been implicated in neuropathies and myopathies, and plasma levels are inversely associated with age-related conditions including cognitive decline \[34\]. Indeed, recent analyses from dietary surveys suggest that the vitamin E intake of \(75\%\) of the populations in the UK and USA may be below recommended...
dietary reference values [10]. Consequently, breeding and metabolic engineering approaches to increase the tocopherol contents of primary plant sources [35] [36] may not only improve oxidative stability of the derived edible oils and therefore a reduction in potentially toxic oxidation products but also may impart important nutritional health benefits to the consumer.

5. Conclusion

Much attention is focused on the role of natural phenolic compounds in relation to the oxidative stability of edible oils as the consumer expresses a preference for natural alternatives to synthetic additives. However, the health benefits of natural phenolic compounds remain poorly understood. Using galvinoxyl, a resonance stabilized free radical, we show that the vitamin E homologs, α- and γ-tocopherol, are major contributors to the antioxidant capacity of several edible oils. As there is strong evidence that tocopherols are implicated in nutritional deficiencies and preventing disease pathogenesis, further research on enhancing the tocopherol content of edible oils may not only increase product shelf-life but also convey a health benefit in populations with habitually low vitamin E intake.

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References


