Journal of Oleo Science
Copyright ©2014 by Japan Oil Chemists' Society
doi : 10.5650/jos.ess13212
J. Oleo Sci. **63**, *(5) 485-496 (2014)*

JOURNAL OF OLEO SCIENCE
Jos

Evolution of Fatty Alcohols in Olive Oils produced in Calabria (Southern Italy) during Fruit Ripening

Angelo M. Giuffrè

Università degli Studi Mediterranea di Reggio Calabria (Italy) - Dipartimento AGRARIA.

Abstract: A study was conducted on olive oils extracted from olives collected in South West Calabria (Southern Italy) over three harvest years 2010-2011-2012. Three autochthonous cultivars were considered: Cassanese, Ottobratica and Sinopolese and seven allochtonous cultivars: Coratina, Itrana, Leccino, Nocellara Messinese, Nociara, Pendolino and Picholine. Thin Layer Chromatography – Gas Chromatograph (TLC-GC) technique permitted the separation and analysis of the fatty alcohol compounds. A general decline in fatty alcohol content was found during the three months of sampling, most evident in hexacosanol. Pendolino showed the greatest decline. A less evident decrease was measured in the odd chained fatty alcohols, mainly in heptacosanol. Both harvest date and cultivar significantly influenced the fatty alcohol content. This is the first report about the fatty alcohol variation during ripening in olive oil produced in South West Calabria (Southern Italy).

Key words: ANOVA, Fatty alcohols, Minor components, Policosanols, Unsaponifiable

1 INTRODUCTION

Virgin olive oil is the most commonly-used oil or fat in the Mediterranean basin and the global consumption of this product is increasing. This use of olive oil in the Mediterranean is a consequence of its easy accessibility and especially its appreciable organoleptic characteristics. In addition, virgin olive oil has been proved to have beneficial effects on human health for the prevention of cardiovascular diseases.

The Region of Calabria (Southern Italy) has an ancient tradition with respect to olive oil production and many cultivars are grown in this territory[1].

Virgin olive oil is mainly composed of glycerides (98-99%) and, for the remaining part, of minor components such as sterols[2,3], waxes[4,5], sesquiterpene hydrocarbons[6], phenols[7-10], squalene[11]. Some minor components are also responsible of the flavour and aroma of the oil such as volatile compounds (aldehydes, alcohols, ketones and esters)[12] which are influenced by extraction conditions[13] and by planting density of the olive trees[14]. Fatty alcohols (FALs), also known as aliphatic alcohols, are contained in the minor component fraction of the olive oil. The short chain compounds (up to C_9) have appreciable water solubility and would not be classified as "fatty" alcohols. Compounds with a chain length greater than 10 carbons are essentially insoluble in water and will partition on the solid phase in the environment[15]. Long chained fatty alcohols are also known as policosanols which are a mixture containing mainly octacosanol and hexacosanol and in minor amounts 22 to 34 chained compounds. Fatty alcohol content is one of the characteristics required by the European regulation to classify olive oil and one of the purity criteria indicated by the International Olive Council regulation. For both regulations, oil with a wax content of between 300 mg/kg and 350 mg/kg are classified as lampante olive oil if the total aliphatic alcohol content is ≤ 350 mg/kg or the erythrodiol and uvaol content is ≤ 3.5%. Oils with a wax content of between 300 mg/kg and 350 mg/kg are classified as crude olive-pomace oil if the total aliphatic alcohol content is > 350 mg/kg and if the erythrodiol and uvaol content is > 3.5%[16,17].

The solvent or non-solvent (mechanical) extraction system determines a different minor component content in olive oil. Solvent-extracted oils require strong treatment for removing acids, the nearly black color and the unpleasant odor and taste. After such treatment, the concentrations of the minor components are strongly reduced, and the concentrations of the fatty alcohols, campesterol and stigmasterol are back at the level of an extra virgin oil[18]. Policosanols are bioactive compounds with healthy benefi-

*Correspondence to: Dr. Angelo M. Giuffrè, Università degli Studi Mediterranea di Reggio Calabria (Italy) - Dipartimento AGRARIA.
E-mail: amgiuffre@unirc.it
Accepted January 21, 2014 (received for review December 6, 2013)
Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online
http://www.jstage.jst.go.jp/browse/jos/ http://mc.manusriptcentral.com/jjocs

cial effects. Octacosanol has the potential to treat numerous conditions without major side effects and thus would be beneficial to many patients. Also, there are opportunities for octacosanol to be taken as a dual-action treatment for hypertension and high cholesterol, with no gastric irritation or muscle problems. This could be an important drug for the future, with the ever-increasing problem of obesity and increased risk of atherosclerosis and coronary heart disease throughout the world[19].

Stüsser *et al.*[20], in a study on a sample of 45 coronary heart disease patients, found decreases in mean percent changes of main cholesterol variables in the group of patients treated with policosanol alone and policosanol plus aspirin therapies compared to the ones treated with placebo plus aspirin.

The administration of 20 and 40 mg/day of policosanol for 30 days significantly inhibited platelet aggregation; in addition policosanols were found to increase the HDL cholesterol and reduce LDL cholesterol content in the blood of 45 hypercholesterolaemic patients[21].

Fatty alcohols are sensible to heat treatments during the physical refining of olive oil and decrease during the heating period[22], also for this reason the FAL analysis can furnish additional information about the chemical quality of the olive oil.

The long chain fatty alcohol fraction, isolated from pomace olive oil, can reduce the release of different inflammatory mediators (eicosanoids, cytokines and nitric oxide) by interfering in different stages of their metabolic pathways, like inducible nitric oxide synthetase expression or phosholipase activity. This suggests that the long chain fatty alcohol fraction may confer a protective role to pomace olive oil against inflammatory damage in different pathologies, including atherosclerosis[23].

The relationship between cultivar and harvest year in the FAL content of olive oil was studied in a previous paper. FAL content in olive oil was highly influenced by cultivar. The combination of cultivar and harvest year showed an effect only in some cases. By and large, the harvest year had no effect on the FAL composition[24].

As Calabria is one of the main Italian Regions for olive oil production, there is a great interest in the study of this oil. Many researches have been conducted in this geographical area to know the best moment (i.e. technological maturity) to collect olives for oil extraction, from the point of view of the phenolic content[25, 26], sterols[27], and triacylglycerols[28].

The aim of this paper was to study the ripening stage effect and the variation during olive ripening of the fatty alcohol composition (considered as total fatty alcohols FALs, as even-chain fatty alcohols ECFALs, as odd-chain fatty alcohols OCFALs, and considered simply: docosanol $C_{22}H_{46}O$ - tricosanol $C_{23}H_{48}O$ - tetracosanol $C_{24}H_{50}O$ - pentacosanol $C_{25}H_{52}O$ - hexacosanol $C_{26}H_{54}O$ - heptacosanol $C_{27}H_{56}O$ - octacosanol $C_{28}H_{58}O$) of pressed olive oil from autochthonous and allochthonous olive cultivars growing in South West Calabria (Southern Italy). All data were also discussed on the basis of the European Union[16] and the International Olive Council[17] regulations. The influence of the cultivar and of the harvest date during olive ripening at biweekly intervals was also discussed. Even and odd chain fatty alcohols were compared also to confirm that the biochemical mechanisms that lead to fatty alcohol formation highlight the differences between bacteria that can produce odd chain and branched compounds, while most other biota produce even chain compounds[15]. This is the first report to show the variation during olive ripening of the fatty alcohol content in olive oils from cultivars grown in South West Calabria (Southern Italy).

2 EXPERIMENTAL

2.1 Plant Materials

Olives were sampled over three harvest years 2010, 2011 and 2012 with the Cassanese, Coratina, Itrana, Leccino, Nocellara Messinese, Nociara, Ottobratica, Pendolino, Picholine and Sinopolese cultivars. Cassanese, Ottobratica and Sinopolese are commonly cultivated in the Region of Calabria (Southern Italy) for oil extraction. The other examined cultivars are allochthonous for this region. The ten studied cultivars have different characteristics in terms of shape and size of the tree, in terms of fruit size and in terms of pulp/seed ratio. Comparing oil from autochthonous and allochthonous cultivars permits the evaluation of the adaptability of the allochthonous ones in a different environment also to increase the biodiversity in this Region, if necessary. Olive trees were well managed and had no nutrient deficiency or pest damage. Fifteen 25-40 year old trees per cultivar were selected and labeled in mono-cultivar groves, situated in the area of Rizziconi (South West Calabria). This area, at an altitude of 100 m above sea level, is characterized by damp and rainy winters and hot summers. Each mono-cultivar grove was at least 3 km from the others. Olive sampling was conducted at biweekly intervals from October, when the pulp and the skin of the fruit were green, until fruit was no longer found on the trees. Freshly and manually harvested drupes (40 kg approximately per cultivar, 2,5 kg approximately per tree) were placed in a plastic container and immediately transported to the laboratory where they were cleaned to eliminate branches and leaves and were washed in fresh water to remove dust. At this point, olives were immediately processed in a laboratory mill "Mini 30" (AGRIMEC Valpesana, Calzaiolo, S. Casciano VP, Florence), with a capacity of 40 kg. First the olives were crushed with a hammer-mill. The resulting paste was mixed at a temperature between 15 and 20℃ for 35 minutes, then placed between a pile of circular metallic grids and pressed using a hydraulic press with a mild and

continuous increase in pressure up to 200 bar. The liquid phase was submitted to separation by centrifugation and the obtained oil was filtered through filter paper. The oil was kept in 100 mL amber glass bottles and maintained in dark conditions at 15-20℃, until analysis.

2.2 Chemicals

Standard samples of 1-docosanol (behenyl alcohol), 1-tricosanol (tricosyl alcohol), 1-tetracosanol (lignoceryl alcohol), 1-heptacosanol, 1-octacosanol (octacosoyl alcohol) from Sigma-Aldrich (St. Louis, MO (USA), and 1-pentacosanol (pentacosoyl alcohol) from GmbH (Germany), and 1-hexacosanol (ceryl alcohol) from J&K scientific Ltd. (China) were used as references. TLC silica gel plates without fluorescence indicator were from Merck S.p.A., (Milan, Italy). All other reagents were from Carlo Erba, (Milan, Italy).

2.3 Determination of fatty alcohols

Analyses were conducted according to the Annex XIX of the CONSLEG 2003[29]. Olive oil (5g) was saponified with 2 M ethanolic potassium hydroxide solution, using the eicosanol ($C_{20\text{-}OH}$) as an internal standard; after boiling, 50 mL of deionized water was added. This mixture was agitated together with diethyl ether three times to extract the unsaponifiable fraction. The three ether extracts were introduced into a separating funnel and washed with distilled water (50 mL each time) until neutral reaction. The organic extracts were dried with anhydrous sodium sulphate and filtered. These extracts were evaporated to dryness using a rotary evaporator. The remaining residue was dissolved in 2 mL of chloroform, and then the FAL fraction was separated by TLC using a plate-developing chamber, which contained hexane/diethyl ether 60:40 (v/v). After TLC separation, the silica plate was sprayed lightly and uniformly with 2,7- dichlorofluorescein. The FAL fraction was separated out by chromatography on a basic silica gel plate. The FALs recovered from the silica gel were transformed into trimethyl silyl ethers and separated and analyzed using a gas chromatograph, Model 8600, Perkin Elmer, Waltham, USA. The oven temperature of the Gas Chromatograph was programmed at 180℃, held for 2 min, then increased to 260℃ at a rate of 2℃/min and maintained for 15 min, then increased to 270℃ at a rate of 6℃/min. Carrier gas was helium at 10 psi of pressure. Auxiliary gases were air at 22 psi of pressure and hydrogen at 15 psi of pressure. The split/splitless injector (operating in the split mode) was set at 280℃; the flame ionization detector (F.I.D.) was set at 290℃. A Mega fused-silica capillary column (SE 54, 30 m length x 0.32 mm ID, 0.5 µm film thickness, Milan - Italy) was used; a 1 µL volume was injected. Peaks were identified by reference to the retention indices of FAL standards and with literature data.

2.4 Statistical analysis

Statistical significance within sets of data was determined by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test; $p \leq 0.05$ was considered to be statistically significant; the effects of harvest date and cultivar were taken into consideration. ANOVA was performed using the Statistical Software Package 15.0 (SPSS Inc., Chicago, IL, U.S.A.). Excel software for Windows (2003 version) was used for standard deviation determination.

3 RESULTS AND DISCUSSION

3.1 Fatty alcohol variation

Each cultivar had a different ripening trend. Cassanese and Pendolino were matured earlier than Coratina, Picholine and Sinopolese (Table 1).

Docosanol variation is described in Table 2. Cassanese, Ottobratica and Sinopolese showed a varying content, whereas almost all the allochthonous cultivars had a constant docosanol content (less than 8 mg/kg) through the three sampling months. Only Leccino and Pendolino showed variations among the allochthonous cultivars, in particular until 1st December when both presented a skin with black coloration (about 50%), (Table 1) and presented values ranging between 5.50 and 16.33 mg/kg (Leccino), and between 6.50 and 23.66 mg/kg (Pendolino). Lazzez *et al.*[30], in oil extracted by centrifugation, found a constant docosanol content from the Chemlali cultivar grown in three different geographical sites of Tunisia during ripening, from September to January; while a small increase was found in February. The docosanol content in olive oil of South West Calabria (2.00-30.67 mg/kg) was always lower than that found by Ranalli *et al.*[31] in a mix of seven major Italian cultivars grown in Central Italy (46 mg/kg).

Tricosanol variation is described in Table 3. The allochthonous cultivars showed a constant content from first to last sampling, always remaining below 2 mg/kg. The autochthonous cultivars had a higher content during ripening and a rapid increase (Sinopolese) or decrease (Cassanese and Ottobratica) in the last two samplings. Krichène *et al.*[32] studied olive oils of Central Tunisia extracted from six minor cultivars: Semni, Jdallou, Chemlali Sfax, Swabaa Alija, El Hor and Oueslati; they found a tricosanol content between 1.27 mg/kg and 5.27 mg/kg, in similar amounts to the South West Calabria cultivars which on 1st December ranged between 0.5 mg/kg for Coratina (75% black skin, 20% purple pulp) to 4.34 mg/kg for Sinopolese found when less than 50% of the skin had turned black.

Tetracosanol variation is described in Table 4. Coratina, Itrana, Nociara and Picholine had a constant content during three months of ripening and remained below 15 mg/kg. Nocellara Messinese had the lowest content in tetracosanol of November and December (7.00 and 5.30 mg/kg

Table 1 Ripening stages of all cultivars.

	2nd October	17th October	1st November	16th November	1st December	16th December	31st December
Cassanese	green skin	green-yellow skin	< 50% reddish skin	> 50% black skin	> 50% black skin <20% purple pulp	>75% black skin 20% purple pulp	100% black skin > 25% purple pulp
Ottobratica	green skin	< 25% reddish > 75% yellow skin	> 50% reddish skin	> 50% black skin	> 75% black skin <50% purple pulp	100% black skin 50% purple pulp	100% black skin 75% purple pulp
Sinopolese	green skin	green skin	green-yellow skin	< 50% reddish skin	< 50% black skin	> 50% black skin 20% purple pulp	100% black skin 75% purple pulp
Coratina	green skin	green-yellow skin	< 25% reddish skin	50% black skin	75% black skin 20% purple pulp	–	–
Itrana	green skin	green-yellow skin	< 25% reddish skin	< 50% black skin	> 50% black skin	–	–
Leccino	green skin	green-yellow skin	< 50% reddish skin	> 50% black skin	> 50% black skin <50% purple pulp	–	–
Nocellara Messinese	green skin	green-yellow skin	< 50% reddish skin	> 50% reddish skin	> 50% black skin	75% black skin 10% purple pulp	>75% black skin 25% purple pulp
Nociara	green skin	green-yellow skin	< 50% reddish skin	> 50% black skin	> 50% black skin <50% purple pulp	>75% black s skin 50% purple pulp	–
Pendolino	green skin	5% reddish 95%yellow skin	< 50% reddish skin	50% black skin	> 50% black skin	> 75% black skin < 50% purple pulp	–
Picholine	green skin	green-yellow skin	< 25% reddish skin	< 50% black skin	> 50% black skin	–	–

Table 2 Variation in docosanol content during ripening. The values, expressed as mg/kg, represent the means of nine replicates, three for each harvest year. Means in the same row with different lowercase letters differ significantly ($p \leq 0.05$). Means in the same column with different uppercase letters differ significantly ($p \leq 0.05$).

	2nd October	17th October	1st November	16th November	1st December	16th December	31st December	± SD
Cassanese	7.99dD	6.67eE	12.67aE	12.50bD	10.01cC	3.00gD	5.00fD	3.68
Ottobratica	13.66eB	13.33fB	20.67dA	23.00bA	30.67aA	21.50cA	10.00gB	7.10
Sinopolese	7.00gE	9.33fC	14.00dC	15.00cC	13.67eB	20.00bB	26.00aA	6.39
Coratina	3.00eI	3.67dG	4.67bI	4.50cI	7.50aE	–	–	1.72
Itrana	5.88dF	6.67cE	7.61aF	7.00bF	5.00eI	–	–	1.01
Leccino	10.67cC	8.33dD	13.33bD	16.33aB	5.50eH	–	–	4.22
Nocellara Messinese	4.00dH	4.20dF	3.50eJ	2.60fJ	7.10aF	6.60bC	5.40cC	1.65
Nociara	2.00fJ	3.33eH	5.87bG	5.33cH	4.71dJ	6.50aC	–	1.68
Pendolino	16.66bA	23.66aA	15.34cB	11.00dE	8.67eD	6.50fC	–	6.25
Picholine	4.00eG	6.67aE	5.33dH	6.00cG	6.50bG	–	–	1.08

respectively). The autochthonous cultivars showed a fluctuating content during ripening with increases and decreases but with values significantly different during olive ripening. Ranalli *et al.*[31], in oil of Central Italy, found a tetracosanol content largely higher (140 mg/kg) in comparison with that found in the South West Calabria oils, in which the maximum content (49.33 mg/kg) was found in the first sampling of Pendolino. García-Gonzales *et al.*[33] in olive oils produced in the province of Seville (Spain), found a lower tetracosanol content (27.7 mg/kg) with respect to the allochthonous cultivars grown in Calabria whereas in olive oils produced in the province of Málaga and Córdoba a $C_{24\text{-OH}}$ content between 51.7 and 52.2 mg/kg was found.

Pentacosanol variation is reported in Table 5. With the exception of Cassanese, in October and November pentacosanol content was less than 8 mg/kg. By and large, most cultivars show a decrease in pentacosanol over the ripening period, but content fluctuated. Pentacosanol increased twice in Sinopolese during December when the skin was largely or completely black and 20-75% of the pulp was purple in color. Cassanese had a varying pentacosanol content during ripening with an overall diminution, but generally showed the highest content.

Hexacosanol variation is described in Table 6. Hexacosanol was one of the most represented FALs and showed a tendency to decrease during ripening in all cultivars. No-

Table 3 Variation in tricosanol content during ripening. The values, expressed as mg/kg, represent the means of nine replicates, three for each harvest year. Means in the same row with different lowercase letters differ significantly ($p \leq 0.05$). Means in the same column with different uppercase letters differ significantly ($p \leq 0.05$).

	2nd October	17th October	1st November	16th November	1st December	16th December	31st December	± SD
Cassanese	2.34cC	1.67dC	2.67aB	2.50bB	1.01eE	1.00fC	1.00gB	0.76
Ottobratica	3.33bA	2.33dB	3.67aA	3.33bA	3.66aB	3.00cB	1.00eB	0.96
Sinopolese	3.00dB	3.00dA	2.33eC	2.33eC	4.34bA	3.50cA	7.99aA	1.98
Coratina	1.00aE	1.00aG	1.00aF	1.00aE	0.50bG	−	−	0.22
Itrana	1.00bE	1.33aD	1.00bF	0.33cG	1.00bE	−	−	0.36
Leccino	1.33bD	1.33bD	1.66aD	1.33bD	1.04cD	−	−	0.22
Nocellara Messinese	0.30fG	1.30bF	0.40eG	0.80dF	1.60aC	0.80dD	1.00cB	0.46
Nociara	0.67cF	1.00bG	1.30aE	1.00bE	0.68cF	1.00bC	−	0.24
Pendolino	1.34aD	1.33aD	1.00bF	1.33aD	1.00bE	0.50cE	−	0.33
Picholine	1.33aD	1.00bG	1.00bF	1.00bE	1.00bE	−	−	0.15

Table 4 Variation in tetracosanol content during ripening. The values, expressed as mg/kg, represent the means of nine replicates, three for each harvest year. Means in the same row with different lowercase letters differ significantly ($p \leq 0.05$). Means in the same column with different uppercase letters differ significantly ($p \leq 0.05$).

	2nd October	17th October	1st November	16th November	1st December	16th December	31st December	± SD
Cassanese	32.00aB	23.33cB	29.67bC	17.00eE	15.50fD	12.00gD	19.00dC	7.44
Ottobratica	17.33gD	18.67fE	40.00cB	40.66bA	48.34aA	32.21dA	20.00eB	12.49
Sinopolese	17.00gE	20.00fC	28.00cD	25.63dC	24.00eB	30.50bB	40.00aA	7.53
Coratina	12.34bF	13.00aF	10.00dH	10.50cG	10.50cG	−	−	1.32
Itrana	11.00cG	12.00aG	11.33bG	9.66eH	10.00dH	−	−	0.96
Leccino	25.33aC	19.33cD	24.00bE	25.33aD	12.50dE	−	−	5.50
Nocellara Messinese	8.10bI	9.80aI	5.80eJ	5.30fJ	6.40dJ	7.00cF	5.30fD	1.65
Nociara	7.66fJ	8.67dJ	8.87bI	8.33eI	8.80cI	9.00aE	−	0.49
Pendolino	49.33aA	47.67bA	46.00cA	34.33dB	20.33eC	19.50fC	−	13.67
Picholine	9.71eH	10.33dH	12.33aF	11.00cF	11.50bF	−	−	1.02

cellara Messinese had the most drastic decline (86.60%) in hexacosanol from the first to the last sampling. In almost all cultivars, except Ottobratica and Nocellara Messinese, a small increase in hexacosanol from the second-last to the last sampling was found. The decreasing trend of the South West Calabrian olive oil is in agreement with the rate found by Lazzez *et al.*[30] in Chemlali cultivar of Tunisia, with an hexacosanol content generally higher at all the ripening stages especially if compared with the allochthonous cultivars grown in South West Calabria. Ranalli *et al.*[31] in olive oil of Central Italy, found 193 mg/kg in hexacosanol: a much higher quantity than that found in Calabrian olive oils. In each cultivar the highest hexacosanol content was found when olives presented a green or green-yellow color (Tables 1, 6). In agreement with our results, Strabbioli *et al.*[34] also found hexacosanol to be the most represented in Leccino oil and in other cultivars grown in Central Italy.

Heptacosanol variation is shown in Table 7. With regard to heptacosanol, different initial total contents were found in the ten cultivars ranging from 4.66 mg/kg found in Cassanese to 1.33 mg/kg found in both Itrana and Picholine. A tendency to decrease was observed during ripening in all cultivars with an exception in the second last sampling of Ottobratica and in the fourth sampling of Cassanese.

Octacosanol variation is described in Table 8. Almost all the allochthonous cultivars showed a general decreasing trend in octacosanol throughout ripening with a small increase in the last sampling. Nocellara Messinese showed the lowest octacosanol content (less than 5 mg/kg) from the second half of November when more than 50% of the olive

Table 5 Variation in pentacosanol content during ripening. The values, expressed as mg/kg, represent the means of nine replicates, three for each harvest year. Means in the same row with different lowercase letters differ significantly ($p \leq 0.05$). Means in the same column with different uppercase letters differ significantly ($p \leq 0.05$).

	2nd October	17th October	1st November	16th November	1st December	16th December	31st December	± SD
Cassanese	12.67aA	8.00dA	10.34bA	8.51cA	5.50fA	5.00gA	6.00eB	2.79
Ottobratica	4.67aD	3.67cE	4.67aB	4.00bB	4.00bB	3.00dC	3.00dC	0.69
Sinopolese	6.00bC	5.33cC	3.68eD	3.33fE	3.67eC	4.50dB	7.01aA	1.38
Coratina	2.00dJ	4.00aD	3.33cF	3.50bD	4.00aB	–	–	0.82
Itrana	3.00aG	2.34bH	1.33dI	1.33dH	2.00cE	–	–	0.71
Leccino	4.00aE	3.00cF	3.39bE	2.66dF	1.50eF	–	–	0.93
Nocellara Messinese	2.30bI	2.60aG	1.40cH	1.30dI	1.40cG	1.20dF	1.00eD	0.60
Nociara	3.33aF	2.33bH	1.65cG	1.67cG	1.01eH	1.50dE	–	0.81
Pendolino	7.67aB	6.00bB	4.33cC	3.67dC	2.67eD	2.50fD	–	2.02
Picholine	2.67aH	2.00bI	1.67cG	1.33eH	1.50dF	–	–	0.53

Table 6 Variation in hexacosanol content during ripening. The values, expressed as mg/kg, represent the means of nine replicates, three for each harvest year. Means in the same row with different lowercase letters differ significantly ($p \leq 0.05$). Means in the same column with different uppercase letters differ significantly ($p \leq 0.05$).

	2nd October	17th October	1st November	16th November	1st December	16th December	31st December	± SD
Cassanese	55.67aD	29.98cF	32.65bD	15.09gF	17.97eE	16.98fD	20.99dB	14.28
Ottobratica	49.00aF	33.34dE	41.33bC	30.34eC	34.34cA	18.30fC	15.00gC	11.99
Sinopolese	71.68aB	58.01bB	48.98cA	34.70fA	23.00gD	36.00eA	40.00dA	16.29
Coratina	60.00aC	51.00bC	24.66eF	26.50dD	30.50cC	–	–	15.95
Itrana	22.12aJ	20.33bI	12.73dH	6.33eI	16.00cF	–	–	6.31
Leccino	51.67aE	36.67bD	27.62dE	26.34eE	31.95cB	–	–	10.24
Nocellara Messinese	32.10aH	28.90bG	7.80dJ	5.80fJ	6.50eJ	8.10cF	4.30gD	11.81
Nociara	35.33aG	17.67bJ	10.52cI	8.33eH	6.73fI	8.50dE	–	10.90
Pendolino	78.51aA	65.33bA	48.67cB	33.00dB	15.33fG	19.50eB	–	25.33
Picholine	28.62aI	21.66bH	15.67cG	9.00dG	9.00dH	–	–	8.46

skin was reddish, until the end of December (>75% black skin and 25% purple pulp). Sinopolese had the highest octacosanol content in the second half of December when olives of this cultivar are usually collected. Lazzez *et al.*[30] in Tunisian olive oils (Chemlali cv.) found a similar decreasing trend during ripening but, more or less, double the amount of octacosanol at all the ripening stages. El Antari *et al.*[35] studied the FAL composition of olive oils from Morocco: in the samples collected in Northern Morocco they found an octacosanol content ranging between 2.3 mg/kg and 2.5 mg/kg whereas in the samples collected in Southern Morocco they found an octacosanol content of 4.3 mg/kg.

The total FAL variation is described in Table 9. There is a decreasing trend during ripening, more evident for the allochthonous cultivars and for Cassanese. The greatest decrease in total FALs for Ottobratica was in December. The total FAL content in Calabrian olive oils, at all the ripening stages was largely lower than that found by Sifi *et al.*[36] in Tunisian olive oils where they found quantities ranging between 125 mg/kg and 599 mg/kg. In olive oils produced in Central Tunisia a total FAL content (102 – 174 mg/kg) was found[36] similar to that of the autochthonous cultivars of South West Calabria and higher than the allochthonous cultivars. El Antari *et al.*[35] in the samples collected in the Southern Morocco, found a total FAL content ranging between 74 mg/kg and 96 mg/kg, whereas in the samples collected in Northern Morocco they found a total FAL content ranging between 46 mg/kg and 65 mg/kg. Ranalli *et al.*[37], in the I-77 cultivar grown in three different

Table 7 Variation in heptacosanol content during ripening. The values, expressed as mg/kg, represent the means of nine replicates, three for each harvest year. Means in the same row with different lowercase letters differ significantly ($p \leq 0.05$). Means in the same column with different uppercase letters differ significantly ($p \leq 0.05$).

	2nd October	17th October	1st November	16th November	1st December	16th December	31st December	± SD
Cassanese	4.66aA	3.67cB	3.33dB	3.87bA	1.01eD	1.00fD	1.00gB	1.59
Ottobratica	3.67bC	2.66dE	3.33cB	2.00eC	2.67dA	6.00aA	1.00fB	1.57
Sinopolese	3.33cD	4.00aA	3.67bA	2.33eB	1.67gC	2.50dB	2.00fA	0.89
Coratina	3.00aE	3.00aC	1.33cE	1.00dF	2.00bB	–	–	0.92
Itrana	1.33aG	1.33aH	0.67cG	0.67cG	1.00bD	–	–	0.33
Leccino	3.00aE	2.00bF	1.67cD	2.00bC	1.00dD	–	–	0.72
Nocellara Messinese	2.20bF	2.70aD	0.60dH	0.40fH	0.70cE	0.50eE	0.20gC	0.99
Nociara	4.00aB	1.67bG	1.30dF	1.67bD	1.01eD	1.50cC	–	1.08
Pendolino	3.33bD	3.67aB	2.67cC	1.34dE	0.67eF	0.50fE	–	1.38
Picholine	1.33aG	1.00bI	0.67cG	0.67cG	1.00bD	–	–	0.28

Table 8 Variation in octacosanol content during ripening. The values, expressed as mg/kg, represent the means of nine replicates, three for each harvest year. Means in the same row with different lowercase letters differ significantly ($p \leq 0.05$). Means in the same column with different uppercase letters differ significantly ($p \leq 0.05$).

	2nd October	17th October	1st November	16th November	1st December	16th December	31st December	± SD
Cassanese	21.01aG	11.67bI	11.00cF	6.51eH	11.50cD	6.00fD	10.00dC	4.95
Ottobratica	42.67aB	30.00cC	38.00bA	26.33eA	28.99dA	14.00fB	11.00gB	11.58
Sinopolese	33.33aC	33.00bA	27.33cB	18.14fB	11.67gC	22.50dA	20.01eA	7.99
Coratina	33.33aC	29.33bD	12.67eD	15.00dD	16.00cB	–	–	9.37
Itrana	14.67bI	15.67aG	8.00dI	5.67eI	11.00cE	–	–	4.26
Leccino	30.67aE	18.33bF	12.33dE	10.33eE	16.00cB	–	–	7.98
Nocellara Messinese	25.60aF	25.60aE	6.80bJ	4.20dJ	3.80eI	4.40cE	3.30fD	10.36
Nociara	32.34aD	15.66bG	9.81cH	7.33dG	5.40fH	6.00eD	–	10.29
Pendolino	37.49aA	32.00bB	21.33cC	16.33dC	7.67fF	9.50eC	–	12.04
Picholine	16.00aH	14.00bH	10.33cG	7.67dF	7.00eG	–	–	3.92

geographical areas of Central and South Eastern Italy, found a total FAL content between 69 and 88 mg/kg. Aparicio and Luna[38], in olive oil extracted with centrifugation systems (two and three phases) from Coratina grown in the region of Apulia (Italy), found a total FAL content of 63 mg/kg and 58 mg/kg, in agreement with results of the South West Calabrian oils from 1st November to 1st December. The total fatty alcohol content of the South Western Calabria olive oil is 2 to 15 times higher than that found in Cornicabra cultivar from Toledo and Ciudad Real (Castilla- La Mancha) Spain[39].

Even chain fatty alcohol variation is described in Table 10. A general decline in ECFALs occurred in almost all cultivars. Only Sinopolese showed an increase in the last two samplings. Pendolino had the sharpest decrease (69.77%).

Odd chain fatty alcohol variation is described in Table 11. A decreasing trend was observed for all cultivars. In Sinopolese the increase in the last two samplings reflected the increase observed in ECFALs. The autochthonous cultivars showed the highest OCFAL content.

The ECFAL/OCFAL ratio was more or less constant during olive ripening for all the cultivars grown in South West Calabria, with the exceptions of the last sampling of Leccino and of the second-last sampling of Sinopolese (Table 12).

Sakouhi *et al*. studied the policosanol content in solvent (petroleum ether) extracted olive oil of Meski cultivar grown in Northern Tunisia. They found the 26th week after flowering as the maximum policosanol accumulation period; hexacosanol and tetracosanol were the major com-

Table 9 Variation in Total FAL content during ripening. The values, expressed as mg/kg, represent the means of nine replicates, three for each harvest year. Means in the same row with different lowercase letters differ significantly ($p \leq 0.05$). Means in the same column with different uppercase letters differ significantly ($p \leq 0.05$).

	2nd October	17th October	1st November	16th November	1st December	16th December	31st December	± SD
Cassanese	136.33aC	85.00cF	102.33bD	66.00dE	62.50fD	45.00gD	63.00eB	30.86
Ottobratica	134.33cD	104.00eD	151.67bA	129.67dA	152.67aA	98.00fB	61.00gB	33.11
Sinopolese	141.33bB	132.67cB	128.00dC	103.33fB	82.00gB	119.50eA	143.00aA	22.05
Coratina	114.67aF	105.00bC	57.67eF	62.00dF	71.00cC	–	–	26.02
Itrana	59.00bJ	59.67aH	42.67dH	31.00eH	46.00cF	–	–	12.02
Leccino	126.67aE	89.00bE	84.00dE	84.33cD	69.50eC	–	–	21.40
Nocellara Messinese	74.60bH	75.10aG	26.30eJ	20.40gI	27.50dH	28.60cF	20.50fC	24.70
Nociara	85.33aG	50.33bJ	39.33cI	33.67eH	28.33fH	34.00dE	–	21.04
Pendolino	194.33aA	179.67bA	139.33cB	101.00dC	56.33fE	58.50eC	–	59.40
Picholine	63.67aI	56.67bI	47.00cG	36.67eG	37.50dG	–	–	11.83

Table 10 Variation in ECFAL content during ripening. The values, expressed as mg/kg, represent the means of nine replicates, three for each harvest year. Means in the same row with different lowercase letters differ significantly ($p \leq 0.05$). Means in the same column with different uppercase letters differ significantly ($p \leq 0.05$).

	2nd October	17th October	1st November	16th November	1st December	16th December	31st December	± SD
Cassanese	116.67aE	71.65cF	86.00bD	51.11eF	54.98dD	37.99fD	54.99dC	26.55
Ottobratica	122.67cC	95.34eD	140.00bA	120.33dA	142.34aA	86.01fB	56.00gB	31.39
Sinopolese	129.01aB	120.34cB	118.32dC	94.93fB	72.33gB	109.00eA	126.00bA	20.17
Coratina	108.67aF	96.99bC	52.00eF	56.50dE	64.50cC	–	–	25.48
Itrana	53.67bJ	54.67aH	39.67dH	28.67eH	42.00cF	–	–	10.78
Leccino	118.34aD	82.66bE	77.28dE	78.33cD	65.95eC	–	–	19.89
Nocellara Messinese	69.80aH	68.50bG	23.90dJ	17.90gI	23.80eI	26.10cF	18.30fD	23.21
Nociara	77.33aG	45.33bJ	35.07cI	29.33eH	25.63fH	30.00dE	–	19.32
Pendolino	181.99aA	168.66bA	131.33cB	94.66dC	52.00fE	55.00eC	–	55.88
Picholine	58.33aI	52.67bI	43.67cG	33.67eG	34.00dG	–	–	11.03

pounds, 3404 mg/kg and about 2500 mg/kg respectively. After this week, a decline in policosanol content was measured[40].

3.2 Analysis of Variance

3.2.1 Data evaluation row by row

Differences are statistically considered for each cultivar ($p \leq 0.05$) and data are evaluated row by row.

The varying docosanol content of the autochthonous cultivars was significantly different during olive ripening. Also all the allochthonous cultivars had values significantly different on all the harvest dates except the first and the second harvest dates of Nocellara Messinese (Table 2).

Tricosanol was very highly significantly different only in olive oil from Cassanese on the seven harvest dates. In all the remaining cultivars tricosanol showed partial differences or not significant differences (Table 3).

Tetracosanol was very highly significantly influenced by harvest date in all cultivars except for Coratina, Leccino and Nocellara Messinese. Pendolino had a constant tetracosanol increase with values significantly different during ripening (Table 4).

Pentacosanol was very highly significantly affected by harvest date in Cassanese, Leccino and Picholine. Pentacosanol constantly decreased in Pendolino with significant differences from early October to the end of December (Table 5).

Hexacosanol was very highly significantly influenced by harvest date and maturation in each cultivar except for the last two samplings of Picholine. Hexacosanol constantly

Table 11 Variation in OCFAL content during ripening. The values, expressed as mg/kg, represent the means of nine replicates, three for each harvest year. Means in the same row with different lowercase letters differ significantly ($p \leq 0.05$). Means in the same column with different uppercase letters differ significantly ($p \leq 0.05$).

	2nd October	17th October	1st November	16th November	1st December	16th December	31st December	± SD
Cassanese	19.67aA	13.35dA	16.34bA	14.88cA	7.52fC	7.01gC	8.01eB	4.96
Ottobratica	11.66bC	8.66eD	11.67bB	9.33dB	10.33cA	11.99aA	5.00fC	2.47
Sinopolese	12.33bB	12.33bB	9.68dC	8.00eC	9.67dB	10.50cB	17.00aA	2.93
Coratina	6.00cF	8.01aE	5.67dF	5.50eF	6.50bD	–	–	1.01
Itrana	5.33aG	5.00bH	3.00dI	2.33eI	4.00cE	–	–	1.28
Leccino	8.33aD	6.34cG	6.72bE	6.00dE	3.55eG	–	–	1.72
Nocellara Messinese	4.80bH	6.60aF	2.40eJ	2.50dI	3.70cF	2.50dF	2.20fD	1.64
Nociara	8.00aE	5.00bH	4.26dG	4.33cG	2.70fH	4.00eD	–	1.78
Pendolino	12.34aB	11.00aC	8.00abD	6.34bcD	4.33bcE	3.50cdE	–	3.56
Picholine	5.33aG	4.00bI	3.33dH	3.00eH	3.50cG	–	–	0.91

Table 12 Variation in ECFAL/OCFAL content during ripening. The values, expressed as mg/kg, represent the means of nine replicates, three for each harvest year. Means in the same row with different lowercase letters differ significantly ($p \leq 0.05$). Means in the same column with different uppercase letters differ significantly ($p \leq 0.05$).

ECFALs/OCFALs	2nd October	17th October	1st November	16th November	1st December	16th December	31st December	± SD
Cassanese	6.40cJ	5.39eI	5.44dJ	3.43fH	7.51aH	5.42dE	6.87bD	1.32
Ottobratica	10.43fG	11.25dE	11.86cF	13.06bD	13.68aB	7.80gD	11.20eA	1.92
Sinopolese	10.89dF	10.56eF	12.28cE	13.02bD	9.75fG	18.42aA	7.41gC	3.45
Coratina	19.92aA	15.54bA	9.08eH	10.64cF	9.93dF	–	–	4.60
Itrana	9.67eI	11.20cE	14.73bB	17.24aA	10.51dE	–	–	3.20
Leccino	14.42bD	13.63cC	12.57eD	13.51dC	26.67aA	–	–	5.91
Nocellara Messinese	14.55aC	10.38cG	9.96dG	7.16fG	6.43gI	10.43bC	8.31eB	2.70
Nociara	10.19aH	9.37cH	8.23dI	7.20fG	9.94bF	7.86eD	–	1.21
Pendolino	15.23dB	15.31cB	16.79aA	15.07eB	12.25fC	15.42bB	–	1.49
Picholine	11.61dE	13.46aD	12.91bC	12.43cE	11.01eD	–	–	0.98

decreased in Pendolino from early October to early December (Table 6).

Heptacosanol values were very highly significantly influenced by harvest date in Cassanese, Sinopolese, Nocellara Messinese and Pendolino; in all other cultivars values were partially different (Table 7).

Octacosanol was very highly significantly affected by harvest date in all cultivars. Nocellara Messinese in October and Cassanese on 1st November and 1st December showed no significant differences (Table 8).

Total FAL values were highly significantly influenced by harvest date in all cultivars. It is worthy of note that Leccino and Pendolino showed a significantly and constant decreasing trend during ripening (Table 9).

ECFALs were partially significantly influenced by harvest date in Cassanese, whereas the maturation very highly significantly influenced all other nine cultivars and Leccino showed a significant and constant trend in diminution (Table 10).

OCFALs were partially significantly influenced by harvest date in Ottobratica, Sinopolese, Nocellara Messinese and Pendolino, whereas the harvest date affected very highly significantly all the other cultivars (Table 11).

The ECFAL/OCFAL ratio was very highly significantly influenced by harvest date in all cultivars except in Cassanese where non-significant differences were found on two sampling dates (1st November and 16th December), (Table 12).

3.2.2 Data evaluation column by column

Differences are statistically considered for each harvest

date ($p \leq 0.05$) and data are evaluated column by column.

Docosanol content was very highly significantly affected by cultivar on 2nd October, in November and at the beginning and end of December. The highest value was found in Ottobratica (30.67 mg/kg), 15.34 times more than in Nociara (2.00 mg/kg). On 17th October the docosanol content was significantly different in all cultivars except for Cassanese, Itrana and Picholine. Pendolino (23.66 mg/Kg) and Nociara (3.33 mg/Kg) had respectively the highest and the lowest value (Table 2).

Tricosanol, heptacosanol, total FALs, ECFALs, OCFALs and ECFALs/OCFALs contents were partially significantly affected by cultivar on all harvest dates (Tables 3, 7, 9, 10, 11, 12) whereas tetracosanol, hexacosanol, were very highly significantly influenced by the cultivar effect (Tables 4, 6).

Pentacosanol content was very highly significantly influenced by cultivar on 2nd October, 16th December and 31st December. In the other harvest dates cultivar partially influenced the pentacosanol content (Table 5).

Octacosanol was partially significantly influenced by cultivar on October and it was very highly significantly influenced by cultivar in November and at the end of December (Table 8).

4 CONCLUSIONS

All cultivars were grown in the same geographical area and with the same microclimatic conditions. No differences were applied to the agronomic practices and consequently the different content of each FAL was exclusively due to the effect of cultivar and harvest date.

The even chain fatty alcohols (ECFALs) were present in olive oil of South West Calabria (Southern Italy) in higher quantity in comparison with the odd-chain fatty alcohols (OCFALs). Hexacosanol had the highest value in all cultivars and throughout the three months of sampling. The highest content of each FAL during olive ripening was often found in Ottobratica, Pendolino or Sinopolese.

From the nutraceutical and technological point of view, a tendency towards total policosanol decrease was observed during ripening (Fig. 1), most evident for hexacosanol and octacosanol. In almost all cultivars the highest policosanol content was found in October at the beginning of ripening when olives were green or yellow-green. Only in Sinopolese after a decrease in policosanol until November, an increase was found in December, this was due to the docosanol, tetracosanol, pentacosanol and hexacosanol contents. It is worthy of note that Sinopolese had the smallest fruit and the smallest ratio pulp/seed among all cultivars. The findings of this paper confirm that to obtain a good nutraceutical virgin olive oil quality it is necessary that olive harvesting is not delayed. No cultivar produced oil with a policosanol content above the legal limit indicated by European Union and by the International Olive Council. Generally the autochthonous cultivars showed a total policosanol content higher than the allochthonous during olive ripening.

At complete physiological maturity, Sinopolese showed the highest content of each policosanol. Hexacosanol, octacosanol, tetracosanol and docosanol were the predominant policosanols in South West Calabrian olive oils.

The policosanol content in olive oil produced in South West Calabria (Southern Italy) was significantly influenced both by the effect of harvest date and by cultivar.

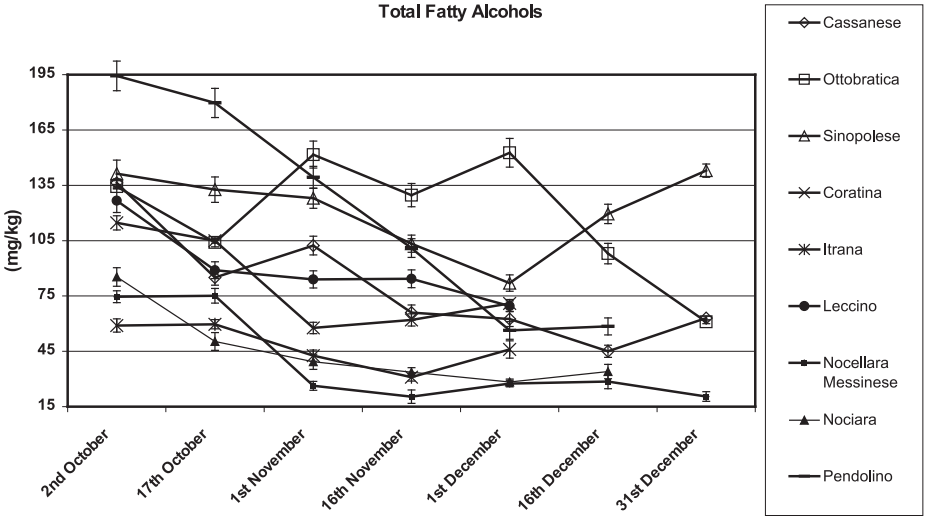

Fig. 1 Evolution in Total Fatty Alcohols (FALs) during fruit ripening. The values represent the means of nine replicates, three for each harvest year.

References

1) Giuffrè, A. M. L'olio di oliva calabrese: aspetti produttivi e tecnologici. *Industrie Alimentari* **42**, 20-25 (2003).
2) Guillaume, C.; Ravetti, L.; Ray, D. L.; Johnson, J. Technological Factors Affecting Sterols in Australian Olive Oils. *J. Am. Oil Chem. Soc.* **89**, 29-39 (2012).
3) Giuffrè, A. M.; Louadj, L. Influence of crop season and cultivar on sterol composition of monovarietal olive oils in Reggio Calabria (Italy). *Czech J. Food Sci.* **31**, 256-263 (2013).
4) Biedermann, M.; Haase-Aschoff, P.; Grob, K. Wax ester fraction of edible oils: Analysis by on-line LC-GC-MS and GCxGC-FID. *Eur. J. Lipid Sci. Technol.* **110**, 1084-1094 (2008).
5) Giuffrè, A. M. Influence of harvest year and cultivar on wax composition of olive oils. *Eur. J. Lipid Sci. Technol.* **115**, 549-555 (2013).
6) Vichi, S.; Lazzez, A.; Grati Kamoun, N.; López-Tamames, E.; Buxaderas, S. Evolution of Sesquiterpene hydrocarbons in virgin olive oil during fruit ripening. *J. Agric. Food Chem.* **58**, 6972-6976 (2010).
7) Jerman Klen, T.; MozetiČčVodopivec B. The fate of olive fruit phenols during commercial olive oil processing: Traditional press versus continuous two- and three-phase centrifuge. *LWT - Food Sci. Technol.* **49**, 267-274 (2012).
8) Gargouri, B.; Ammar, S.; Zribi, A.; Ben Mansour, A.; Bouaziz, M. Effect of growing region on quality characteristics and phenolic compounds of chemlali extra-virgin olive oils. *Acta Physiol Plant* **35**, 2801-2812 (2013).
9) Migliorini, M.; Cherubini, C.; Checchi, L.; Zanoni, B. Degradazione dei composti fenolici durante la conservazione dell'olio extra vergine di oliva. *Riv. Ital. Sostanze Grasse* **90**, 71-79 (2013).
10) Criado, M. N.; Ramón Morelló, J.; Motilva, M. J.; Paz Romero, M. Effect of growing area on pigment and phenolic fractions of virgin olive oils of the Arbequina variety in Spain. *J. Am. Oil Chem. Soc.* **81**, 633-640 (2004).
11) Rigane, G.; Bouaziz, M.; Sayadi, S.; Ben Salem, M. Effect of storage on refined oil composition: stabilization by addition of chlorophyll pigments and squalene. *J. Oleo Sci.* **62**, 981-987 (2013).
12) Dabbou, S.; Dabbou, S.; Selvaggini, R.; Urbani, S.; Taticchi, A.; Servili, M.; Hammami, M. Comparison of the chemical composition and the organoleptic profile of virgin olive oil from two wild and two cultivated tunisian Olea europaea. *Chem. Biodivers.* **8**, 189-202 (2011).
13) Conde, C.; Delrot, S.; Gerós, H. Physiological, biochemical and molecular changes occurring during olive development and ripening. *J. Plant Physiol.* **165**, 1545-1562, (2008).
14) Guerfel, M.; BenMansour, M.; Ouni, Y.; Guido, F.; Boujnah, D.; Zarrouk, M. Triacylglycerols composition and volatile compounds of virgin olive oil from Chemlali cultivar: comparison among different planting densities. *ScientificWorldJournal* **2012**, 1-6 (2012).
15) Mudge, S. M.; Belanger, S. E.; Nielsen, A. M. Fatty Alcohols - Anthropogenic and natural occurrence in the environment. Published by Royal Society of Chemistry, Thomas Graham House, Science Park, Milton Road, Cambridge, CB4 OWF, UK (2008).
16) EU, 2013. Commission Implementing Regulation (EU) No 299/2013 of 26 March 2013. Official Journal of the European Union.
17) IOC, 2013. IOC/T.15/NC No 3/Rev.7. May 2013. Trade Standard Applying to Olive Oils and Olive-Pomace Oils.
18) Grob, K.; Lanfranchi, M.; Mariani, C. Evaluation of olive oils through the fatty alcohols, the sterols and their esters by coupled LC-GC. *J. Am. Oil Chem. Soc.* **67**, 626-634 (1990).
19) Taylor, J. C.; Rapport, L.; Lockwood, G. B. Octacosanol in human health. *Nutrition* **19**, 192-195 (2003).
20) Stüsser, R.; Batista, J.; Padrón, R., Sosa, F.; Pereztol, O. Long-term therapy with policosanol improves treadmill exercise-ECG testing performance of coronary heart disease patients. *Int. J. Clin. Pharmacol. Ther.* **36**, 469-473 (1998).
21) Arruzazabala, M. L.; Molina, V.; Mas, R.; Fernández, L.; Carbajal, D.; Valdés, S.; Castaño, G. Antiplatelet effects of policosanol (20 and 40 mg/day) in healthy volunteers and dyslipidaemic patients. *Clin. Exp. Pharmacol. P.* **29**, 891-897 (2002).
22) Tubaileh, R. M.; Garrido-Fernández, A.; Ruiz-Méndez, M.V.; León-Camacho, M., Graciani-Constante, E. Effects of physical refining on contents of waxes and fatty alcohols of refined olive oil. *J. Am. Oil Chem. Soc.* **79**, 101-104 (2002).
23) Fernández-Arche, A.; Marquez-Martín, A.; de la Puerta Vazquez, R.; Perona, J. S.; Terencio, C.; Perez-Camino, C.; Ruiz-Gutierrez, V. Long-chain fatty alcohols from pomace olive oil modulate the release of protoinflammatory mediators, *J. Nutr. Biochem.* **20**, 155-162 (2009).
24) Giuffrè, A. M. The effects of cultivar and harvest year on fatty alcohol composition of olive oils from South West Calabria (Italy), *Grasas Aceites* (in press).
25) Giuffrè, A. M.; Piscopo, A.; Sicari, V.; Poiana M. The effects of harvesting on phenolic compounds and fatty acids content in virgin olive oil (cv Roggianella). *Riv. Ital. Sostanze Grasse* **87**, 14-23 (2010).
26) Sicari, V.; Giuffrè, A. M.; Piscopo, A.; Poiana M. Effect of the "Ottobratica" variety ripening stage on the phenolic profile of the obtained oil. *Riv. Ital. Sostanze*

Grasse **86**, 215-219 (2009).
27) Giuffrè, A. M.; Louadj, L.; Poiana, M.; Macario, A. Composition en sterols des huiles extraites d' olives de cultivars de la province de Reggio Calabria (Sud d'Italie). *Riv. Ital. Sostanze Grasse* **89**, 177-183 (2012).
28) Giuffrè, A. M. Variation in triacylglycerols of olive oils produced in Calabria (Southern Italy) during olive ripening. *Riv. Ital. Sostanze Grasse* (in press).
29) Consolidated text, CONSLEG: 1991R2568 - 01/11/2003, on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis, Annex XIX.
30) Lazzez, A.; Perri, E.; Caravita, M. A.; Khlif, M.; Cossentini, M. Influence of olive maturity stage and geographical origin on some minor components in virgin olive oil of the Chemlali variety. *J. Agric. Food Chem.* **56**, 982-988 (2008).
31) Ranalli, A.; Pollastri, L.; Contento, S.; Di Loreto, G., Iannucci, E.; Lucera, L.; Russi, F. Sterol and alcohol components of seed, pulp and whole olive fruit oils. Their use to characterise olive fruit variety by multivariates. *J. Sci. Food Agric.* **82**, 854-859 (2002).
32) Krichène, D.; Allalout, A.; Salvador, M. D.; Fregapane, G.; Zarrouk, M. Fatty acids, volatiles, sterols and triterpenic alcohols of six monovarietal Tunisian virgin olive oil. *Eur. J. Lipid Sci. Technol.* **112**, 400-409 (2010).
33) García-Gonzáles, D. L.; Tena, N.; Aparicio, R. Describing the chemical singularity of the Spanish protected designations of origin for virgin olive oils in relation to oils from neighbouring areas. *Grasas Aceites* **63**, 26-34 (2012).
34) Strabbioli, R.; Bocci, F.; Frega, N. G. Qualità e stabilità dell' olio estratto dalle olive delle varietà marchigiane Piantone di Mogliano e Raggia a confronto con la cv Leccino. *Riv. Ital. Sostanze Grasse* **84**, 125-132 (2007).
35) El Antari, A.; Hilal, A.; Boulouha, B.; El Moudni, A. Influence of variety, environmental and cultural techniques on the characteristics of olive fruits and the chemical composition of extra virgin olive oil in Morocco. *Olivae* **80**, 29-36 (2000).
36) Sifi, S.; Ben Ammar, K. ; Chekir, S. ; Amamou, T. Etude de composé mineurs libre et esterifiés dans la fraction insaponifiable des huiles d'olives tunisiennes. *Riv. Ital. Sostanze Grasse* **80**, 229-231 (2003).
37) Ranalli, A. ; Modesti, G. ; Patumi, M., Fontanazza, G. The compositional quality and sensory properties of virgin olive oil from a new olive cultivar - I-77. *Food Chem.* **69**, 37-46 (2000).
38) Aparicio, R.; Luna, G. Characterisation of monovarietal virgin olive oils. *Eur. J. Lipid Sci. Technol.* **104**, 614-627 (2002).
39) Rivera del Álamo, R. M.; Fregapane, G.; Aranda, F.; Gómez-Alonso, S.; Salvador, M. D. Sterol and alcohol composition of Cornicabra virgin olive oil: the campesterol content exceeds the upper limit of 4% established by EU regulations. *Food Chem.* **84**, 533-537 (2004).
40) Sakouhi, F.; Boukhchina, S.; Absalo, C.; Fouquet, E,; Kallel, H. Policosanol characterization and accumulation during ripening of Tunisian *Olea europaea* L. fruits. *Eur. J. Lipid Sci. Technol.* **112**, 373-379 (2010).