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Qualitative traits and peel essential oil profiles of 24 Italian and international lemon varieties

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A B S T R A C T

Lemon (*Citrus limon* L. Burm. f.) is used for fresh consumption because it is rich in several nutraceutical products especially vitamin C. The physicochemical properties and the bioactive compounds of the peel, such as the essential oil, are strictly depended on the genotype. In this context, peel and juice qualitative traits of 24 lemon cultivars were described: 20 varieties of the Italian germplasm, mostly belonging to Femminello (F.) lines, and 4 international varieties were studied. This study which may be considered one of the few examples carried out on a large number of varieties of a single Citrus species, was conducted over two years and the oils collected by hydro-distillation of the flavedo were analyzed by GC-FID and GC-MS. Differences were observed in the oil yields. For an easier comparison the essential oils' component were subdivided in five classes: monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes and others (not terpenoidic compounds). Among the monoterpene hydrocarbons, in all the cultivars the most abundant compounds were limonene, followed by β-pinene and γ-terpinene. In particular, Monachello showed the highest content of monoterpene hydrocarbons, while the lowest value was observed in F. Carrubaro, Fino and Akragas. Femminello lines are interesting cultivars for qualitative traits of the juice (organic acids and vitamin C) and for the highest content of volatile compounds in the peel, such as limonene, myrcene, β-pinene, α-pinene, γ-terpinene and sabinene. Significant differences in morphological and physicochemical traits were observed: fruits of Lemox, F. Adamo and F. Scandurra showed to be seedless, while high acidity content was observed in F. 2Kr, which also showed the highest vitamin C content.

1. Introduction

Lemon (*Citrus limon* L. Burm.f.), belonging to the Rutaceae family, is one of the most spread *Citrus* species cultivated in the countries characterized by a subtropical and tropical climate. In Italy, the cultivation is mostly concentrated in coastal areas of Southern regions due to its sensitivity to cold temperatures, representing one of the main fruit tree crops for its economic and commercial importance. In detail, Italian production reached 476.311 t in 2022, approximately 88% of which are produced in Sicily (Istat, 2024).

For several decades, the study of the effect of the genetic variability of lemons on the qualitative parameters of the fruit has played an important role in the research activities. The most cultivated varieties in Sicily are Femminello, Monachello and Interdonato, which are characterized by several marked differences in plant growth, disease tolerance and qualitative traits. The Femminello cultivar is extremely prone to spontaneous mutation; indeed, it could be considered a cultivar-population as different lines have been selected in the last century, such as Femminello comune, F. Adamo, F. Continella, F. Siracusano, F. Santa Teresa, and F. Zagara bianca (Amenta et al., 2015) with the aim of obtaining better production and tolerance to mal secco disease (Oliveri et al., 2022).

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All cultivars differ in terms of morphological and biochemical compounds. The size and the shape of the fruits are one of the most important traits influencing consumer preferences. Previous study reported that the Sicilian cultivars F. Adamo and F. Cerza showed the highest fruit weight in comparison with Ovale di Sorrento and Sfusato Amalfitano, cultivars original of Campania region (Di Matteo et al., 2021). In addition, a group of researchers showed that fruits of Monachello, cultivar characterized by low productivity and poor-quality juice, reached commercial size for the fresh market later than Femminello varieties (Amenta et al., 2015).

Spain is the main lemon producing country in the Mediterranean basin and the production is mainly located in the coastal area of Murcia and Alicante provinces. The lemon production is dominated by Fino and Verna varieties. The first produces high quality fruit appropriate for export between October and February, while Verna production is extended from March to July (Aguilar-Hernández et al., 2020).

Outside the Mediterranean basin, the most cultivated cultivars are Eureka, originated in California, and Lisbon in Portugal with the latter showing a higher yield than Eureka and a good resistance to frost. Previous studies investigated the physico-chemical characteristics of Eureka lemon showing that its fruit were smaller, with higher content of titratable citric acid and sugars than Fino and Verna cultivars (Aguilar-Hernández et al., 2020; Perez-Perez et al., 2005).

Citrus peel essential oils represent one of the most important products of citrus fruits transformation. The worldwide production of citrus essential oils is estimated to be about 16,000 tons. Today the economic value of the global citrus oil market is estimated at US$ 3.3 Bn and, as it is constantly expanding, the aforementioned market is expected to stand at US$ 5.3 Bn in 2031. Orange oil sweet type is the main product representing 62.9% of total oils, lemon oil with 16.2% ranks second; in 2021 the total market of these two products share US$ 2072 Mn and US$ 534 Mn, respectively. However, it is to be underlined the large difference of the market price of orange sweet and lemon oils, whose values are 2–4 euro/kg and 30–50 euro/kg, respectively.

From a compositional point of view, phenolic compounds and essential oils represent the main phytochemical classes in lemon peels. Recent comprehensive reviews highlight the peculiar features of lemon peel, such as the phytochemical components, the nutritional and nutraceutic properties, as well as the industrial applications (Klimek-Szczykutowicz et al., 2020).

Lemon oil is considered safe being enclosed in the GRAS list and broadly used as natural food additives (Dosoky & Setzer, 2018). Terpenes are the main components of citrus oils, being subdivided in monoterpenes and sesquiterpenes; both classes are represented by hydrocarbons and oxygenated derivatives, being the first class the main represented. D-limonene, a monoterpene hydrocarbon is the main component of citrus essential oils, being characterized by a large list of biological positive effects (Anandakumar et al., 2021), this compound is considered one of the most spread and cheapest chiral starting material useful for many synthetic purposes (Thomas & Bessiere, 1989). Concerning the market value of only D-limonene, it has been estimated about US$ 480 Mn in 2021, being expected to reach about 700 Mn by 2029.

The main aim of this study was to carry out the characterization and the qualitative comparison of different lemon varieties cultivated in a collection field located in the experimental agricultural farm of the University of Catania. From an analysis of literature data, the work here reported may be considered one of the few if not the only one reporting a study on an large number of varieties of a single Citrus species, in this case the lemon.

We would like to underline that similar studies on citrus correlating the chemical composition of essential oils with the citrus genus taxonomy or their genetic types (Azam et al., 2013; Jing et al., 2015), as well as the studies of the varieties of a single citrus species, as the determination of the profile of the volatile components affected by the rootstock or the analysis of biomarkers has been carried out on a few number of varieties of a single species like the lemon (Aguilar-Hernandez et al., 2020; Haq & Wang, 2023).

The 24 different varieties have been selected in order to evaluate the qualitative features in a unique environment.

2. Materials and methods

2.1. Plant material and fruit sampling

All fruits were collected from a germplasm collection field belonging to the University of Catania, located in the plain of Catania (37°24′33″N; 15°03′20″E). The recorded rainfall was 681 mm per year and the average monthly temperature was between 11.4 °C in January and 25.8 °C in August. The texture components of the soil were 68.2% sand, 18.3% loam, and 13.5% clay. In the experimental field, the pH value was 7.2. The orchard was subjected to standard cultural practices.

All plants were 10 years old, grafted onto sour orange (*C. aurantium* L.). A total of 24 cultivars were used for the characterization (Table S1), 20 of the Italian germplasm, mostly belonging to Femminello lines, and 4 international varieties. Sixty fruits per cultivar were harvested in February of two consecutive years from 4 trees per cultivar, commercially known as "Primofiore" or winter lemon, the most important production: 30 fruits were used for morphological and physico-chemical analyses and 30 for essential oil extraction.

2.2. Morphological and physico-chemical traits

Individual fruit weight was measured using an electronic balance and rind thickness using an electronic digital gauge (model CD-15 DC, Mitutoyo Ltd, Telford, Shropshire, UK). The number of seeds per fruit was counted for each variety and the seed frequency (%) was evaluated.

Fruit juice was extracted with a commercial juice extractor (Kenwood Citrus Juicer JE290, Havant, Hampshire, UK). Three juice samples, each obtained from the pooled juice of 10 fruits per genotype, were used for biochemical analyses. Juice was weighted and expressed as juice yield (mL of juice per 10 fruits). The total soluble solid (TSS) content was determined using a digital refractometer (Atago CO., LTD, model PR-32 α, Tokyo, Japan), with the results expressed as °Brix. Titratable acidity (TA) and the pH were determined by potentiometric titration (Hach Company, TitraLab AT1000 Series, Loveland, Colorado, USA) of the juice with 0.1 N NaOH beyond pH 8.1 according to the AOAC method, with the results expressed as g L^{-1} of citric acid equivalent. Vitamin C (L-ascorbic acid) was determined using an automatic titration apparatus (702 SM Titrino, Metrohm, Herisau, Appenzello Esterno, Switzerland) with 0.001 M I_2, and the results were expressed as mg L^{-1}.

2.3. Isolation of essential oils

Fresh rind tissue (flavedo, 100 g) of each sample was subjected to hydrodistillation until there was no significant increase in the volume of oil collected (3 h). The oils, whose average yields from both years samples are reported in Table S1, were dried over anhydrous sodium sulphate and stored under N_2 in a sealed vial at −20 °C until required, normally from few days to a week.

2.4. Essential oil analysis

Gas chromatographic (GC) analyses were run on a Shimadzu gas chromatograph, Model 17-A equipped with a flame ionization detector (FID, Shimadzu, Milan, Italy), and with an operating software Class VP Chromatography Data System version 4.3 (Shimadzu, Milan, Italy). Analytical conditions: SPB-5 capillary column (15 m × 0.10 mm x 0.15 μm), helium as carrier gas (1 mL/min). Injection in split mode (1:200), injected volume 1 μL (4% essential oil/CH_2Cl_2 v/v), injector and detector temperature 250 e 280 °C, respectively. Linear velocity in column 19 cm/s. The oven temperature was held at 60 °C for 1 min, then programmed as reported previously (Saija et al., 2016). Percentages of compounds were determined from their peak areas in the GC-FID profiles.

Gas-chromatography-mass spectrometry (GC-MS) was carried out in the fast mode on a Shimadzu GC-MS mod. GCMS-QP5050A, with the same column and the same operative conditions used for analytical GC-FID, operating software GCMS solution version 1.02 (Shimadzu, Kyoto, Japan). Ionization voltage 70 eV, electron multiplier 900 V, ion source temperature 180 °C. Mass spectra data were acquired in the scan mode in *m/z* range 40–400. The same oil solutions (1 μL) were injected with the split mode (1:96).

2.5. Identification of components of essential oils

The identity of components was based on their GC retention index (relative to C_9–C_{22} *n*-alkanes on the SPB-5 column), computer matching of spectral MS data with those from NIST MS libraries, the comparison of the fragmentation patterns with those reported in the literature (Adams, 2007) and, whenever possible, co-injections with authentic samples.

2.6. Statistical analyses

Statistical analyses were performed using STATISTICA 6.0 (StatSoft, Inc., Tulsa, Oklahoma, USA) and used to test the significance of each variable ($P \leq 0.05$). A basic descriptive statistical analysis was followed by an analysis of variance test for mean comparisons. The method used to discriminate among the means (Multiple Range Test) was Fisher's Least Significant Difference (LSD) procedure at a 95.0% confidence level. Principal component analysis (PCA) was were carried out using Statistica, version 6.0 (StatSoft, Inc., Tulsa, Oklahoma, USA). Linear Discriminate Analyses (LDA) analysis was performed using the "lda" function embedded in the 'mass' R package. The results were displayed using the "ggplot" function embedded in the 'ggrepel' R package.

3. Results and discussion

3.1. Morphological and physico-chemical characteristics

Several differences of morphological and chemical parameters of the fruits were detected between the 24 cultivars (Fig. 1) in the two years of observation. The size of citrus fruit, that is an important benchmark for citrus market, and the shape were mainly affected by the genotype. The highest fruit weight was observed in fruits of Interdonato and Lemox (hybrid of Femminello lemon x Pera del commendatore (2x) x Doppio Lentini lemon (4x)), 223 and 224 g, respectively (Table 1), while the lowest size was found in F. Carrubaro (131 g), F. Scandurra (124 g) and F. S. Teresa (122 g). These results are different from Di Vaio et al. (2010), where F. Scandurra showed the greatest weight.

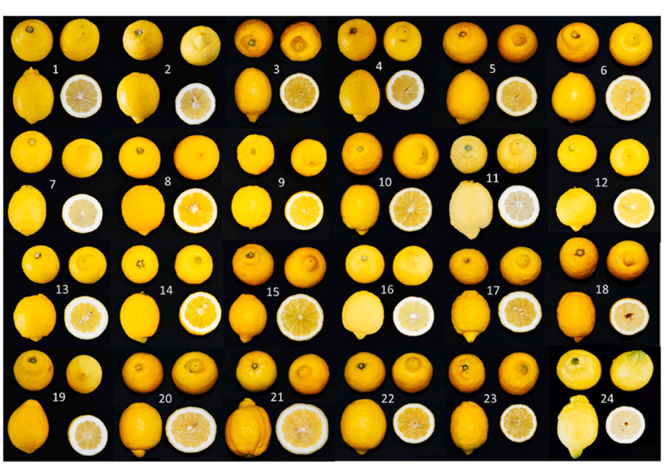

Fig. 1. Fruits of lemons genotype. From left to right: Chaparro (1), Femminello 2Kr (2), Femminello Adamo (3), Femminello Akragas (4), Femminello Carrubaro (5), Femminello Cerza (6), Femminello CNR L58 (7), Femminello Cocuzzaro (8), Femminello Continella (9), Femminello Dosaco (10), Femminello Fior d'arancio (11), Femminello Fragalà (12), Femminello Incappucciato (13), Femminello S. Teresa (14), Femminello Scandurra (15), Femminello Siracusano (16), Fino 49 (17), Interdonato (18), Lemox (19), Lisbon (20), Monachello (21), Ovale Sorrento (22), S. Amalfitano (23) and Verna (24).

Another important parameter for citrus fruits is peel thickness (Table 1) that in lemon contribute to rind hardness limiting weight loss and fruit decay (D'Aquino et al., 2020). Indeed, peel thickness is also related to the total amount of essential oils (Di Matteo et al., 2021). Fruit of Lemox is distinguished from all the other genotypes by its statistically greater peel thickness (11.0 mm), while Interdonato (5.8 mm), F. Adamo (5.7 mm) and F. S. Teresa (5.7 mm) had the lowest value. Even if Interdonato and Lisbon had significantly similar values of fruit weight, Lemox was the only with a thick peel; F. Fior d'arancio and F. Akragas were the cultivars with the second main peel thickness (8.8 and 8.7 mm, respectively).

Seedlessness of lemon fruit is one of the goal of the genetic improvement (Catalano et al., 2022). Among the cultivars examined, Lemox, F. Adamo, F. Scandurra and F. Cerza showed a low frequency of fruits with seeds (<35%), confirming previous results (Catalano et al., 2022; Di Matteo et al., 2021; Di Vaio et al., 2010). Furthermore, the frequency of seeds per fruit is an important feature for the characterization of each cultivar. In detail, we observed that about 75% of the fruits of Lemox had no seeds, while in F. 2Kr, F. Siracusano and F. Cocuzzaro more than 97% of the fruit presented more than 10 seeds per fruit (Fig. 2). Other studies reported several cultivars with a few number of seeds per fruit (Di Vaio et al., 2010), among which F. Continella, F. Scandurra and F. Cerza are described. In our results, Lemox, F. Adamo and F. Scandurra could be considered practically seedless cultivars since more than 90% of the fruits had no seeds or, in low percentages, 1–2 seeds.

Lemon juice has a high consumption per capita, even if other citrus fruits, such as orange or mandarin are mostly used for soft drinks and squeezed juices; however, it is mostly used in culinary procedures for its richness in bioactive compounds and it is added to food matrices not only for taste amending, but also to preserve shelf-life acting as a natural antioxidant (De Carvalho et al., 2021). The highest quantitative of juice yield was observed in fruits of Interdonato (629 mL) that also had the highest fruit weight together with Lemox (Table 1) that, however, had a fair juice yield due to the high peel thickness.

The flavor and the taste of citrus fruits is considerably influenced by the concentration of TSS and organic acids (TA) in the juice (Frederick & Larry, 2009). Vitamin C is a water-soluble vitamin that carries out different preventive activities in human body (Liu et al., 2022). It was reported that the concentrations of ascorbic acid in lemon juice showed significant differences among cultivars and ripening stages (Di Matteo et al., 2021). In our study, the TSS content showed statistical difference among the cultivars under observation, with values between 6.4 and 8.5 °Brix (Table 1). The organic acid of the juice changed from a minimum of 49.4 g L^{-1} in Lemox to 74.6 g L^{-1} in F. 2Kr, which also had the highest vitamin C content (815.6 mg L^{-1}) in the juice. In our results, fruits of F. Scandurra had the lowest vitamin C content, even if no statistical difference was observed with F. Carrubaro and Interdonato genotype.

3.2. Identification of components of essential oils

Citrus essential oils are characterized by a very high amount of monoterpenes, being the hydrocarbons the main components followed by the oxygenated derivatives. Until now, about 400 different compounds have been characterized in the *Citrus* essential oils (Agarwal et al., 2022).

Limonene, a monoterpene hydrocarbon ($C_{10}H_{16}$), in almost all *Citrus* essential oils is the main component ranging between 32 and 98% of the

Table 1
Physico-chemical analyses performed on the fruits of 24 lemon cultivars.

Cultivar	Weight (g)[a]	Peel thickness (mm)[a]	Juice yield (mL)[a,b]	pH[a]	TSS (°Brix)[a]	TA (g L^{-1})[a]	Vitamin C (mg L^{-1})[a]
Chaparro*	192 cde	7.5 egh	584 ab	2.0 a	7.9 def	65.2 bcde	555.0 defgh
F. 2Kr	164 gh	7.4 gh	390 hilm	2.7 a	7.8 ef	74.6 a	815.6 a
F. Adamo	134 lm	5.8 n	521 abcdfe	2.8 a	7.7 f	70.2 abc	544.9 efgh
F. Akragas	152 hi	8.7 bc	365 lm	2.7 a	8.0 cde	60.2 defg	545.7 efgh
F. Carrubaro	131 m	6.0 mn	505 bcdfegh	2.8 a	6.7 no	61.0 defg	442.4 il
F. Cerza	199 bcd	6.5 ilm	528 abcdf	2.7 a	7.0 lmn	59.8 efg	602.9 cdef
F. CNR L58	158 hi	7.1 hi	399 ghilm	2.5 a	7.3 hil	74.5 a	742.9 ab
F. Cocuzzaro	183 ef	6.5 ilm	508 bcdfeg	2.7 a	8.1 bcd	63.6 cdef	619.6 cde
F. Continella	175 fg	8.1 df	541 abcd	2.7 a	6.9 mno	66.5 bcde	477.2 hil
F. Dosaco	166 gh	6.5 lm	580 ab	2.8 a	7.3 il	64.6 bcdef	597.4 cdef
F. Fior d'arancio	187 def	8.8 b	447 cdfeghil	2.8 a	7.9 def	61.7 def	558.4 defgh
F. Fragalà	156 hi	8.3 bcd	553 abc	2.8 a	7.7 f	67.2 bcd	669.8 bc
F. Incappuciato	135 lm	6.2 lmn	302 m	3.0 a	6.9 mno	70.8 ab	648.8 bcd
F. S. Teresa	122 m	5.7 n	422 dfeghilm	2.6 a	8.5 a	71.6 ab	648.8 bcd
F. Scandurra	124 m	7.5 egh	385 ilm	2.8 a	7.6 fg	66.4 bcde	418.2 l
F. Siracusano	153 hi	6.6 il	416 feghilm	2.8 a	8.4 ab	64.3 bcdef	631.1 cde
Fino 49*	204 bc	8.2 cd	603 ab	2.0 a	7.4 ghi	64.6 bcdef	649.8 bcd
Interdonato	223 a	5.8 n	629 a	3.0 a	6.7 op	57.7 fgh	494.5 ghil
Lemox	224 a	11.0 a	407 eghilm	2.8 a	6.4 p	49.4 i	619.0 cde
Lisbon*	211 ab	7.4 gh	593 ab	2.4 a	7.6 fgh	67.1 bcd	558.4 defgh
Monachello	158 hi	8.0 dfe	382 ilm	2.8 a	6.9 mno	52.3 hi	540.6 efghi
Ovale Sorrento	146 il	7.4 gh	493 bcdfeghi	2.7 a	8.3 abc	64.3 bcdef	520.7 fghi
S. Amalfitano	161 ghi	7.2 gh	456 cdfeghil	2.8 a	8.5 a	62.4 def	582.4 cdefg
Verna*	186 def	7.6 feg	533 abcd	2.7 a	7.1 ilm	54.4 ghi	539.6 efghi

[a] Average of two years.
[b] mL of juice per 10 fruits (n = 6); * international genotypes; values followed by the same lowercase letter, within the same column, are not significant different according to Fisher's LSD procedure at 95% confidence level.

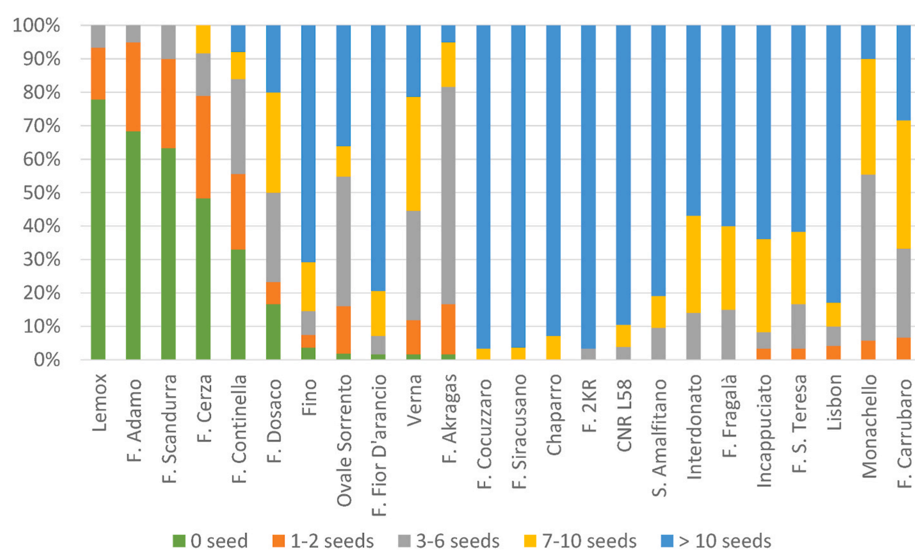

Fig. 2. Frequency (%) of seed number per fruit observed in the 24 lemon genotypes. Average of two years (n = 60).

total peel oil, depending on the species, variety, genotype of *Citrus* sample (Agarwal et al., 2022).

The two years' study here reported on 24 cultivars of lemon mostly belonging to the Femminello population has been carried out with the aim to establish a genetic variability inside the aforesaid group. In particular, 20 cultivars belong to the Italian germplasm, whereas the remaining 4 cultivars are international varieties (Table S1) of different origin cultivated in the Experimental Field of the University of Catania.

An important phytochemical aspect of lemon from a characterizing and commercial points of view is represented by the peel essential oil, which in the general panorama of the citrus essential oils shows a higher commercial value than, for example, those obtainable from orange. In Table S1 are reported the yields of essential oils in the two years of sampling: the samples collected in the first year showed a lower yield than those collected in the second. In the first year, the highest value was observed in F. Dosaco (1.44%), while the lowest was recorded in F. Cocuzzaro (0.47%); in the second the yield of the essential oil ranged from 0.32% to 1.93%, respectively, in Lemox and Monachello. The highest yields were observed for the cultivars. Monachello, Femminello Cocuzzaro and Ovale Sorrento, with values close to 2% (v/w).

Table S2 (in Supplementary) list the fully characterized components in the essential oils from both years collections, respectively. For their easier comparison, the components have been grouped into five classes: monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesuiterpenes and others (not terpenoidic compounds).

The main difference between the profiles of the two years is the higher number of components individuated in the first year samples, namely 63 against the 54 of the second. However, these differences between the two years of analysis are to be ascribed to very minor compounds present in amounts below 0.05%.

The compositional features of the 24 lemon varieties/genotypes of this study confirm that monoterpene hydrocarbons are the main components of the essential oils and that limonene is the most abundant compound with a content ranging between 58.16 and 75.99% in the two years of analysis. β-pinene and γ-terpinene are the other two more significant hydrocarbons with amounts comprised between 6.37% and 10.32% for the first compound, and 5.98% and 9.57% for the second one.

A peculiar aspect of the lemon peel essential oil is the content and the quality of the oxygenated monoterpenes, which are different by the essential oil of many other citrus peel oil. In particular, the two couple: neral/geranial and nerol/geraniol, which have been defined as the lemon odor trigger, to underline their importance in the definition and characterization of the flavor of lemon peel essential oil (Kvittingen et al., 2021).

Moreover, the analysis of the content of the aforesaid compounds highlights a consistent quantitative difference among the analyzed samples, representing a decisive element of diversification and characterization of the 24 samples subjected to this study.

Table 2 lists the content of the main classes of the essential components as average of the two years, together with the results of variance analyses. Concerning the monoterpene hydrocarbons, the main class of *Citrus* essential oil, the cultivar/genotype Monachello with 94.9% shows the high amount of these components, whereas Femminello Carrubaro, with 81.2%, shows the lowest value. The oxygenated monoterpenes are present in the highest amount in the Femminello Carrubaro, Fino and Akragas cultivars (16.7%, 14.6% and 14.1% respectively), whereas the Monachello cultivar showed the lowest value of these components. Sesquiterpenes and others with values rarely slightly over 1% play a secondary role in the phytochemical characterization of the 24 cultivars. Fig. S1 shows a selection of the lemon essential oil GC profiles, together with a comparison of the same samples collected in the two years.

Table 2
Content of the main classes of essential oil component.

Cultivar	Monoterpene hydrocarbons[b]	Oxygenated monoterpenes[b]	Sesquiterpenes[b]	Others[b]
	%[a]	%[a]	%[a]	%[a]
Chaparro*	86.7 ghilm	11.5 bcdef	1.1 ab	0.6 cde
F. 2Kr	91.9 abcd	6.8 hilmn	0.9 defg	0.3 ilmn
F. Adamo	87.7 efghil	10.0 cdefgh	0.8 ghil	0.5 cdef
F. Akragas	83.9 lmn	14.1 ab	1.1 bc	0.7 bc
F. Carrubaro	81.2 n	16.7 ab	1.1 bc	0.8 ab
F. Cerza	92.5 abcd	6.2 ilm	0.8 efghi	0.3 ilmn
F. CNR L58	86.5 hilm	12.2 bcd	0.7 hilm	0.5 defg
F. Cocuzzaro	86.8 fghilm	11.9 bcde	0.8 hil	0.4 fghil
F. Continella	94.3 ab	4.6 mn	0.7 hil	0.3 ilmn
F. Dosaco	93.4 abcd	5.6 lmn	0.8 fghil	0.2 n
F. Fior d'arancio	91.2 abcde	7.9 fghilm	0.6 mn	0.3 lmn
F. Fragalà	90.2 cdefgh	8.4 efghil	0.9 def	0.4 ghilmn
F. Incappuciato	85.7 ilm	12.4 bcd	1.0 bcd	0.5 cdefg
F. S. Teresa	86.8 fghilm	12.1 bcde	0.6 n	0.5 cdef
F. Scandurra	92.9 abcd	6.0 ilmn	0.7 ilmn	0.3 ilmn
F. Siracusano	87.8 efghil	11.0 dcbefg	0.7 lmn	0.4 ghilmn
Fino*	83.6 nm	14.6 ab	0.9 efgh	0.8 a
Interdonato	93.8 abc	4.5 mn	1.3 ab	0.3 lmn
Lemox	90.7 bcdef	7.6 ghilm	1.1 ab	0.4 efghi
Lisbon*	89.9 cdefgh	7.1 hilmn	1.0 cde	0.5 efgh
Monachello	94.9 a	3.9 n	1.0 de	0.2 mn
Ovale Sorrento	89.4 defghi	9.4 defghi	0.8 hil	0.3 hilmn
S. Amalfitano	84.7 lmn	13.5 abc	1.1 ab	0.6 bcd
Verna*	90.6 bcdefg	8.0 gfhilm	0.9 de	0.4 fghilm

[a] % average of two years.
[b] Values followed by the same lowercase letter, within the same column, are not significant different according to Fisher's LSD procedure at 95% confidence level; *international genotypes.

3.3. Principal component analysis (PCA) and Linear Discriminate Analyses (LDA)

To achieve a better understanding the different performance of cultivars in oil phenotyping, principal component analysis was applied considering only selected compounds listed in Table S3 representing more than 95% of essential oils of lemon.

According the principal component analysis (PCA) reported in Fig. 3, the two functions accounted for 76.99% of the total explained variance, with the first function (factor 1), which representing 52.17% of the total variance, being related to the variable limonene, myrcene, β-pinene, α-pinene, and γ-terpinene and sabinene (Fig. 3A). These compounds are monoterpene hydrocarbons and are the main component of oil lemon, as previously reported (Di Rauso et al., 2020; Di Vaio et al., 2010). The first function showed that cultivars F. Dosaco, F. 2Kr, F. Cerza, F. Continella, Monachello and Interdonato were the ones with a high content of limonene and myrcene in the peel (Fig. 3B). Additionally, we observed that Ovale di Sorrento differed from the other genotypes for its higher amount of β-pinene. The second function (factor 2) representing 24.82% of the total variance was highly correlated with oxygenated monoterpenes, especially geranial. The second principal function was positively correlated also with nerol and geraniol, whereas α-terpineol was negatively correlated with most of the cultivars. The genotypes F. Carrubaro and Fino 49 were highly linked to geranial and neral compounds. The cultivar Incappucciato was related to neryl acetate and geranyl acetate. On the other hand, all cultivars were positively correlated with monoterpene hydrocarbons, and especially limonene. Instead, only some were particularly influenced by the presence of oxygenated monoterpenes.

A linear discriminant analysis (LDA) was performed using as discriminant variables the selected components of lemon essential oils reported in Table S3 to discriminate the different lemon cultivars. LDA method is one of the most used in aroma classifications (Fabroni et al., 2012).

The presence and influence of six monoterpene hydrocarbons and nine oxygenated monoterpenes compounds involved in the separation of cultivars emerged in this study. The result of a linear discriminant analysis can be visualized in two dimensions by a scatter plot (Fig. 4), in which the x-axis plot showed the values of discriminant function 1 (LD1) and the y-axis plot represented the values of discriminant function 2 (LD2). LD1 explained 42.0% of the variance and LD2 17.8%; therefore, the combination of the first and the second function gave 59.8% of the variance.

The scatter plot allowed to discriminate clearly that the LDA allowed the cultivars to be grouped into a macro cluster represented by the Femminello lines while a clear separation is shown by national and international genotypes e.g. Fino 49, Lemox, Ovale di Sorrento, Verna, Monachello and Interdonato. We also observed that this discrimination was mostly influenced by LD1 and LD2 and it was mostly related by α-Pinene, Sabinene, β-Pinene, Myrcene, Limonene and γ-Terpinene compounds. The LDA results contained sufficient information to allow us to discriminate Femminello lines from other Italian and International genotypes by searching for the presence of the discriminating compounds through the use of gas-chromatography-mass spectrometry

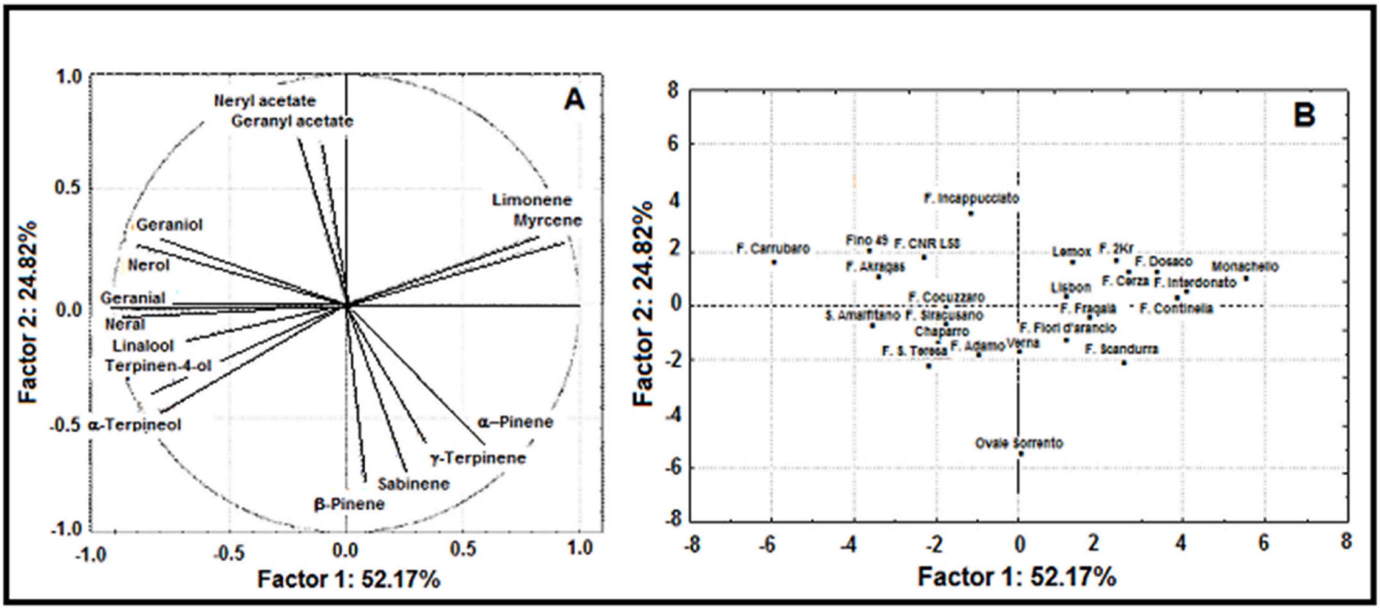

Fig. 3. Principal component analysis (PCA) of 24 lemon genotypes related with the selected compounds: Monoterpene hydrocarbons (α-Pinene, Sabinene, β-pinene, Myrcene, Limonene and γ-Terpinene) and Oxygenated monoterpenes (Linalool, Terpinen-4-ol, α-Terpineol, Nerol, Neral, Geraniol, Geranial, Neryl acetate, Geranyl acetate). Average of two years (n = 6).

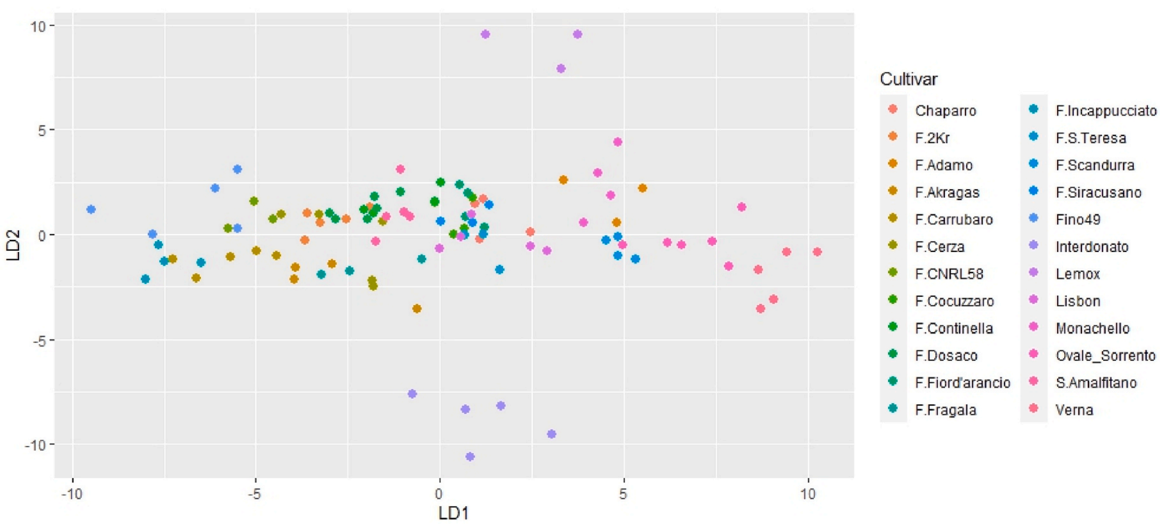

Fig. 4. Linear Discriminate Analyses (LDA) of 24 lemon genotypes related with selected compound: Monoterpene hydrocarbons (α-Pinene, Sabinene, β-pinene, Myrcene, Limonene and γ-Terpinene) and Oxygenated monoterpenes (Linalool, Terpinen-4-ol, α-Terpineol, Nerol, Neral, Geraniol, Geranial, Neryl acetate, Geranyl acetate). Cultivar under study: Chaparro, Femminello 2Kr, Femminello Adamo, Femminello Akragas, Femminello Carrubaro, Femminello Cerza, Femminello CNR L58, Femminello Cocuzzaro, Femminello Continella, Femminello Dosaco, Femminello Fior d'arancio, Femminello Fragalà, Femminello Incappucciato, Femminello Santa Teresa, Femminello Scandurra, Femminello Siracusano, Fino 49, Interdonato, Lemox, Lisbon, Monachello, Ovale Sorrento, Sfusato Amalfitano, Verna (n = 6).

analysis.

On the whole, it is known that external part of lemon fruits is mostly used to produce regional traditional sweets and that the fruit is also used for the preparation of liqueurs by hydroalcoholic maceration (Motti et al., 2022; Rosa et al., 2019). LDA results suggest that lemon peel can be used by industries due to its potential interest in functional foods and cosmetics. In this context, the GC-MS methodology could be useful to identify biochemical markers and distinguish Femminello lines from other genotypes by analyzing the profile of essential oils. After all, several studies have shown that the use of GC-MS to identify the possible presence of biochemical markers in fresh and squeezed citrus juice. Additionally, it can be used to distinguish products of a specific protected geographical indication (PGI) and geographical origin (Crupi et al., 2007; Centonze et al., 2019).

4. Conclusion

In view of broad evaluation fruit parameters (morphological and physico-chemical properties) among lemon cultivars, we observed that fruits of Lemox and Interdonato performed a good average weight, even if the first had low content of juice yield. The growing interest for seedless fruits implies that Lemox, F. Adamo and F. Scandurra may be considered as interesting cultivars for the fresh market. Nevertheless, chemical analysis showed good qualitative performance in F. 2Kr cultivar.

The main classes of compounds in all samples were monoterpenes:

hydrocarbons and oxygenated, the first ones ranged between 78 and 93% and the second ones between 5 and 18% in the first year samples; whereas in the samples collected in the second year the first ones ranged between 82% and 96%, and the second ones between 2 and 15%. As previously mentioned the quality of a lemon peel essential oil depends also on the amount and the typology of oxygenated monoterpenes, and in particular, on the presence of neral/geranial and nerol/geraniol couples, which most of all contribute to building the typical lemon aroma. Also, in this case significant differences are observed in the content of these compound in the two years of sampling, being the samples of the first year richer in these compounds than those of the second. In particular, under this aspect F. Carrubbaro, F. Cucuzzaro, Sfusato Amalfitano, and F. Siracusano appear as the best samples. This study, which as previously mentioned may considered one the few studies carried out on a very large number of cultivars of a single species, also demonstrated that the selected compounds of lemon essential oils could be considered lemon biochemical markers and in this context GC-MS methodology could be useful to distinguish Femminello lines from other lemon genotypes.

CRediT authorship contribution statement

Giulia Modica: Writing – original draft, Investigation, Formal analysis. **Tonia Strano:** Investigation. **Edoardo Napoli:** Writing – original draft. **Sebastiano Seminara:** Investigation. **Marlene Aguilar-Hernández:** Investigation. **Pilar Legua:** Writing – review & editing. **Alessandra Gentile:** Writing – review & editing, Funding acquisition. **Giuseppe Ruberto:** Writing – review & editing. **Alberto Continella:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2024.103881.

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