Journal of Applied Biological Sciences 3 (1): 23-27, 2009
ISSN: 1307-1130, www.nobel.gen.tr

Antioxidant Properties of Various Olive Cultivars

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Received: June 05, 2008
Accepted: August 17, 2008

Abstract

Virgin olive oil is a typical component of the Mediterranean diet, consumed unrefined and rich in important molecules, such as minor polar compounds (tyrosol, ferulic acid and caffeic acid). These molecules not only influence the sensorial properties of both olives and virgin olive but they are also important markers for cultivar identification, biodiversity and quality determination of this product. In this study, levels of phenolics, that have antioxidant activity, such as α-tocopherol, caffeic acid, ferulic acid, and tyrosol, have been determined using HPLC. This research examined the phenolic fraction of virgin olive oil samples of five different olive (*Olea europaea L.*) cultivar grown in the region of Akhisar, Turkey. The cultivar Uslu has been found to contain the highest levels of these compunds. The qualitative and quantitative HPLC analyses of the extracts showed that α-tocopherol was the most abundant compound (from 6.35 mg/kg to 33.7 mg/kg).

Keywords: Olive oil, HPLC, Antioxidants, Quality

INTRODUCTION

It has been postulated that the components in olive oil in the Mediterranean diet, a diet which is largely vegetarian in nature, can contribute to the lower incidence of coronary heart disease, prostate and colon cancers. The Mediterranean diet includes the consumption of large amounts of olive oil. Recently there has been a surge in the number of publications that has investigated their biological properties. The phenolic compounds present in olive oil are strong antioxidants and radical scavengers [1].

In the context of coronary heart disease, it is known that experimental feeding of olive oil enriched in polyphenols to humans or rabbits increases the resistance of low density lipoprotein to oxidation in vivo [2,3], emphasising the known capacity of certain phenolics present in virgin olive oil act as antioxidant. This has been shown previously using several chemical systems [4,5,6].

The biological properties of olive oil are related to its antioxidant composition, namely, tocopherols and minor polar compound, in particular phenols and secoiridoids [7]. Phenolic compounds are of great importance for several characteristics of the olive oil, such as flavour, shelf-life and resistance against oxidation [8]. Phenolics from virgin olive oil are also powerful scavengers of superoxide anions and hydrogen peroxide are capable of preventing the generation of reactive oxygen species by intact leukocytes as it has been demonstrated by other authors [9].

In virgin olive oil, **α**-tocopherol dominates with its composition reaching 95% of the total tocopherol [10]. Both phenols and tocopherol contribute to the remarkable stability of the olive oil [6]. **α**-Tocopherol and phenolic compounds have been reported as having beneficial biological activity [11]. The isolation and quantification of the above compounds are therefore of high importance. The major phenolic found in virgin olive oil is tyrosol (4-hydroxyphenylethanol), caffeic acid and ferulic acid (Fig.3). The concertration of the total phenol content varies from 100 to 800 mg/kg in olive oil [12], and depends on many factors, including the species, location, climate, maturation of the drupes, proccesing and storage conditions. Amounts are greatest in the first pressing of extra virgin olive oil.

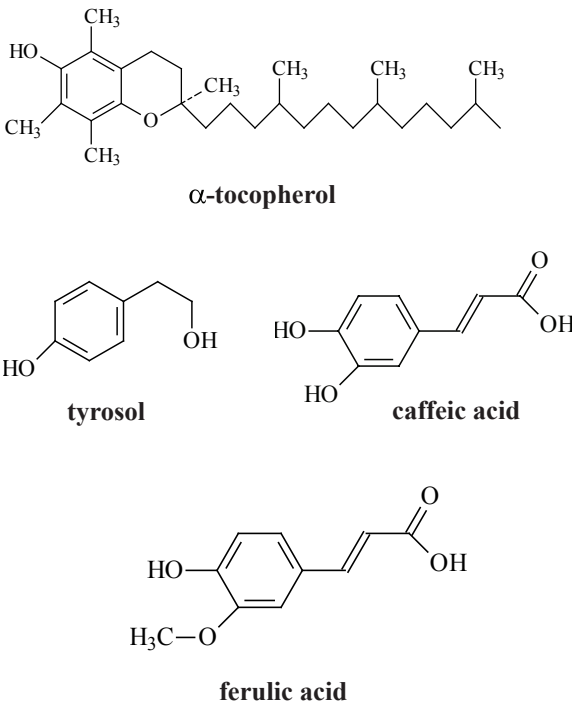

Fig.3 Phenolic compounds from virgin olive oil

Quantitative determination of phenolic compounds in olive oil usually performed accordin to Folin-Ciocalteau colorimetric method [8]. However, this method is not specific, as it gives no indication of the nature of the phenolic compounds present. Reversed-phase HPLC currently represents the most popular and reliable technique for the analysis of phenolic compounds. The technique has been mainly used with UV detection [13]. A simple procedure was used for the extraction and HPLC determination of phenols in olive oil by Maria Tasioula et al. The extraction was carried out using methanol and an isopropanol –methanol mixture. Separation was achieved on a reversed phase C18 column with acetic acid/water-methanol-acetonitrile-isopropanol mixture under gradient elution. Detection was accomplished with UV detection at **α**=280 nm.

Cultivar, degree of maturation, climate and type of extraction method selected are among the factors affecting the phenolic content of virgin olive oil. Studies on different olive oil varieties indicate that cultivar has a significant impact on the phenolic composition of virgin olive oil [14]. No reported literature was found concerned with antioxidant content of these cultivars (Domat, Edremit, Gemlik, Kiraz and Uslu). The virgin olive oil samples used in this study were obtained from plants growing in a specific Akhisar area.

The oxidative stability, sensory quality and health properties of virgin olive oil stem from a prominent and well-balanced chemical composition [15].

This is the first evaluation of chemical composition of five cultivars (Domat, Edremit, Gemlik, Kiraz and Uslu) from Akhisar (Turkey). Because of the importance of these five cultivars for Turkey oil production, the aim of this work was to characterize Domat, Edremit, Gemlik, Kiraz and Uslu virgin olive oils based on the study of minor compounds (tocopherols and phenols) as well as on the oxidative stability. This research examined the phenolic fraction of virgin olive oil samples of five different olive (*Olea europaea L.*) cultivar grown in the region of Akhisar, Turkey.

MATERIALS AND METHODS

Materials

Olive oil samples from different variety olives grown in the region of Akhisar, Turkey were used. These variety were not registered since they have been widespread amoung farmers for years. The samples were stored under-20 oC prior to analysis.

Reagents and standards

Acetonitrile, methanol, hexane isopropanol (2-propanol) acetic acid and water were all of HPLC grade and were purchased from Merck (Germany). Methanol and hexane for oil extraction were pro-analysis grade and were purchased from Merck (Germany). The standards dl-**α**-tocopherol, caffeic acid, tyrosol and ferulic acid were purchased from Sigma (Germany).

Extraction of phenolic compounds

Phenolic compounds were extracted from olive oil according to the method described by Gutfinger [8]. Oil (10 g), were dissolved in 50 ml hexane and the solution was extracted successively with three 20-ml portions of 60% aqueous methanol. The combined extracts were brought to dryness in a rotary evaporator at 40 oC and the residue was dissolved in 5 ml methanol, and submitted to chromatographic analysis.

Extraction of tocopherols

Tocopherols were extracted from olive oil according to the method described by Maria et al…2001 [14]. Samples of 10 g olive oil were extracted at room temperature with two 25-ml portions of absolute methanol. The residue was extracted again under the same conditions with two 25-ml portions of methanol/isopropanol (80:20, v/v). The extracts were combined and brought to dryness in a vacuum rotary evaporator at 40 oC. The residue was dissolved in 5 ml of a methanol and analysed for tocopherol content by HPLC.

HPLC apparatus

A Agilent model HPLC system (Agilent 1100 Series) consisting of a solvent delivery module (LC-3D) with a double plunger reciprocating pump, UV-VIS detector (G1314A), column oven (G1316A) and 20 µl injection loop was used. The column used was an Apex octadecyl 104 C18 (25x0.4 cm ID) with 5-µm packing (Agilent Technologies, USA).

HPLC conditions

Detection was performed at 280 nm for both phenols and a-tocopherol. The elution solvents used were A (2% acetic acit in water), B (methanol), C (acetonitril) and D (isopropanol). The samples were eluted according to the following gradient: 95%A / 5%B in 2 min; 60%A /10%B / 30%C in 8 min; 25%B / 75%C in 22 min, and this percentage was maintained for 10 min; 40%c /60%D in 10 min; andthis percentage was maintained for 15 min; 25%B / 75%C in 2 min, and finally, 95%A /5%B in 3 min. Flow ratewas 1 ml/min and run time, 70 min [14]. The run was performed at 32 oC. The sample injection volume was 20 ml. Identification of compounds was achieved by comparing their retention time values with those of standards. Data was collected and processed using G2170AA single instrument, Agilent ChemStation software (Agilent Technologies).

Quantitation of α-tocopherol and phenols

The **α**-tocopherol content of samples was determined by diluting approximately 200 mg of olive oil in 1 ml methanol/isopropanol mixture (1:4, v/v) and analyzing the sample solution by HPLC. Concentrations of **α**-tocopherol contents were then calculated from integrated peak areas of the samples and calibration curve of **α**-tocopherol Standard. Good linearity was achieved in the range 10-220 mgkg-1 (r2 = 0,988).

RESULTS and DISCUSSION

Generally, essential oils of spices possess strong antibacterial properties against foodborne pathogens and contain high concentrations of phenolic compounds [16]. These compounds exhibit a wide range of biological effects, including antioxidant properties. Phenolic compounds contribute to the overall antioxidant activities of herbs and spices. Generally, the mechanisms of phenolic compounds for antioxidant activity are inactivating lipid free radicals and preventing decomposition of

hydroperoxides into free radicals. [17].

Oxidative deterioration of fat components in foods is responsible for the rancid odors and flavors which decrease nutritional quality. The addition of antioxidants is required to preserve food quality [18].

Polyphenols may exert their beneficial effects at several stages. At the primary step, as strong antioxidants, they have the potential to scavenge free radicals and reduce the incidence of damage to nucleic acids, blocking the initiation of cancer cell formation. As a secondary step, they can modulate cellular biochemical processes such as the maintenance of calcium homeostasis and mitochondrial function [19].

There are several studies which show the beneficial effect of fruits and fruit products leading to disease prevention. A recent study showed that polyphenol components of several commonly consumed berries that include blackberry, black- and red raspberry, blueberry, cranberry and strawberry could inhibit the growth of human oral, breast, colon and prostate tumour cell lines under in vitro conditions [20].

HPLC analysis

Olive oil phenols are usually extracted with water/methanol mixture from hexane solution. On the other hand, extraction of tocopherol cannot be achieved from oil/hexane solution as tocopherol is retained in the hexane solution. Extraction was consequently applied directly to oil samples to oil without dissolution in hexane. Isopropanol/methanol ratio of 20:80 (v/v) was used for extraction of tocopherol. It has been demonstrated that the conctration of phenols in extracts were dependent on extraction solvents and the class of phenolic compounds present in olive oil [21]. Montedore et al. reported a mixture of CH3OH/H2O (80:20, v/v) as the most efficient extraction solvent for simple and hydrolyzable phenolic compounds [13].

The HPLC profiles of phenolic compounds present in olive oil were determined. The retention times for tocopherol, tyrosol, caffeic acid and ferulic acid are 38.0 min, 6.2 min, 7.2 min and 9.0 min, respectively. Antioxidant contents of different olive cultivars were determined and are shown in table 1.

Tocopherols are typical and important antioxidants in humans. a-tocopherol, which is present at the ratio of 1 to 1000 lipid molecules, is the most abundant among tocopherol. Tocopherols can protect polyunsaturated fatty acids within the membrane and LDL, and inhibit smooth muscle cell proliferation and protein kinase C activity. Tocopherol has been associated with the reduction of heart disease, delay of Alzheimer's disease, and prevention of cancer [22]. Tocopherol has been mostly found in Uslu olive oil. The values of tocopherol reported in this study (Table 1) are lower than those found by other authors [23]. The high variability in the amount of tocopherol in olive oil has been widely reported and depends on several factors, such as genetic, agronomic, environmental, and extraction procedures [24].

The ferulic acid has an active oxygen erasing function and the effect has been reported to be similar to superoxide dismutase, known as the enzyme, which protects living bodies from the toxicity of active oxygen. It is also revealed to powerfully absorb harmful long wave ultraviolet light. Thus, the ferulic acid has a wide variety of applications because it has radical and active oxygen erasing effects, absorbs ultraviolet causing active oxygen generation and is a natural substance. It seems much effective for cosmetic use as whitening agent and sunscreen making use of its powerful long wave ultraviolet absorbing function [25]. The ferulic acid has been mostly found in Uslu olive oil (3.68 mg/kg).

Fig. 1. HPLC chromatogram of tocopherol for uslu olive oil.

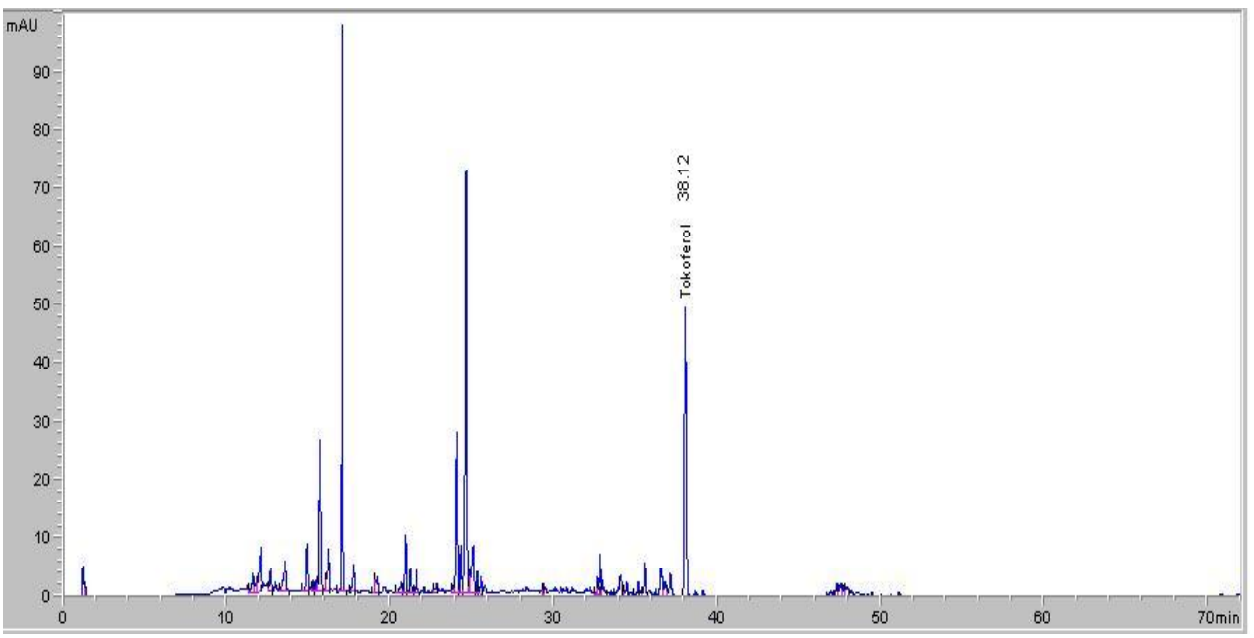

Fig. 2 HPLC chromatogram of tyrosol, caffeic acid and ferulic acid for uslu olive oil

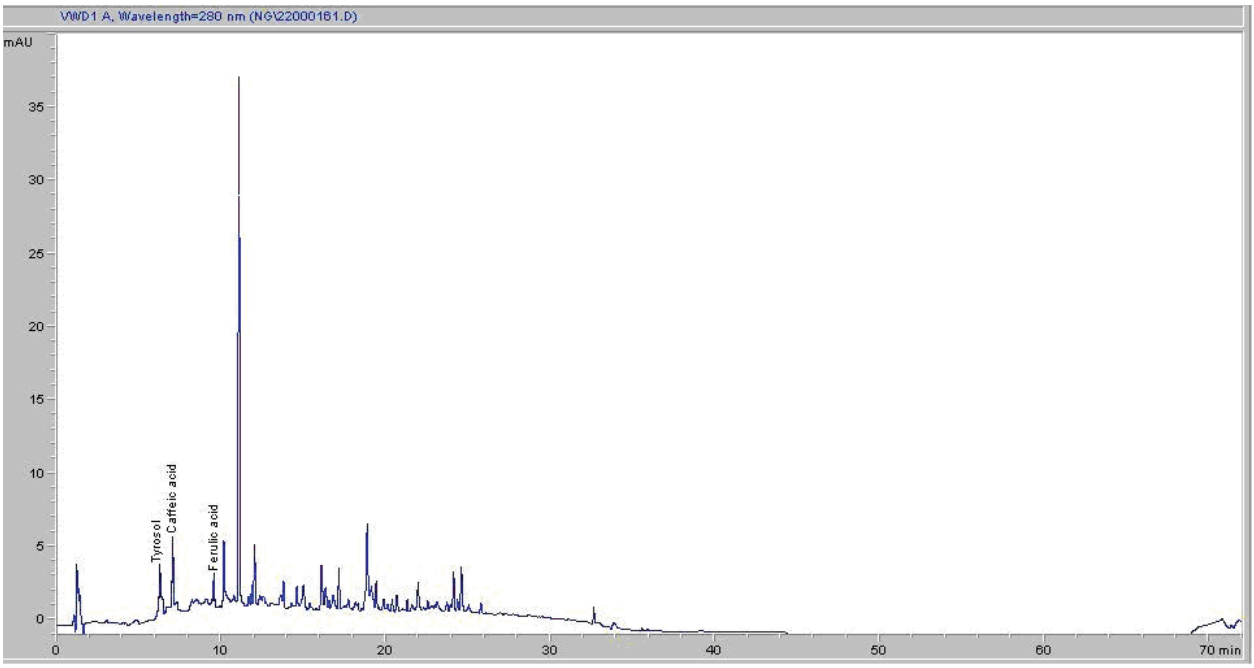

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Table 1. Antioxidant concentrations at different olive varieties

Olive Oil variety	Tyrosol (mg/kg±S.D.)[a]	Caffeic acid (mg/ kg±S.D.)[a]	Ferulic acid (mg/kg±S.D.)[a]	-tocopherol (mg/kg±S.D.)[a]
Domat Olive Oil	1.34±0.18	1.69±0.01	3.43±0.11	6.35±1.23
Edremit Olive Oil	-	1.56±0.06	-	11.3±1.76
Gemlik Olive Oil -	-	-		22.0±2.43
Kiraz Olive Oil	1.1±0.70	-	3.54±0.18	26.5±1.62
Uslu Olive Oil	9.84±2.25	4.23±0.11	3.68±0.12	33.7±2.37

a n = 3

The ferulic acid has an active oxygen erasing function and the effect has been reported to be similar to superoxide dismutase, known as the enzyme, which protects living bodies from the toxicity of active oxygen. It is also revealed to powerfully absorb harmful long wave ultraviolet light. Thus, the ferulic acid has a wide variety of applications because it has radical and active oxygen erasing effects, absorbs ultraviolet causing active oxygen generation and is a natural substance. It seems much effective for cosmetic use as whitening agent and sunscreen making use of its powerful long wave ultraviolet absorbing function [25]. The ferulic acid has been mostly found in Uslu olive oil (3.68 mg/kg).

Tyrosol is the antioxidant that decreases with the lowest rate, providing the oil with the less antioxidant activity [26]. The concentration of tyrosol in olive oil has been reported to be 4.69 ± 0.77 mg/kg [27]; and 27.45 mg/kg in extra-virgin olive oil and 2.98 ± 1.33 mg/kg in refined virgin oil [28]. Tyrosol has been mostly found in Uslu olive oil (9.84 mg/kg).

Caffeic acid was the most effective antioxidant in the lipophilic system and it is oxidized during the chemical oxidation of olives, whereas tyrosol, vanillic acid, and p-coumaric acids are unreactive [29]. Caffeic acid has been mostly found in uslu olive oil (4.23 mg/kg).

As with the total phenol content of olive oil the content of ferulic acid, tyrosol and caffeic acid in olive oil has varied in the literature. These compounds are quite different in olive oil because of environmental and extraction procedures.

Acknowledgements

This study was supported by The Scientific and Technical Research Council of Turkey (TUBITAK). The authors thank to The Scientific and Technical Research Council of Turkey and Balikesir University, Research Center of Applied Sciences (BURCAS / Balikesir, Turkey) for providing the research facilities.

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