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# Influence of Cultivar on Lemon Oil Composition

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**Abstract**

In order to evaluate the differences in the qualitative and quantitative composition of lemon essential oils obtained from different lemon cultivars, the oils were analyzed by HRGC and HRGC/MS. The components were identified in each oil on polar and apolar capillary columns. The samples analyzed were obtained from lemon plants cultivated in Sicily in an experimental field. The selection reflected the diversity of these cultivars in the Italian productive areas.

The results obtained are informative of the different oils quality and explain the variation of the Italian industrial lemon essential oil composition.

**Key Word Index**

*Citrus limon*, Rutaceae; lemon cultivars, essential oil composition, limonene, γ-terpinene.

## Introduction

In Italy, the highest density of lemon cultivation is found in Sicily and Calabria. In particular Sicily produces over 90% of Italian lemon, gaining a quasi-monopoly of the production. The main productive areas are in the vicinity of Catania, Palermo, Siracusa, and Messina. It has been observed that in each small Italian lemon productive area, there are different cultivars while some specific cultivars are predominant in other countries such as "Fino" and "Verna" in Spain, "Eureka" and "Lisbon" in the US., etc. "Malsecco", caused by the fungus *Phoma tracheiphila*, and which locates in the wooden part of the lemon tree, has forced Italian producers and researchers to select different clones, for their resistance to the fungus.

The most common cultivars present in the Sicilian areas are "Femminello" which represents 84% of the whole lemon production, "Monachello" and "Interdonato". "Femminello" is a very heterogeneous cultivar giving rise to a large number of clones. These clones differ in plant development, productivity, fruit shape, resistance to "malsecco", etc. (1,2).

The data reported in literature do not refer to the influence of each cultivar on the composition of Italian lemon essential oils, with the exception of some papers published in the 1960s and 1970s (3-4), and some specific papers on experimental cultivars not representative of the real cultivation panorama (5-7). A great deal of information is, otherwise, available on the composition of industrially produced lemon oil (8-10).

Given the large number of cultivars, it was necessary to carry out a study on the composition of lemon oil obtained from different cultivars representative of the current reality of the Sicilian production, in order to evaluate the dependency of oil quality on cultivar.

## Experimental

This study was carried out on 18 samples of laboratory extracted Sicilian lemon oils obtained from fruits harvested in February 1999 in an experimental field of the "Istituto di Coltivazione Arboree" of the University of Catania, situated in the South-East part of the province of Catania (Sicily, Italy). The fruits were picked in February since the Italian winter lemon fruits give the most valuable oils. All the fruits were collected in the same experimental field in order to avoid possible differentiation due to production area. The oils analyzed belonged to the following cultivars: "Femminello siracusano", "Femminello S. Teresa", "Femminello Continella", "Femminello Dosaco", "Femminello fior d'arancio",

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"Femminello incappucciato", "Monachello", "Interdonato" and "Fino". The cultivars analyzed represented about 90% of the plants grown in Sicily. "Fino" is a Spanish cultivar, which has been recently introduced in Italy. Two samples of each cultivar were analyzed. Each sample was obtained from the peel of 10 fruits. The oils were isolated in the laboratory by applying manual pressure on the rind thereby breaking of the utricles and release of the oil which was collected on a watch glass, transferred to a test tube, centrifuged and analyzed. The qualitative and quantitative volatile fraction composition was studied by HRGC and HRGC/MS.

## HRGC Analysis

Each oil was analyzed by HRGC on a Gas Chromatograph 5160 Mega series (Fisons Instrument, Milan, Italy) equipped with a Shimadzu data processor C-R3A using the following experimental conditions: SE-52 fused silica capillary column, 30 m x 0.32 mm; 0.40-0.45 μm, film thickness (Mega, Legnano (MI), Italy); column temperature, 45°C (6 min) to 250°C at 3°C/min; injection mode, split; split ratio, 1:100; detector, FID; injector and detector temperature, 250°C; carrier gas, He 100 kPa; injected volume, 1 μL of net whole oil. The quantitative composition was obtained by peak area normalization and the response factor for each component was considered equal to one.

## HRGC-MS Analysis

Samples were analyzed by HRGC/MS (EI) on a Fisons MD 800 (Fisons Instrument, Milan, Italy) system coupled with Adams' library (11). Two different columns were used: 1) Mega 5 MS fused silica capillary column, 30 m x 0.25 μm i.d.; 0.25 mm, film thickness (Mega, Legnano (MI) Italy); carrier gas, He, 90 kPa; linear velocity, 42.7 cm/sec at 40°C; column temperature, 40°C (2 min) to 240°C at 3.0°C/min. 2) Megawax fused silica capillary column, 30 m x 0.32 mm; 0.40-0.45 μm, film thickness (Mega, Legnano (MI), Italy); carrier gas, He 90 kPa; linear velocity, 34.7 cm/sec at 40°C; column temperature, 40°C (6 min) to 220°C at 2°C/min. For both columns: injector temperature, 250°C; injection mode, split; split ratio, 1:30; volume injected, 1 μL of a solution 1:100 in pentane of the oil; interface temperature, 250°C; acquisition mass range, 41-300 amu; solvent cut, 2 min.

Linear retention indices of the sample components were determined on the basis of homologue n-alkane hydrocarbons analyzed under the same GC conditions. The compound identification was confirmed by comparison of mass spectra of compounds with published spectra and of retention indices with published index data.

## Results and Discussion

Sixty-seven components were identified in each oil. The components identified and their linear retention indices calculated on Mega 5 MS and Megawax can be seen in Table I.

For each sample, the quantitative composition (as a relative percentage of peak area) for each component, as well as the total amount of monoterpenes and sesquiterpenes hydrocarbons, carbonyl compounds, alcohols and esters was calcu-

**Table I. Linear retention indices calculated on Mega 5MS and Megawax capillary columns for the components identified in the lemon oils analyzed**

| Compound | Linear retention indices | |
|---|---|---|
| | Mega 5MS | Megawax |
| tricyclene | 913 | - |
| α-thujene | 920 | 1012 |
| α-pinene | 925 | 1008 |
| camphene | 936 | 1043 |
| sabinene | 964 | 1105 |
| β-pinene | 964 | 1085 |
| 6-methyl-5-hepten-2-one | 981 | - |
| myrcene | 987 | 1152 |
| α-phellandrene | 997 | 1146 |
| octanal | 997 | 1270 |
| δ-3-carene | 1002 | 1128 |
| α-terpinene | 1009 | 1160 |
| p-cymene | 1016 | - |
| limonene | 1021 | 1181 |
| (Z)-β-ocimene | 1036 | - |
| (E)-β-ocimene | 1045 | 1239 |
| γ-terpinene | 1051 | 1225 |
| *cis*-sabinene hydrate | 1058 | 1447 |
| octanol | 1071 | 1546 |
| terpinolene | 1079 | 1260 |
| *trans*-sabinene hydrate | 1088 | - |
| linalol | 1094 | 1537 |
| nonanal | 1098 | 1372 |
| heptyl acetate | 1111 | - |
| *cis*-limonene oxide | 1122 | 1403 |
| *trans*-limonene oxide | 1127 | 1423 |
| camphor | 1127 | - |
| citronellal | 1146 | 1457 |
| borneol | 1152 | - |
| terpinen-4-ol | 1165 | 1575 |
| α-terpineol | 1179 | 1670 |
| decanal | 1199 | 1476 |
| octyl acetate | 1210 | 1461 |
| nerol | 1222 | 1830 |
| citronellol | 1222 | - |
| carvone | 1229 | 1684 |
| neral | 1231 | 1645 |
| piperitone | 1239 | - |
| geraniol | 1251 | 1783 |
| perillaldehyde | 1257 | 1729 |
| geranial | 1262 | 1697 |
| bornyl acetate | 1275 | - |
| undecanal | 1300 | 1580 |
| nonyl acetate | 1308 | - |
| methylgeranate | 1316 | - |
| citronellyl acetate | 1349 | - |
| neryl acetate | 1360 | 1705 |
| geranyl acetate | 1379 | 1738 |
| dodecanal | 1402 | - |
| decyl acetate | 1404 | 1662 |
| β-caryophyllene | 1404 | 1555 |
| *trans*-α-bergamottene | 1427 | 1560 |
| α-humulene | 1437 | 1624 |
| β-santalene | 1449 | - |
| (Z)-β-farnesene | 1452 | 1649 |
| germacrene D | 1466 | 1664 |
| γ-muurolene | 1469 | - |
| valencene | 1479 | - |
| bicyclogermacrene | 1482 | 1688 |
| (Z)-α-bisabolene | 1495 | - |
| β-bisabolene | 1500 | 1701 |
| (E)-α-bisabolene | 1504 | - |
| tetradecanal | 1605 | - |
| 2,3-dimethyl-3-(4-methyl-3-pentenyl)-2-norbornanol | 1638 | - |
| campherenol | 1654 | - |
| β-bisabolol | 1657 | - |
| nootkatone | 1780 | 243 |

**Table II. Percentage composition (mean values X with standard deviations s) as single compounds and as classes of substances for the different lemon cultivar oils analyzed**

| | Cultivars | | | |
|---|---|---|---|---|
| | Feminello siracusano | Feminello continella | Feminello S. Teresa | Feminello fior d'arancio |
| tricyclene | 0,03 | t | t | t |
| α-thujene | 0,32 | 0,33 | 0,28 | 0,29 |
| α-pinene | 1,38 | 1,50 | 1,18 | 1,21 |
| camphene | 0,04 | 0,04 | 0,03 | 0,03 |
| sabinene + β-pinene | 8,48 | 8,49 | 8,50 | 8,52 |
| 6-methyl-5-hepten-2-one | t | t | t | t |
| myrcene | 1,72 | 1,75 | 1,54 | 1,62 |
| octanal | 0,04 | 0,03 | 0,03 | 0,04 |
| α-phellandrene | 0,06 | 0,06 | 0,12 | 0,04 |
| δ-3-carene | t | t | t | t |
| α-terpinene | 0,19 | 0,16 | 0,19 | 0,17 |
| p-cymene | 0,04 | 0,02 | 0,04 | 0,06 |
| limonene | 72,23 | 75,99 | 69,58 | 72,38 |
| (Z)-β-ocimene | 0,06 | 0,04 | 0,05 | 0,03 |
| (E)-β-ocimene | 0,12 | 0,08 | 0,11 | 0,07 |
| γ-terpinene | 9,27 | 7,42 | 9,43 | 8,70 |
| *cis*-sabinene hydrate | 0,07 | 0,05 | 0,07 | 0,07 |
| octanol | t | t | t | t |
| terpinolene | 0,42 | 0,32 | 0,42 | 0,39 |
| *trans*-sabinene hydrate | 0,07 | 0,04 | 0,10 | 0,06 |
| linalool | 0,17 | 0,13 | 0,20 | 0,18 |
| nonanal | 0,06 | 0,05 | 0,11 | 0,05 |
| heptyl acetate | t | t | t | t |
| *cis*-limonene oxide | t | t | t | t |
| *trans*-limonene oxide | t | t | t | t |
| camphor | 0,01 | 0,01 | 0,01 | 0,01 |
| citronellal | 0,15 | 0,08 | 0,18 | 0,21 |
| borneol | 0,01 | 0,01 | 0,03 | 0,02 |
| terpinen-4-ol | 0,03 | 0,02 | 0,03 | 0,03 |
| α-terpineol | 0,26 | 0,16 | 0,32 | 0,26 |
| decanal | 0,04 | 0,04 | 0,06 | 0,04 |
| octyl acetate | t | t | t | t |
| nerol+ citronellol | 0,03 | 0,02 | 0,07 | 0,05 |
| neral + carvone | 0,92 | 0,48 | 1,72 | 1,06 |
| piperitone | t | t | t | t |
| geraniol | 0,02 | 0,01 | 0,04 | 0,03 |
| geranial + perillaldehyde | 1,51 | 0,78 | 2,86 | 1,76 |
| bornyl acetate | t | 0,01 | 0,01 | 0,01 |
| undecanal | 0,01 | 0,01 | 0,02 | 0,02 |
| nonyl acetate | t | t | t | t |
| methylgeranate | 0,01 | 0,01 | 0,01 | 0,01 |
| citronellyl acetate | 0,04 | 0,03 | 0,03 | 0,06 |
| neryl acetate | 0,30 | 0,31 | 0,46 | 0,53 |
| geranyl acetate | 0,22 | 0,17 | 0,31 | 0,35 |
| dodecanal | 0,01 | 0,01 | 0,01 | 0,01 |
| decyl acetate | 0,03 | 0,02 | 0,03 | 0,02 |
| β-caryophyllene | 0,22 | 0,16 | 0,23 | 0,31 |
| *trans*-α-bergamotene | 0,40 | 0,32 | 0,48 | 0,31 |
| α-humulene | 0,02 | 0,02 | 0,02 | 0,03 |
| (E)-β-farnesene | 0,04 | 0,03 | 0,04 | 0,03 |
| β-santalene | 0,02 | 0,01 | 0,02 | 0,01 |
| γ-muurolene | 0,01 | 0,01 | 0,01 | 0,01 |
| germacrene D | 0,01 | 0,01 | 0,01 | 0,01 |
| valencene | 0,02 | 0,01 | 0,02 | 0,02 |
| bicyclogermacrene | 0,07 | 0,04 | 0,05 | 0,09 |
| (Z)-α-bisabolene | 0,04 | 0,04 | 0,05 | 0,03 |
| β-bisabolene | 0,58 | 0,47 | 0,71 | 0,46 |
| (E)-α-bisabolene | 0,02 | 0,01 | 0,02 | 0,01 |
| tetradecanal | 0,01 | 0,01 | 0,01 | 0,02 |
| 2,3-dimethyl-3-(4-methyl-3-pentenyl)-2-norbornanol | 0,02 | 0,02 | 0,02 | 0,02 |
| campherenol | 0,03 | 0,02 | 0,03 | 0,02 |
| α-bisabolol | 0,03 | 0,02 | 0,03 | 0,02 |
| nootkatone | t | t | t | t |
| hydrocarbons | 95,77 | 97,36 | 93,15 | 94,85 |
| monoterpenes | 94,32 | 96,22 | 91,47 | 93,52 |
| sesquiterpenes | 1,45 | 1,14 | 1,68 | 1,34 |
| oxygenated compounds | 4,07 | 2,55 | 6,80 | 4,94 |
| alcohols | 0,73 | 0,51 | 0,94 | 0,76 |
| carbonyl compounds | 2,75 | 1,50 | 5,01 | 3,21 |
| esters | 0,59 | 0,55 | 0,84 | 0,96 |

**Table II. Continued**

| | Cultivars | | | | |
|---|---|---|---|---|---|
| | Feminello Dosaco | Feminello incappucciato | Monachello | Interdonato | Fino |
| tricyclene | t | t | t | t | t |
| α-thujene | 0,27 | 0,26 | 0,39 | 0,33 | 0,29 |
| α-pinene | 1,12 | 1,13 | 1,51 | 1,41 | 1,44 |
| camphene | 0,03 | 0,03 | 0,04 | 0,04 | 0,04 |
| sabinene + β-pinene | 6,66 | 8,33 | 9,77 | 8,44 | 10,81 |
| 6-methyl-5-hepten-2-one | t | t | t | t | t |
| myrcene | 1,65 | 1,45 | 1,52 | 1,54 | 1,60 |
| octanal | 0,03 | 0,04 | 0,04 | 0,03 | 0,03 |
| α-phellandrene | 0,07 | 0,10 | 0,09 | 0,03 | 0,11 |
| δ-3-carene | t | 0,01 | t | t | t |
| α-terpinene | 0,17 | 0,19 | 0,23 | 0,20 | 0,15 |
| p-cymene | 0,05 | 0,08 | 0,04 | 0,04 | 0,06 |
| limonene | 74,34 | 65,96 | 67,01 | 70,20 | 70,95 |
| (Z)-β-ocimene | 0,04 | 0,04 | 0,07 | 0,20 | 0,03 |
| (E)-β-ocimene | 0,08 | 0,10 | 0,13 | 0,29 | 0,07 |
| γ-terpinene | 8,55 | 9,82 | 11,11 | 9,61 | 7,16 |
| *cis*-sabinene hydrate | 0,06 | 0,08 | 0,08 | 0,06 | 0,07 |
| octanol | | t | | | t |
| terpinolene | 0,39 | 0,46 | 0,48 | 0,42 | 0,31 |
| *trans*-sabinene hydrate | 0,07 | 0,09 | 0,10 | 0,05 | 0,06 |
| linalool | 0,18 | 0,22 | 0,14 | 0,13 | 0,15 |
| nonanal | 0,06 | 0,11 | 0,12 | 0,01 | 0,09 |
| heptyl acetate | t | t | t | t | t |
| *cis*-limonene oxide | t | t | t | t | t |
| *trans*-limonene oxide | t | 0,01 | t | t | t |
| camphor | 0,01 | 0,01 | 0,01 | 0,01 | t |
| citronellal | 0,17 | 0,33 | 0,10 | 0,07 | 0,19 |
| borneol | 0,02 | 0,03 | 0,02 | 0,01 | 0,02 |
| terpinen-4-ol | 0,03 | 0,04 | 0,03 | 0,03 | 0,02 |
| α-terpineol | 0,26 | 0,37 | 0,31 | 0,21 | 0,24 |
| decanal | 0,05 | 0,08 | 0,22 | 0,02 | 0,05 |
| octyl acetate | t | t | t | t | t |
| nerol + citronellol | 0,04 | 0,07 | 0,03 | 0,01 | 0,03 |
| neral + carvone | 1,05 | 2,47 | 1,44 | 0,41 | 1,34 |
| piperitone | t | t | t | t | |
| geraniol | 0,02 | 0,05 | 0,05 | 0,01 | 0,03 |
| geranial + perillaldehyde | 1,74 | 4,16 | 2,42 | 0,68 | 2,20 |
| bornyl acetate | 0,01 | 0,01 | 0,01 | 0,02 | t |
| undecanal | 0,02 | 0,04 | 0,02 | 0,01 | 0,02 |
| nonyl acetate | t | t | t | t | t |
| methylgeranate | 0,01 | 0,01 | t | 0,02 | t |
| citronellyl acetate | 0,05 | 0,06 | 0,02 | 0,06 | 0,04 |
| neryl acetate | 0,42 | 0,60 | 0,39 | 0,38 | 0,40 |
| geranyl acetate | 0,34 | 0,45 | 0,54 | 0,49 | 0,31 |
| dodecanal | 0,01 | 0,01 | 0,01 | 0,02 | 0,01 |
| decyl acetate | 0,03 | 0,04 | 0,03 | 0,04 | 0,03 |
| β-caryophyllene | 0,25 | 0,44 | 0,28 | 0,35 | 0,17 |
| *trans*-α-bergamotene | 0,45 | 0,62 | 0,42 | 0,61 | 0,38 |
| α-humulene | 0,02 | 0,04 | 0,03 | 0,03 | 0,02 |
| (E)-β-farnesene | 0,04 | 0,06 | 0,04 | 0,05 | 0,03 |
| β-santalene | 0,02 | 0,02 | 0,02 | 0,02 | 0,01 |
| γ-muurolene | 0,01 | 0,01 | 0,01 | 0,01 | 0,01 |
| germacrene D | 0,01 | 0,01 | 0,01 | t | 0,02 |
| valencene | 0,01 | 0,06 | 0,01 | 0,03 | 0,01 |
| bicyclogermacrene | 0,05 | 0,11 | 0,02 | 0,02 | 0,05 |
| (Z)-α-bisabolene | 0,05 | 0,07 | 0,05 | 0,06 | 0,04 |
| β-bisabolene | 0,67 | 0,91 | 0,63 | 0,88 | 0,54 |
| (E)-α-bisabolene | 0,02 | 0,03 | 0,02 | 0,03 | 0,02 |
| tetradecanal | 0,01 | 0,02 | 0,01 | 0,05 | 0,01 |
| 2,3-dimethyl-3-(4-methyl-3-pentenyl)-2-norbornanol | 0,02 | 0,03 | 0,02 | 0,03 | 0,02 |
| campherenol | 0,03 | 0,04 | 0,03 | 0,04 | 0,02 |
| α-bisabolol | 0,03 | 0,05 | 0,03 | 0,04 | 0,02 |
| nootkatone | t | 0,01 | t | t | t |
| hydrocarbons | 95,04 | 90,38 | 93,95 | 96,27 | 94,33 |
| monoterpenes | 93,42 | 87,97 | 92,40 | 94,15 | 93,03 |
| sesquiterpenes | 1,62 | 2,42 | 1,55 | 2,12 | 1,31 |
| oxygenated compounds | 4,74 | 9,49 | 6,18 | 2,90 | 5,41 |
| carbonyl compounds | 3,14 | 7,25 | 4,38 | 1,31 | 3,95 |
| esters | 0,85 | 1,16 | 0,98 | 1,02 | 0,78 |
| alcohols | 0,75 | 1,07 | 0,83 | 0,58 | 0,68 |

t = trace

lated. Table II reports the average composition as single components and as classes of substances for the different cultivar oils analyzed. The components were reported according to their retention times on SE-52 column. The data obtained for the two samples of each cultivar were in agreement. The values obtained for each single component and, therefore for the classes of substances, were very close for each pair of samples within the same cultivar.

The laboratory-isolated oils usually have a slightly higher content of oxygenated compounds than that of industrial oils due to the different isolation processes (12). By observing the composition of each cultivar clear quantitative differences resulted. First we can observe that the different cultivars of "Femminello" had a different quantitative composition, which is moreover different from the "Monachello", "Interdonato" and "Fino" cultivars. With regard to the "Interdonato" oils, the data here obtained were in agreement with those previously obtained (13).

A first analysis of data on the class of substances showed that the monoterpenes reached a maximum value in the "Femminello Continella" (96.22%) oil and a minimum in "Femminello Incappucciato" (87.97%). This behavior was particularly due to the different limonene and γ-terpinene contents. With regard to the sesquiterpene hydrocarbons, "Femminello Incappucciato" and "Interdonato" oils had values higher than 2%, while the other cultivar oils had similar values of about 1.5%. Among the oxygenated compounds, "Femminello Incappucciato" had the highest value (9.49%) while "Interdonato" (2.90%) and "Femminello Continella" (2.55%) the minimum. These differences were mainly due to carbonyl compounds whose average content varied from 7.25% for "Femminello Incappucciato" to 1.31% for "Interdonato". The alcohol and ester contents varied in a more restricted range. Alcohols varied from 0.51% for "Femminello Continella" to 1.07% for "Femminello Incappucciato", while the esters ranged from 0.55% for "Femminello Continella" to 1.16% for "Femminello Incappucciato". Among the carbonyl compounds, the values obtained for the oils analyzed mainly regarded the content of neral and geranial; these two components named citral characterize the olfactive notes of lemon oil giving it a particular fragrance; their content determines the quality and the commercial value of a lemon oil. The maximum total value of neral and geranial was reached in the oils of "Femminello Incappucciato" (6.63%) and "Femminello S. Teresa" (4.55%) which can be considered the more interesting oils.

In conclusion, because of the noticeable cultivar influence on the lemon oil composition, in order to improve the quality of Italian lemon oil, it would be of great help if only some selected cultivars were industrially processed.

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