Quality indices, polyphenols, terpenic acids, squalene, fatty acid profile, and sterols in virgin olive oil produced by organic versus non-organic cultivation method

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Abstract

The aim of this study was to evaluate the influence of cultivation method on the quality indices of Koroneiki cv. virgin olive oil. Drupes of organic and of non-organic cultivation were sampled at four successive ripening periods in crop years 2000 and 2004. Quality indices, total and simple polyphenols, terpenic acids, squalene, fatty acid profile, and sterols were measured. Statistical analysis demonstrated that peroxide value differed according to cultivation method and according to crop year. Organic Koroneiki cv. olive oils exhibited higher total phenols content. Total phenols differed mainly according to cultivation method, also according to crop year and maturation phase. Total unsaturated and saturated fatty acids differed according to cultivation method, crop year and maturation. Oleic and palmitoleic acids varied according to cultivation method. Cholesterol, campesterol and stigmasterol differed according to maturity, while β-sitosterol differed according to crop year, however not according to cultivation method. Overall, olive oil from organic cultivation was of superior quality compared to non-organic.
Keywords: olive oil, organic cultivar, phenols, terpenic acids, squalene, sterols.

1 Introduction

Virgin olive oil is typical of the Mediterranean diet. Although the composition of olive oil is complex, the fatty acid composition, especially the monounsaturated

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fatty acids content, and the natural antioxidants are those mainly credited with advantages for health, such as the low incidence of coronary heart disease, and even cancer, particularly breast cancer [1].

Many advantages, both from an environmental and a social-economic point of view, are offered through the organic cultivation of the olive tree, which firstly occurred in the 1980s in Greece. The most significant objectives are protection of the environment, protection of producers' and consumers' health and economic advantages. However, conventional cultivations still exist in Greece and the most common organophosphate pesticides applied are dimethoate and fenthion. Among chlorinated organic compounds, endosulfan is most frequently detected in conventional oils.

This work aims to investigate alterations in qualitative characteristics and bioactive microconsituents of monovarietal Koroneiki cv. olive oil of organic and conventional cultivation during successive ripening phases and in different crop years. In order to evaluate these differences, several chemical parameters as well as fatty acid profile, sterols, squalene, terpenic acids and polyphenols were monitored.

2 Materials and methods

2.1 Samples

This study included olive oil samples produced from the Koroneiki variety, of standard and of organic cultivar, produced in Messinia, Peloponnesus, Greece. The climate in Messinia is characterized by a combination of excellent conditions, such as mild winters and extensive hot summer times, long periods of sunlight, optimum rainfall height (roughly 600 mm) and winds of moderate intensity, which allow airing of olive groves. The soil in olive tree farms – located in low hills– is argillaceous with neutral to alkaline pH, with satisfactory stockings of phosphorus, potassium and boron, moderately permeable in water and soil solutions, however with excellent water movement so that no water retention occurs. Olive fruits were harvested during different harvesting periods: November 21st, (phase 1, n=4), December 5th (phase 2, n=4), December 21st (phase 3, n=4) and January 7th (phase 4, n=4), for the crop seasons 2000 and 2004. Olives were processed by a two-phase decanter under the same conditions. Analyses were carried out after processing.

2.2 Organic cultivation method

Organic cultivation in Greece was adopted according to the EU Regulation 2092, which has been amended in 2007 and 2008 by the EU Regulations 834 and 889 respectively. Nowadays, organic cultivation in Greece is being validated from Control and Certification private bodies accredited by the National Accreditation System. Control and Certification private bodies are responsible for the accurate implementation of this system, in compliance to European directives. These certification organizations are authorized from AgroCert® (Organization for

Certification and Inspection of Agricultural Products) [2], which is a Private Law Legal Entity operating for the public benefit under the supervision of the Ministry of Rural Development and Food. Similar structure is applied (or operative) in the rest of the European countries [3]. Organic olive objectives set are: i) olive oil production of high nutritional value, without residues of pesticides and fertilizers -healthy, safe and of high quality, ii) protection of the health of olive oil producers as a result of their exposure to harmful chemicals used during the production of the conventional olive oil, iii) protection of the Environment and conservation of the ecosystem (birds, reptiles etc.) which are dramatically affected by the misuse of chemicals in conventional cultivations. According to the above Regulations, prior to integrate an olive oil yard in organic farming, strict standards for both the olive tree yard and the olive oil are set during a transition period of at least 3 years. The fertility of soil is regulated by culture of legumes (green fertilization) and incorporation of organic substances in soil, such as manure and compost, while cleansing of parasites, illnesses and weeds is achieved by mechanic methods and application of hedges, nests, predatory birds, however no sprayings are used. Systematic controls and analyses, in olive trees and in olive oil produced precede certification of "Biological Olive Oil".

2.3 Reagents and standards

Standards of β-sitosterol, stigmasterol, campesterol, squalene, 5-α-cholestane, and a mixture of fatty acid methyl esters (FAME) were purchased from Sigma Chemicals Co. (St. Lewis, MO, USA). Butylated hydroxytoluene (BHT), boron trifluoride in methanol solution (14% BF_3/MeOH), and bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) were provided by Sigma (Steinheim, Germany). 3-(4-hydroxyphenyl)-1-propanol, homovanillic acid, cinnamic acid and oleanolic acid were obtained from Aldrich (Steinheim, Germany). Tyrosol, protocatechuic acid, 3,4-dihydroxyphenylacetic acid and caffeic acid were purchased from Fluka (Steinheim, Germany); *p*-hydroxy-benzoic acid, ursolic acid, vanillin, *p*-coumaric acid, syringic acid, ferulic acid, and ursolic acid were obtained from Sigma (Steinheim, Germany); vanillic acid was obtained from Serva (Heidelberg, Germany), hydroxytyrosol was from Extrasynthése (Genay-Sedex, France). All the solvents used were of analytical grade and were obtained from Aldrich (Steinheim, Germany). Folin-Ciocalteu reagent and sodium carbonate were purchased from Merck (Darmstadt, Germany). All other reagents were of HPLC grade and were purchased from Merck or Aldrich.

2.4 Free acidity, peroxide value and UV spectrophotometric indices

Free acidity, expressed as percent of oleic acid (% w/w C18:1), peroxide value, given as milliequivalents of active oxygen per kilogram of oil (mequiv O_2 kg^{-1}) and UV absorption characteristics (K_{232} and K_{270}) were determined according to the European Communities official methods [4].

2.5 Colorimetric determination of total phenolic content

Total polyphenol content was measured in methanol / water extracts according to Gutfinger [5]. Results were expressed as mg caffeic acid kg^{-1} oil.

2.6 Gas chromatography (GC) analysis of fatty acids

The fatty acid profile of oil samples (30-50mg) was determined after transesterification of the oil triacylglycerols (TG) by 14% BF_3 / MeOH (EEC, 2003). The resulting FAME were separated on a 50 m x 0.22mm internal diameter BPX 70 capillary column, coated with a 0.25μm thickness of cyanopropyl silicone provided by SGE (Melbourne, Australia), using an Agilent Tech. (formerly Hewlett Packard, HP) HP-6890 gas chromatograph (Avondale, PA, USA), equipped with MSD-6890 mass ionization detector. HP MS Chemstation software was used for quantitation and identification of peaks. Peak identities were verified by reference to a mass spectra library. The analytical conditions were as follows: carrier gas He, flow/rate 1.0 mL min^{-1}, inlet 230°C, oven from 150 to 260°C in 40 min, mass detector transfer line 280°C. The injection volume was 1 μL.

2.7 Sterols and squalene determination

Cholesterol, phytosterols and squalene were determined after hot saponification of 100 mg of oil samples with 2 mL KOH/MeOH 0.5 M for 15 min at 90ºC, followed by methylation with 1.5 mL BF_3/MeOH for 2 min at 90ºC as described by Salta et al. [6]. Saturated sodium chloride solution (5 mL) was then added and the nonsaponifiable fraction was extracted with hexane (2 mL). After vortexing and centrifuging at 3000 rpm for 10 min, aliquots (0.5 mL) of the hexane extracts together with 0.1 mL of internal standard solution (5-α-cholestane, 1 mg/mL) were evaporated to dryness under nitrogen, derivatised to trimethylsilyl ethers (TMS ether) by the addition of 0.25 mL BSTFA at 70ºC for 20 min, and injected into the gas chromatograph. The Agilent HP series GC 6890 (Avondale, PA, USA) equipped with flame ionisation detector, split–splitless injector, and an HP 6890 autosampler were employed. Separation of the sterols and squalene was achieved on a SGE (Melbourne, Australia), BPX50 capillary column (30 m long, 0.25 mm internal diameter), coated with a 0.25 mm thick film of 50% PH phenylmethylpolysiloxane as previously described [6]. The identification and quantification of squalene, cholesterol, and phytosterols was performed by using standard solutions and by constructing the respective standard curves employing 5-α-cholestane as internal standard. The peak corresponding to Δ^5-avenasterol was recognised as the first major peak eluting after β-sitosterol in chromatograms obtained from sesame oil, which has been reported to be relatively rich in the specific phytosterol, and was quantified according to β-sitosterol reference curve [6].

2.8 Simple polyphenol and terpenic acid extraction and derivatisation

The phenolic and terpenic compounds were extracted from oil samples with methanol, which has been reported to be superior to the mixtures of methanol/water [7]. Oil (1 g) was extracted with 3 x 5 mL methanol, the extracts were combined, methanol was evaporated under vacuum and the residue was dissolved in 2 mL acetonitrile, followed by washing with 3 × 3 mL hexane. The acetonitrile was evaporated under vacuum and the residue was dissolved in 1 mL methanol. Aliquots of 0.1 mL of the final methanol solutions were transferred to GC vials, internal standard was added (50 μL of 3-(4-hydroxyphenyl)-1-propanol solution, 19.2 μg/mL), then they were evaporated to dryness under nitrogen, derivatized by addition of 250μL BSTFA at 70°C for 20 min [8] and injected into the gas chromatograph.

2.9 Gas chromatography/mass spectrometry (GC/MS) of simple polyphenols and terpenic acids

An Agilent (Wallborn, Germany) HP series GC 6890N coupled with a HP 5973 MS detector (EI, 70 eV), split–splitless injector and an HP 7683 autosampler were used for the determination of simple polyphenols and terpenic acids. An aliquot (1μL) of each silylated extract was injected into the gas chromatograph at a split ratio 1:20. Separation of sample was achieved using an HP-5 MS capillary column (5% phenyl–95% methyl siloxane, 30m x 0.25mm x 250μm). For the determination of simple polyphenols and the triterpenic acids, which have been detected in olive oils [9], a selective ion monitoring (SIM) GC/MS method was applied as previously reported [10]. Quantification was carried out by employing 3-(4-hydroxyphenyl)-1-propanol as internal standard and constructing reference curves for every polyphenol and terpenic acid by means of standard solutions. Due to the lack of appropriate standard, the quantification of maslinic acid was based on oleanolic acid response factors.

2.10 Statistical analysis

Chemical data were analyzed using the SPSS r.13.0.0 statistical software (SPSS Inc., Chicago, IL, USA). Univariate treatments of the data were performed by analysis of variance (ANOVA). Bonferroni test was performed to evaluate significant differences on the studied parameters. Differences were considered statistically significant when $P \leq 0.05$.

3 Results and discussion

Most parameters considered in this work of studied olive oils were within estimated limits of (EC) Reg No 1989/2003 and in most cases samples fell within the range of values set for extra virgin olive oil.

Univariate analysis of samples showed that free acidity does not differ according to the cultivation method (organic or non-organic) (Table 1). In total, free acidity was lower to olive oil obtained from the organic cultivar compared to

the non-organic (ranging from 0.3 ± 0.0 to 1.2 ± 0.7 in organic vs 0.4 ± 0.1 to 1.3 ± 0.7 in non-organic cultivation). Previous studies on the effect of organic conditions in free acidity do not exist and as for the effect of ripening stage in free acidity it has been shown that drupe maturity increases free acidity [11].

The changes in peroxide index were not significant, while univariate analysis showed that peroxide value differs according to cultivation method (P=0.003), also according to crop year (P=0.001). The changes in peroxide index were comparable in the two cultivation methods.

Measurements of absorbance at specific wavelengths in the UV region provide information on the quality of olive oil. In most cases, these coefficients did not exceed the respective limits for extra virgin olive oil category (K232<2.50, K270<0.22). Univariate analysis did not demonstrate the effect of the cultivation method on K_{232} and K_{270} absorption (Table 1).

Table 1: The impact of cultivation method, crop year and maturation on quality parameters of olive oils obtained from non-organic Koroneiki cv. and organic Koroneiki cv. from four different harvesting periods in two crop seasons (2000 and 2004).

Differences according to	F.A.	PV	K232	K270	TP
cultivation method	-	+ (P=0.003)	-	-	+ (P=0.005)
crop year	-	+ (P=0.001)	-	-	+ (P=0.001)
maturation	-	-	-	-	+ (P=0.001)

F.A: free acidity, PV: peroxide values, TP: total polyphenols. (+) points out significant effect, (-) points out insignificant effect.

The amount of phenolic compounds in extra virgin olive oil is an important factor when evaluating olive oil quality, given that the natural phenols improve its resistance to oxidation, and are responsible for its sharp bitter taste [12]. In general, organic Koroneiki cv. olive oils exhibited higher levels of total phenols compared to the respective conventional oils (organic ranging from 224.7 ± 27.8 to 408.5 ± 6.9 versus conventional ranging from 202.5 ± 45.4 to 322.0 ± 34.7 mg/kg). Univariate analysis demonstrated that total phenols differed according to cultivation method (P=0.005), also according to crop year (P=0.001) and maturation phase (P=0.001). With regard to crop year, differences could be due to water availability, as the level of phenolic compounds is higher in oils obtained from drought-stressed crops than in those from irrigated crops [13, 14]. The different water content of the paste could imply a different solubilization of phenols which are more soluble in water than in oil [15]. The activity of enzymes responsible for phenolic compound synthesis, such as L-phenylalanine ammonia-lyase, differs according to water conditions [16]. In the present study, phenolic acids did not differ according to crop year. Vanillic acid differed according to

cultivation system (P=0.0001) and occurred higher in organic samples, while levels of homovanillic alcohol were significantly higher in non-organic compared to organic samples in harvesting period 1. Tyrosol concentrations differed according to crop year (P=0.035) and according to ripening stage (P=0.027), however not according to cultivation system (P=0.05). Univariate analysis demonstrated that levels of hydroxytyrosol did not differ according to cultivation method. In general, both organic and non-organic oils had higher content of hydroxytyrosol in crop year 2004 compared to crop year 2000. 3,4 di OH-phenyl acetic acid was detected in traces. Identified oleanolic, maslinic and ursolic acid did not differ according to cultivation system.

Identified fatty acids were myristic (14:0), palmitic (16:0), palmitoleic (16:1ω9), (16:1ω7), margaric (17:0), stearic (18:0), oleic (18:1ω9), cis-vaccenic (18:1ω7), linoleic (18:2ω6), linolenic (18:3ω3), arachidic (20:0), gadoleic (20:1ω9), behenic (22:0) and lignoceric (24:0) acids. Palmitic acid (ranging from 14.6 ± 0.8 to 19.4 ± 0.3% total in organic vs conventional ranging from 14.6 ± 0.8 to 18.0 ± 0.3% total), stearic acid (ranging from 3.5 ± 0.2 to 4.1 ± 0.1% total in organic vs conventional ranging from 3.5 ± 0.2 to 3.9 ± 0.1% total), oleic acid (ranging from 65.9 ± 0.1 to 69.4 ± 0.3% total in organic vs conventional ranging from 66.8 ± 0.5 to 69.4 ± 0.6% total) and linoleic acid (ranging from 5.7 ± 0.2 to 8.7 ± 0.1% total in organic vs conventional ranging from 5.7 ± 0.7 to 7.8 ± 0.9% total) were major fatty acids. Palmitoleic acid (ranging from 0.9 ± 0.0 to 0.9 ± 0.1% total in both organic and conventional), linolenic acid (ranging from 0.7 ± 0.0 to 0.8 ± 0.1% total in both organic and conventional), and arachidic acid (ranging from 0.6 ± 0.0 to 0.7 ± 0.0% total in both organic and conventional) were also present. Margaric, behenic, gadoleic and lignoceric were present at less than 0.4% in the studied monovarietal olive oils. In all the samples, the oleic acid was always predominant, never less than 59% of total fatty acids. Univariate analysis illustrated that palmitoleic and oleic acids varied according to cultivation method (P=0.003 and P=0.001, respectively). Total unsaturated (ranging from 75.3 ± 0.9 to 80.0 ± 0.9% total in organic and 77.0 ± 3.2 to 80.0 ± 0.9% total in conventional cultivation) and saturated fatty acids (ranging from 19.6 ± 0.5 to 24.1 ± 0.3% total in organic and 19.2 ± 0.9 to 22.7 ± 3.2% total in conventional cultivation) differed according to all parameters; according to cultivation method P=0.004 and P=0.004, respectively, according to crop year P=0.001 and P=0.001, respectively, and according to maturation P=0.001 and P=0.001, respectively.

Cholesterol, campesterol and stigmasterol did not differ according to cultivation method.

Squalene is the major olive oil terpenoid hydrocarbon, making up more than 90% of the hydrocarbon fraction [7]. Univariate analysis showed that squalene did not change according to cultivation method.

Conclusively, the olive oils of the present study were of good quality according to the examined characteristics. Samples of organic cultivation were overall of superior quality compared to non-organic, especially as far as phenolic content is concerned. Also, maturation and crop year affect the composition of

olive oil. Differences depend on enzymatic activity and environmental parameters, such as climate and water.

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