



Biochemical discrimination and market-oriented qualitative traits of a 24 lemon cultivar collection

Giulia Modica^a, Luana Pulvirenti^b, Alessandra Gentile^a, Giuseppe Ruberto^b,
Laura Siracusa^{b,*}, Alberto Continella^{a,**,1}

^a Department of Agriculture, Food and Environment, University of Catania, Via Santa Sofia, 100, 95123, Catania, Italy

^b Istituto di Chimica Biomolecolare del CNR, Via P. Gaifami, 18, 95126, Catania, Italy

ARTICLE INFO

Keywords:

Citrus limon
fruit quality
Morphological traits
specialized metabolites
Varietal discrimination
Market placement

ABSTRACT

Lemon [*Citrus limon* (L.) Burm. f.] is an important species cultivated in several citrus-producing countries. Lemon fruits are usually used for fresh consumption and for processed juice due to their excellent nutraceutical properties attributed to their high levels of bioactive compounds, mainly specialized (secondary) metabolites. A total of 24 cultivars were studied (20 belonging to the Italian germplasm plus 4 international varieties) for their morphological parameters, specialized metabolite content evaluated by HPLC/Uv-Vis/DAD and UPLC/ESI/Orbitrap MS analyses and antioxidant activity of juices assessed by ABTS⁺ and DPPH[•] tests. State of the art statistical tools were used to rationalize the data, specifically Principal Component Analysis (PCA) and clustering analysis, Random Forest and Heatmap were performed. The results obtained allowed us to assess biochemical variability and to identify a commercial placement (fresh market vs industrial transformation) for the analyzed genotypes. The results indicated that F. Adamo, F. Scandurra, F. Continella, F. Cerza and F. Akragas could be suitable for the fresh consumption due to their favorable morphological parameters. Conversely, F. Siracusano, F. Cocuzzaro and F. S.Teresa, F. 2Kr and F. Fragalà were distinguished by their high levels of metabolic compounds. Lemox and Fino 49 exhibited the largest fruit weight, suggesting their potential use for processed juice production.

1. Introduction

Lemon [*Citrus limon* (L.) Burm. f.], family Rutaceae, is the third most important citrus species after orange and mandarin; its production is traditionally concentrated in temperate areas such as the Mediterranean basin (Italy, Spain, Greece), and in other climatic regions such as North/South Africa, Argentina, Türkiye, USA, and Australia [1,2]. Currently, FAO statistics aggregate lemon and lime species amounting approximately to 23 M tons in the world, with Italy covering the 12th position among worldwide producers [3]. In detail, Italian production reached 476.311 t in 2022, and approximately 88 % of which are produced in Sicily [4]. The most cultivated genotypes in Sicily are represented by Femminello, Monachello and Interdonato varieties, all possessing interesting peculiarities [5]. The cultivar Femminello is extremely prone to create mutations; different Femminello lines have been selected in the last century [1] with the aim of obtaining better results in terms of

production, fruit qualitative traits and plant tolerance to “mal secco” (*Plenodomus tracheiphilus*) disease [5,6]. In this context, the selected cultivars represent the main Italian lemon germplasm, encompassing both traditional and commercially relevant varieties, and capturing the genetic and phenotyping variability present in Sicilian lemon production.

Lemons are usually consumed as fresh fruits, for producing juice and decorating dishes [7]. The juice contains many important biochemical components, including phenolic compounds, vitamins, minerals, dietary fiber, essential oils and carotenoids [8]. All these compounds have beneficial properties for health and provide various human biological functions, including antiviral, antiallergic, anti-inflammatory, anticarcinogenic and lipid-lowering properties [9]. Lemon cultivars exhibited variegated morphological and biochemical characteristics. Among these, fruit size and shape are key factors influencing consumer preferences. Considering just the Italian production, Femminello Adamo and F. Cerza

* Corresponding author.

** Corresponding author.

E-mail addresses: laura.siracusa@icb.cnr.it (L. Siracusa), alberto.continella@unict.it (A. Continella).

¹ These authors share senior authorship.

(Sicilian cultivars) showed the highest fruit weight compared to Ovale di Sorrento and Sfusato Amalfitano, two cultivars native to the Campania region. Previous researches also indicated that Monachello, that is known for its low productivity and poor-quality juice, reached commercial size for the market later than Femminello lines [1,10]. Another nowadays requested trait in the market is the absence of seeds. Both fresh fruit consumers and the processing industry prefer seedless fruits, as the seeds contain bitter compounds such as limonene and naringin. Some citrus cultivars possess desirable quality characteristics, but have not achieved commercial diffusion due to their high seed content [11]. Consequently, the selection of seedless fruits has become a key objective of citrus research worldwide. Spain is the first lemon producer in the Mediterranean area and the most widespread Spanish varieties are Fino and Verna. Among the foreign varieties Eureka and Lisbon are worth mentioning; the first originated in California (USA) and the second in Portugal [5,12]. Specialized metabolites, formerly known as secondary metabolites, are small molecules produced and accumulated in plants mainly for defense and communication purposes. These compounds have a peculiar distribution in the whole plant kingdom as they are often found only in one plant species or in a taxonomically related group of species. The species belonging to the genus *Citrus* are broadly reported to accumulate high amounts of a class of secondary metabolites, namely flavanones, which are considered as taxonomic markers for the genus itself. Additionally, each *Citrus* species has its peculiar flavanone pattern, which is used also for authentication purposes. *C. limon*, apart from hesperidin, is in fact characterized by the considerable presence of another flavanone, eriodictyol, as well as the dominance of derivatives (mainly 3'-4' methoxy) of the flavone luteolin [13,14].

The purpose of the present work is to search for peculiar similarities and differences in qualitative traits and nutritional properties within a broad collection of lemon varieties, with the aim of operating a varietal discrimination and identifying a commercial placement for the analyzed genotypes. This study therefore contributes to filling this gap by providing an updated characterization of Italian and Mediterranean lemon germplasm. To this purpose, 20 Italian varieties were studied, including a group belonging to the Femminello lines as well as Monachello, Interdonato, Lemox, Ovale di Sorrento and Sfusato Amalfitano. The Spanish varieties Verna, Fino 49 and Chaparro and the Portuguese cultivar Lisbon were also included in the collection for comparison purposes.

2. Materials and methods

2.1. Fruit sampling

Fruits of lemon cultivars were collected from the germplasm field collection of the University of Catania (37°24'33"N; 15°03'20"E) located in the plain of Catania. The study included 24 genotypes: 20 Italian germplasm mostly belonging to Femminello lines, and 4 international varieties, as elsewhere described [5].

Ten years old plants were grafted onto sour orange (*Citrus aurantium* L.). Sixty fruits of the most important blooming, known commercially as "Primofiore" [1], were collected in February from 10 trees per cultivar for two consecutive years. For each genotype, three replicates samples, each consisting of 20 fruits, were used for physical determinations (n = 30) and for liquid chromatography (n = 30).

2.2. Physico-chemical determinations

Fruits were individually weighed with digital balance (accuracy of 0.01 g) (model BL-600; Sartorius, Göttingen, Germany). Fruit height, height of nose and equatorial diameter were measured using an electronic digital slide gauge (model CD-15 DC; Mitutoyo (UK) Ltd, Telford, UK) to within 0.01 mm accuracy. The number of seeds was determined by manual counting for each genotype. Fruit juice was extracted with a commercial juice extractor (Kenwood Citrus Juicer JE290, Havant,

Hampshire, UK). Juice was weighed and expressed as a percentage of the total fruit weight. The content of organic acids (g L^{-1}) was quantified by HPLC/Uv-vis/DAD and HPLC/ESI-MS.

2.3. Determination and quantification of lemon specialized metabolites via HPLC/Uv-Vis/DAD and HPLC/ESI/Orbitrap MS analyses

Thirty fruits were collected and used for HPLC analysis. All solvents and reagents used in this study were high purity laboratory solvents from VWR (Milan, Italy); HPLC grade water and acetonitrile were also obtained from VWR. High purity standards rutin, ferulic acid, sinapic acid and p-coumaric acid were purchased from Sigma (VWR chemicals, Milan, Italy), whilst quercetin 7-O-rutinoside, narirutin, eriocitrin, diosmin and vicenin-2 were from Extrasynthese (Lyon, France). Small portions (2 mL) of the juices were put in 15 mL plastic sample tubes and 100 μL of formic acid (98 %) were added. Samples were shaken for 5 min, then centrifuged at 4000 rpm for 10 min to separate the solid portion of the juices. 1 mL of the clear supernatants were transferred into 2 mL HPLC amber vials and immediately analyzed. Chromatographic analyses were carried out on an Ultimate3000 UHPLC focussed instrument equipped with a binary high-pressure pump, a Photodiode Array detector, a Thermostatted Column Compartment and an Automated Sample Injector (Thermo Fisher Scientific, Inc., Milan, Italy). Collected data were processed through a Chromeleon Chromatography Information Management System v. 6.80. Chromatographic runs and DAD acquisitions were all performed according to Pannitteri et al. [15]. A series of HPLC/ESI/MS analyses were also performed on a selected number of representative samples to confirm spectrophotometry-based peak assignments. In this case, aliquots (5 mL) of previously centrifuged juices were freeze dried (Lyoquest-85, Telstar Italy, Legnano, Milan, Italy) then re-dissolved in 2 mL HPLC grade water and transferred into 2 mL HPLC amber vials ready to ESI/MS analyses. ESI mass spectra were acquired by a Thermo Scientific Exactive Plus Orbitrap MS (Thermo Fisher Scientific, Inc., Milan, Italy), using a heated electrospray ionization (HESI II) interface. LC/ESI/MS settings and mass spectra acquisition were performed according to our previous works ([15,16]). All analyses were carried out in triplicate; results are reported in milligram (mg) of compound per liter (L) of juice. Quantifications were carried out using the commercial high purity standards reported above to build to corresponding calibration curves as follows: eriocitrin ($R_2 = 0,9998$) was used at 280 nm to quantify itself and hesperidin; sinapic acid ($R_2 = 0,9997$), ferulic acid ($R_2 = 0,9999$) and p-coumaric acid ($R_2 = 0,9998$) were used at 330 nm to quantify the corresponding hexoses; diosmin (the rutinoside derivative of diosmetin, 4-methoxy-luteolin) was used to quantify all luteolin derivatives at 350 nm ($R_2 = 0,9998$). Finally, rutin (quercetin 3-O-rutinoside) was used to quantify quercetin and isorhamnetin (3' methoxy-quercetin) derivatives ($R_2 = 0,9997$), except quercetin 7-O-rutinoside that was quantified using the corresponding external standard ($R_2 = 0,9999$). All flavonols were quantified using 350 nm as wavelength.

2.4. Antioxidant activity

The antioxidant activity was measured by different methods for all cultivars, as described by Legua et al. [17]. In detail, ABTS⁺ and the DPPH• methods were performed according to Modica et al. [16] and Legua et al. [17].

2.5. Statistical analysis

Statistical analyses were performed using STATISTICA 6.0 (Statsoft Inc., Tulsa, OK, USA). 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. All data were standardized for statistical analysis. Principal component analysis (PCA) and clustering analysis, Random Forest and Heatmap were performed using

R software (v. 4.3.1) ([18–20]).

3. Results

3.1. Profile and content of individual and total specialized metabolites in *C. limon* juices

A representative chromatogram visualized at diagnostic wavelengths (280, 330 and 350 nm) of lemon juice from Femminello Carrubbaro (see paragraph 2.1 and Table S1) is reported in Fig. S1. Overall, nineteen specialized metabolites were detected and tentatively identified on the basis of their Uv–vis and mass spectra (pseudomolecular ions and diagnostic fragments) and retention times (see Table S1). An exhaustive comparison with data already presents in literature corroborated the assignments. According to our results, *C. limon* secondary metabolic pool comprised three hydroxycinnamic acids (compounds C1-C3, see Fig. S1 and Table S1) and sixteen flavonoids (F1-F16), of which three flavanones (F1-F3), eight flavones (compounds F4-F7, F11, F12, F13 and F15) and finally five flavonols (compounds F8-F10, F14 and F16 in Table S1). Table S2 reports the individual contents for all the metabolites identified; when considering the whole genotype collection, the flavanone hesperidin (F3) is by far the most abundant metabolite

present in lemon juices (104,4 mg L⁻¹ juice as mean value over 24 cultivars), followed by another flavanone, eriocitrin (F1) with 33,6 mg L⁻¹ juice). Compound F7 (diosmetin 6,8 di-C-hexoside, see Table S1) resulted as the third most abundant with ca. 22,8 mg L⁻¹ juice, followed by a hydroxycinnamic acid derivative, feruloyl-hexose (compound C2, see Table S1) with a mean value of 11,6 mg L⁻¹ juice.

Considering instead the total content of specialized metabolites for each single genotype studied (Fig. 1), cultivar F. Siracusano showed the highest amount in flavonoids and hydroxycinnamic acids (263.77 mg L⁻¹ and 37.33 mg L⁻¹, respectively). Genotype Interdonato exhibited the lowest value of total flavonoids (89.29 mg L⁻¹), while in F. Continella the lowest amount of hydroxycinnamic acids (16.32 mg L⁻¹) was registered. As mentioned, hesperidin was the most abundant compound in lemon juice, followed by eriocitrin and diosmetin 6,8 di-C-glucoside (Table S2). The highest amount of hesperidin was recorded again in F. Siracusano (154.14 mg L⁻¹), while the flavanone eriocitrin peaked in Lemox (53.04 mg L⁻¹) and F. S. Teresa exhibited the highest content of diosmetin 6,8 di-C-glucoside (39.78 mg L⁻¹). Within flavonoids, the less abundant compounds were luteolin 7-O-rutinoside and lucenin-2. Feruloyl hexose, the principal compound recorded among hydroxycinnamic acids, ranged from 9.01 mg L⁻¹ (genotype F. CNRL58) to 15.90 mg L⁻¹ of the cultivar Verna. These data were processed into a heatmap

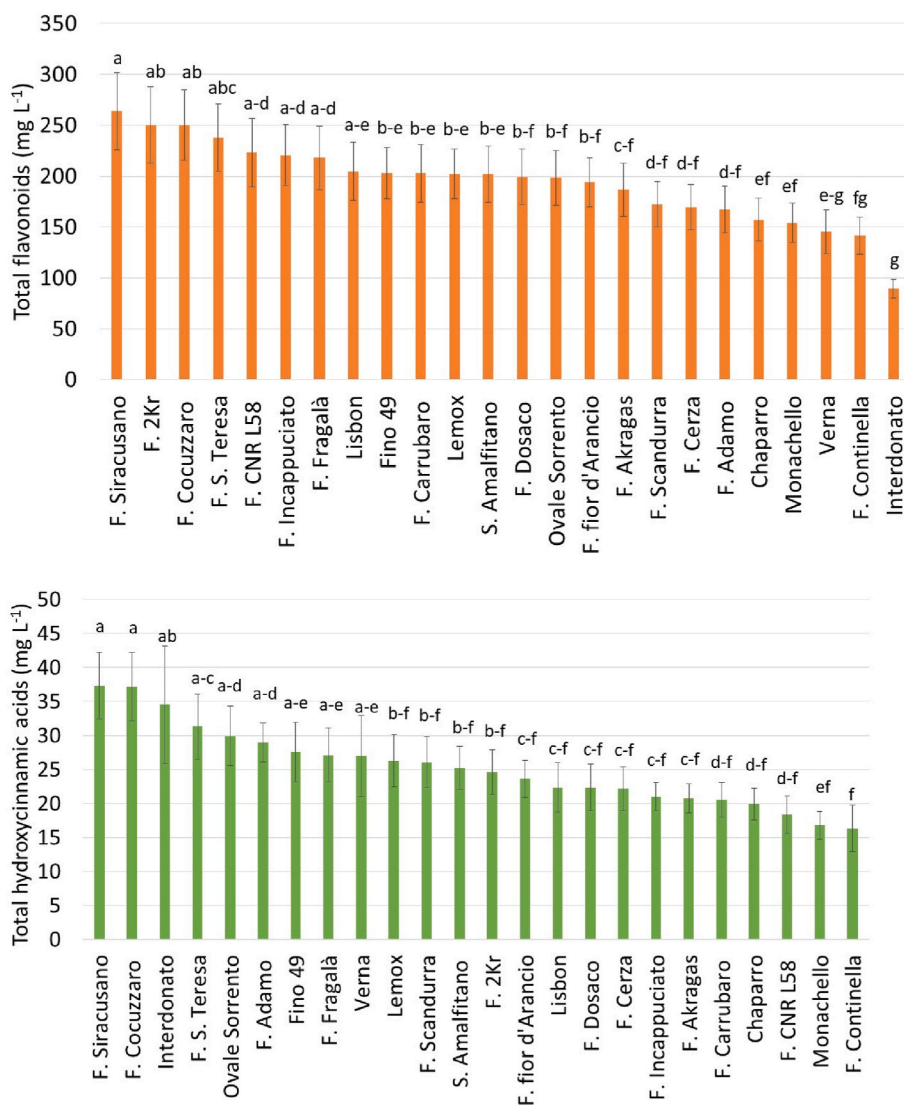


Fig. 1. Flavonoids and hydroxycinnamic acids content in the juice of 24 lemon cultivars. Means \pm standard deviation of the treatments analyzed in two years are reported. Values without letters have no significant differences according to Fisher's LSD procedure at a 95 % confidence level.

of the Random Forest analysis depicted in Fig. 2 (Table S3), where each column represents a different lemon cultivar and each row a different compound belonging to the metabolic pool. Overall, hesperidin (F3) and lucenin-2 (F5) were the most significant metabolites in all cultivars analyzed. On the other side, metabolites F8 (quercetin 7-O-rutinoside), F10 (rutin), F11 (diosmetin 6-C-glucoside), F12 (diosmetin 8-C-glucoside) and F9 (quercetin hexoside-deoxyhexoside) are the less significant compounds in terms of cultivar differentiation. In detail, F. Akragas and Ovale di Sorrento were affected by compound F16 (isorhamnetin 3-O-rutinoside), the first cultivar was also related with metabolite F15 (diosmin). Monachello and Interdonato were related with hydroxycinnamic acids, more specifically with C2 and C3 (feruloyl and sinapoyl hexose, respectively). Genotype Femminello S. Teresa was affected by compound F15 (diosmin), while Ovale di Sorrento was associated with luteolin 7-O-rutinoside (F13). Interestingly, some flavonoids such as quercetin 7-O-rutinoside, rutin, quercetin hexoside-deoxyhexoside, diosmetin 8-C-glucoside and diosmetin 6-C-glucoside were clustered close together.

3.2. Physico-chemical determinations

Morphological and physical traits were assessed on 30 fruits per cultivar for two years (Fig. 3 and Table 1). The highest number of seeds were recorded in Femminello Cocuzzaro, F. Siracusano, F. CNR L58 and F. 2Kr which showed an average of more than 18 seeds per fruit. It was observed that the fruits of F. Dosaco, Monachello, Verna, F. Carrubaro, Ovale di Sorrento, F. Incappucciato, F. Fragalà, Interdonato, F. Santa Teresa, Fino 49, Sfusato Amalfitano, F. Fior d'arancio and Lisbon showed fruits with an average number of seeds ranging from 6 to 16 respectively (Fig. 3). It was noted that F. Akragas and F. Continella had 5 and 3 seeds per fruit, while F. Cerza, F. Scandurra, Lemox and F. Adamo had less than 2 seeds per fruit (Fig. 3). Lemox (224 g), Interdonato (223 g), Lisbon (211 g) and Fino 49 (204 g) showed the highest fruit weight, while F. S. Teresa (122 g) had the lowest value (Table 1). The diameter ranged from 61.0 mm (F. Incappucciato) to 75.0 mm in fruits of Lemox that also showed the highest height of the fruits. Verna had the highest length of nose (15 mm), while Lemox showed the lowest value (5 mm). The percentage of juice ranged from 24.3 % in Lemox to 39.0 % in F. Adamo.

The content of organic acids was described in Table 2. The malic acid measured in the juice ranged from 0.14 (Lemox) to 1.16 g L⁻¹ (F. 2Kr),

while the content of ascorbic acid varied from 0.13 g L⁻¹ in juice of Ovale di Sorrento to 0.33 g L⁻¹ in F. Cocuzzaro. No significant differences were found for ascorbic acid between all the varieties studied, with the exception of Ovale di Sorrento. Citric acid was the most abundant organic acid in the juice. It ranged from 27.59 g L⁻¹ in Ovale di Sorrento to 63.61 g L⁻¹ in the juice of F. fior d'arancio, although no statistical difference was observed again except for Ovale di Sorrento. The highest value of total organic acids was noted in F. fior d'arancio (64.95 g L⁻¹).

The results of the Principal Component Analysis (PCA) explained 54.48 % of the total variation (Fig. S2). PC1 (29.41 %) was negatively correlated with height (-0.481), diameter (-0.461), weight (-0.475) and total organic acids (-0.381). PC2 (25.07 %) was mostly related with the number of seeds (0.834). It was noted that Fino 49 was negatively related with PC1, while F. Incappucciato and Ovale di Sorrento were largely correlated with PC1 as they presented low value of weight and diameter of fruit (Table 1) and the lowest content of organic acids (Table 2). Indeed, fruit weight (0.233), diameter (0.288) and height (0.289) were correlated with PC2, while malic acids (-0.532) and citric acids (-0.421) were negatively related with PC2. It was noticed that Interdonato and Monachello showed the correlation with PC2, while F. 2Kr was negatively correlated with PC2 because it had the highest content of malic acids (Table 2).

3.3. Antioxidant activity

The antioxidant activity was performed using ABTS + [2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] method and DPPH• radical method (2,2-diphenyl-1-picrylhydrazyl). DPPH• ranged from 10.51 mM Trolox to 17.41 mM Trolox in F. Fior d'arancio and Lemox, respectively (Table 3). It was also observed that Lisbon and Chaparro did not show statistical differences with Lemox fruits. Femminello 2Kr reported the highest value of ABTS+ (7.64 mM Trolox), while F. Scandurra (3.51 mM Trolox) had the lowest antioxidant potential (Table 3).

3.4. Analysis of the morphological and biochemical parameters of lemon juices via principal component analysis (PCA)

Principal component analysis (PCA) was performed with the aim of studying the effect of secondary metabolites and physico-chemical parameters on 24 lemon cultivars. PCA showed that the first two main

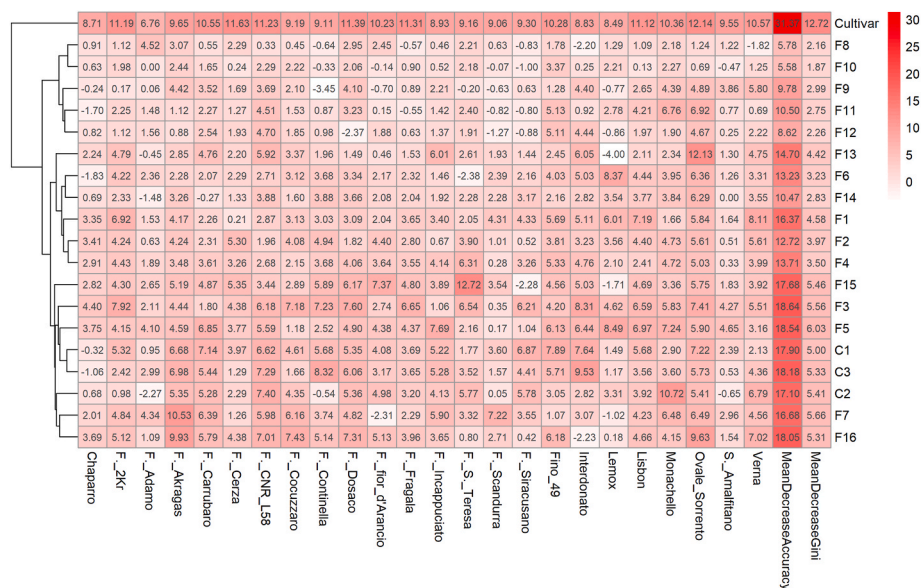


Fig. 2. Heatmap showing the statistical clustering (by Random Forest) of the secondary metabolites identified in the 24 lemon cultivars object of this study. A higher value indicates the importance of that metabolite in predicting group. See also Table S1 for metabolite coding and text for details.

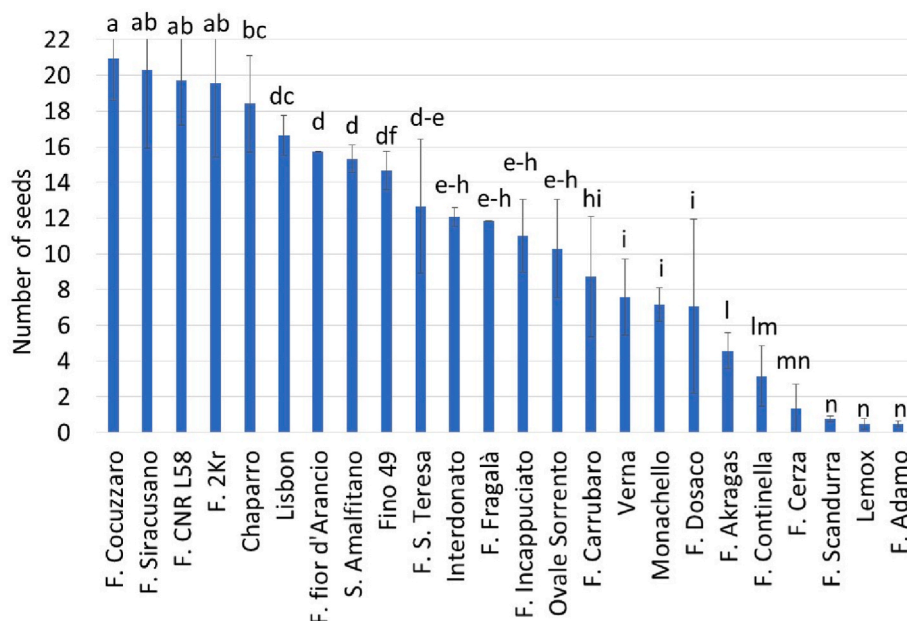


Fig. 3. Number of seeds per fruit in 24 lemon cultivars. Means \pm standard deviation of the treatments analyzed in two years are reported. Values without letters have no significant differences according to Fisher's LSD procedure at a 95 % confidence level.

Table 1

Morphological and physical parameters measured in 24 lemon cultivars; data are reported as mean of 2 years cultivation. Values without letters have no significant differences according to Fisher's LSD procedure at a 95 % confidence level.

Genotype	Weight (g)		Diameter (mm)		Height (mm)		Nose (mm)		Percentage of juice (%)	
Lemox	224	a	75.0	a	84.0	a	5	n	24.3	m
Interdonato	223	a	71.5	b	82.7	ab	11	bcdf	37.9	abc
Lisbon	211	ab	71.5	b	79.0	cdf	11	bcdf	31.6	dfeghi
Fino 49	204	bc	69.6	bc	80.3	abcd	11	cdf	33.0	dfeg
F. Cerza	199	bcd	69.5	bcd	79.8	bcdf	11	cdf	32.9	dfeg
Chaparro	192	cde	69.8	b	77.6	cdfe	9	hil	32.6	dfegh
F. fior d'Arancio	187	def	69.4	bcd	76.6	feg	11	cdfe	30.4	feghil
Verna	186	def	66.9	defg	80.8	abc	15	a	30.1	eghil
F. Cocuzzaro	183	ef	74.3	a	77.0	dfeg	7	m	32.7	dfegh
F. Continella	175	fg	70.7	b	74.2	eghi	10	dfegh	33.8	dfe
F. Dosaco	166	gh	65.4	efg	73.7	ghi	11	bcdf	35.1	bcd
F. 2Kr	164	gh	54.7	l	59.0	p	13	b	28.8	hil
S. Amalfitano	161	ghi	64.8	fg	79.4	bcdf	11	cdfe	31.6	dfeghi
Monachello	158	hi	66.4	efg	69.6	lmn	12	bc	26.8	lm
F. CNR L58	158	hi	64.9	efg	71.9	ilm	10	feghi	28.3	il
F. Fragalà	156	hi	67.3	cde	72.7	hil	9	hil	35.5	abcd
F. Siracusano	153	hi	67.2	cdef	69.5	lmn	10	eghi	29.4	ghil
F. Akragas	152	hi	64.6	gh	75.4	egh	11	cdfe	24.0	m
Ovale Sorrento	146	il	65.3	efg	68.6	mn	10	ghi	35.4	abcd
F. Incappuciato	135	lm	61.0	i	63.8	o	10	dfeghi	30.7	feghil
F. Adamo	134	lm	61.5	i	68.5	mn	11	bcd	39.0	a
F. Carrubaro	131	m	61.2	i	69.9	lmn	8	lm	38.8	ab
F. Scandurra	124	m	61.6	i	66.7	no	9	il	31.0	feghi
F. S. Teresa	122	m	62.3	hi	67.2	no	7	m	34.4	cdf

components explained 57.8 % of the total variation (Fig. 4). PC1 (45.1 % of variance explained) was positively correlated mainly with F3 and F4. PC1 was negatively associated with physical parameters such as weight and number of seeds. PC2 (12.7 % of variance explained) was affected by F15 and A.A., while it was negatively correlated with F10 and F12 (Fig. 4A). Fig. 4B showed four clusters. An analysis of variance (ANOVA) was conducted on the several variables, which showed that the differences between the four clusters are mainly explained by F8, F10, F5, F12, F1, and F4 ($p < 0.001$), which are therefore the main discriminating factors between the groups. Furthermore, it was observed that F3, C3, N.S., and F14 showed significant but less marked differences ($p < 0.01$), while M.A., N., F2, and F11 contributed more marginally ($p < 0.05$).

Overall, the variable F8 was the main discriminating factor, followed by F10, F5, and F12, which together explained most of the differences between the identified clusters.

4. Discussion

4.1. Citrus limon specialized metabolites: tool for biochemistry-based varietal discrimination

As mentioned, flavanones represent the dominant class of phenolic compounds in Citrus fruits and are increasingly studied for their chemotaxonomic significance including their potential in varietal discrimination due to their stability, structural diversity, and tissue-

Table 2

Organic acids (g L^{-1}) measured in 24 lemon cultivars; data are reported as mean of 2 years cultivation. Values without letters have no significant differences according to Fisher's LSD procedure at a 95 % confidence level.

Genotype	Malic acid	Ascorbic acid	Citric acid	Total organic acids
F. fior d'Arancio	1.02 abc	0.32 a	63.61 a	64.95 a
F. Cocuzzaro	1.10 ab	0.33 a	59.30 ab	60.73 ab
F. Dosaco	0.94 abc	0.31 a	57.07 ab	58.32 ab
F. Fragalà	0.76 abcd	0.30 a	56.44 ab	57.50 ab
Chaparro	0.48 abcd	0.29 a	55.20 abc	55.98 abc
F. Akragas	0.44 bcd	0.30 a	55.17 abc	55.91 abc
Lisbon	0.73 abcd	0.28 a	54.68 abc	55.70 abc
F. Cerza	0.55 abcd	0.31 a	54.51 abc	55.37 abc
S. Amalfitano	0.77 abcd	0.30 a	54.28 abc	55.35 abc
F. Carrubaro	0.45 bcd	0.29 a	54.55 abc	55.30 abc
F. S.Teresa	0.77 abcd	0.30 a	54.20 abc	55.27 abc
F. Siracusano	1.13 ab	0.30 a	53.75 abc	55.18 abc
Fino 49	0.78 abcd	0.29 a	53.95 abc	55.03 abc
F. 2Kr	1.16 a	0.29 a	53.54 abc	55.00 abc
F. Scandurra	0.79 abcd	0.25 ab	53.53 abc	54.57 abc
F. Continella	0.90 abc	0.25 ab	50.27 abc	51.42 abc
F. Adamo	0.67 abcd	0.28 a	50.42 abc	51.37 abc
Monachello	0.73 abcd	0.27 a	48.55 abc	49.55 abc
Verna	0.73 abcd	0.25 ab	45.58 abc	46.55 abc
Interdonato	0.34 cd	0.22 abc	44.56 abc	45.12 abc
F. CNRL58	0.67 abcd	0.26 a	44.09 abc	45.02 abc
Lemox	0.14 d	0.27 a	43.04 abc	43.46 abc
F. Incappuciato	0.36 cd	0.23 abc	38.35 abc	38.94 abc
Ovale Sorrento	0.42 bcd	0.13 c	27.59 c	28.14 c

Table 3

Antioxidant activity (DPPH and ABTS, mM Trolox) measured in 24 lemon cultivars in two years. Values without letters have no significant differences according to Fisher's LSD procedure at a 95 % confidence level.

Genotype	DPPH	ABTS		
Chaparro	16.39	abc	5.44	bcd
CNR L58	16.03	abcd	5.89	ab
F. 2Kr	11.03	hi	7.64	a
F. Adamo	12.78	fghi	4.06	Bcd
F. Akragas	13.03	fghi	5.05	Bcd
F. Carrubaro	12.92	fghi	3.93	cd
F. Cerza	13.15	defghi	5.03	bcd
F. Cocuzzaro	14.00	bcdefgh	5.84	ab
F. Continella	14.12	bcdefg	4.61	bcd
F. Dosaco	13.58	cdefgh	4.58	bcd
F. Fior D'arancio	10.51	i	4.87	bcd
F. Fragalà	16.17	abc	5.69	abc
F. S. Teresa	12.30	ghi	4.79	bcd
F. Scandurra	12.38	ghi	3.51	d
F. Siracusano	13.05	efghi	5.81	abc
Fino	15.59	abcdef	4.76	bcd
F. Incappuciato	15.23	abcdefg	4.98	bcd
Interdonato	15.18	abcdefg	4.36	bcd
Lemox	17.41	a	4.55	bcd
Lisbon	16.67	ab	4.74	bcd
Monachello	13.77	bcdefgh	4.60	bcd
Ovale Sorrento	14.25	bcdefg	4.54	bcd
S. Amalfitano	14.96	abcdefg	5.34	bcd
Verna	15.98	abcde	4.17	bcd

specific distribution. In *Citrus*, they are almost exclusively present as glycosides, where the sugar moiety is typically linked at position C7 of the aglycone [21]. Two major disaccharide conjugation patterns are found: rutinosides (e.g., hesperidin and eriocitrin), where the sugar unit is α -L-rhamnopyranosyl-(1 \rightarrow 6)- β -D-glucopyranose, and neohesperidosides (e.g., naringin and neohesperidin), where the linkage is α -L-rhamnopyranosyl-(1 \rightarrow 2)- β -D-glucopyranose. Although these compounds may share the same flavanone backbone (e.g., hesperetin, naringenin, eriodyctiol), the different glycosylation patterns profoundly influence solubility, bitterness, and metabolic behavior, making them powerful chemotaxonomic markers [22]. Notably, lemon tissues, especially peels, are also enriched in C-glycosyl flavones such as lucenin-2 (a

luteolin derivative) and stellarin-2 (a chrysoeriol, that is, a 3'-methoxy-luteolin derivative), which are relatively rare in other citrus species and exhibit enhanced stability due to the carbon-carbon bond between sugar and aglycone [23]. These flavonoids are often involved in photoprotection and defense, while water-soluble rutinosides and neohesperidosides are more concentrated in the pulp [24]. In this study, we analyzed the juice of 24 *C. limon* genotypes cultivated over two consecutive harvest seasons with the aim of using its peculiar metabolic pool to discriminate among the various cultivars and to identify, if present, the most discriminant metabolites. Fig. 1 presents the average content of hydroxycinnamic acids (HCAs) and flavonoids, two major classes of specialized metabolites known to contribute to the nutritional value of citrus juices [22,25]. The graphs highlight substantial inter-genotypic variability in the accumulation of both flavonoids and HCAs, with some genotypes exhibiting notably high average levels of one or both classes. Fig. 2 examines the same dataset at individual compound level (see Table S.

2 for details) using a Random Forest classification model. The resulting variable importance plot and heatmap identify a subset of discriminant metabolites, mainly flavanone rutinosides and neohesperidosides, as key varietal markers, in line with previous reports [26]. Hierarchical clustering reveals consistent groupings of genotypes based on similar metabolite fingerprints, providing a data-driven framework for chemotaxonomic classification [27,28]. These data highlight the utility of phytochemical profiling as a discriminating strategy in *C. limon* cultivar selection, at the same time promoting for the first time the valorization of these particular genetic resources [29,30].

4.2. Physico-chemical determinations

Citrus fruits such as lemon are recognized as products whose demand in the international market is growing because they positively influence human health due to their quality and nutraceutical characteristics [31]. Fruit qualitative parameters were mostly affected by different agronomical practices [32,33]. The most relevant aspects that are considered by the consumer are the physical attributes, including seedlessness, fruit size and weight, and juice percentage [31]. The production of seedless citrus fruits is considered a major enhancement in fruit quality, particularly for fresh market consumption [34]. Previous studies reported as seedless cultivars F. Continella, F. Scandurra and F. Cerza [10,35]. Actually, the results showed that F. Cocuzzaro, F. Siracusano, F. CNR L58 and F. 2Kr had the highest content of seed per fruit, while F. Akragas and F. Continella, F. Cerza, F. Scandurra, Lemox and F. Adamo could be considered as seedless fruit, according with previous results [36]. Fruits size and shape, along with juice percentage, are important traits for fresh consumption and industrial processing. Small sized fruits are primarily processed for juice production. In contrast, medium to large-sized fruits typically offer the highest profitability in the fresh consumption market [32,37].

Fruit weight ranged from 122 (F. S. Teresa) to 224 g (Lemox). It was observed that Fino 49 presented a higher weight than reported in other previous studies [32]. Fruit diameter and height confirmed previous results [10,35]. Furthermore, it was observed that Interdonato presented a medium/large size with an oval shape and pronounced nose, as previously described [38]. Overall, it was observed that the juice percentage was higher in all varieties compared to Di Vaio et al. [35] and similar to Di Matteo et al. [10]. However, our results indicated that F. Adamo had the highest percentage of juice, contrarily to what reported by Di Matteo et al. [10]. Citric acid is an essential component of lemons, and it is the most abundant organic acid in the juice [32,39,40]; our results confirmed what is reported in the literature [32]. The concentration of ascorbic acid (or vitamin C) was similar to what was found in a previous study [10]. However, previous researches reported that the vitamin C content in fruits of the Fino and Verna varieties was significantly influenced by weather conditions [41,42]. Regarding the antioxidant activity of the juice, it was found that vitamin C contributes only

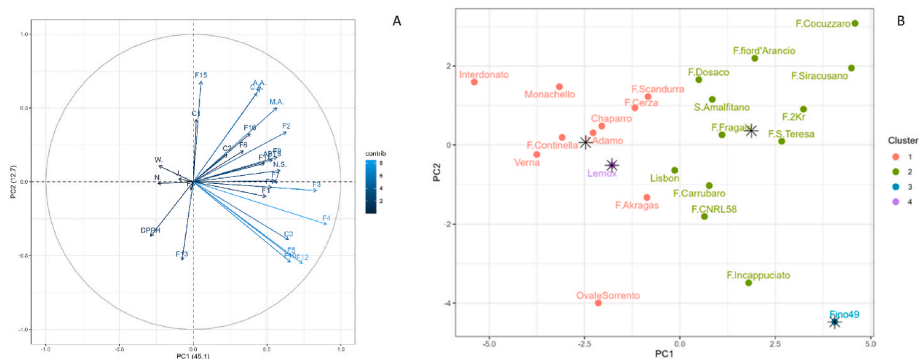


Fig. 4. Principal component analysis (PCA) and clustering showing the distribution of 24 lemon cultivars based on biochemical and pomological parameters: for metabolite coding see [Table S1](#); DPPH, ABTS, weight (W.), nose (N.), percentage of juice (J.), number of seeds (N.S.), citric acid (C.A.), ascorbic acid (A.A.) and malic acid (M.A.). Data are presented as the mean of two years of cultivation. A: PCA loading plot of biochemical and pomological parameters. B: PCA score plot of biochemical and pomological parameters.

8.60 % of the total antioxidant activity in grapefruit, followed by orange (8.16 %) and lemon (6.15 %). Therefore, the main contribution to the total antioxidant activity is due to the presence of various bioactive compounds, and vitamin C plays a partial role [17,43].

The PCA results discriminated the different cultivars: it was found that F. Incappucciato and Ovale di Sorrento were largely affected by weight and diameter of fruit and exhibited the lowest quantitative of total organic acids.

4.3. Antioxidant activity

Lemon juice is known for its health benefits due to its antioxidant potential and anti-inflammatory activity [33]. To evaluate the antioxidant potential of its different components, it is necessary to consider that the antioxidant capacity depends on plant extracts and their chemical composition. The various methods used to determine the antioxidant properties of the juice were not directly comparable, as highlighted in previous studies [44]. However, both methodologies are considered reliable for assessing antioxidant potential [16]. The results showed that F. fior d'arancio had the highest DPPH[•] value, while Lemox exhibited the lowest. The antioxidant potential measured by ABTS⁺ ranged from 3.51 mM Trolox (F. Scandurra) to 7.64 mM Trolox (F. 2Kr). Overall, the results were incompatible with previous observations reported by Di Vaio et al. (2010). In particular, the antioxidant potential measured in Femminello lines, Interdonato, Monachello, Ovale di Sorrento e S. Amalfitano differed from values obtained using ABTS + assay in other studies. This discrepancy suggests that antioxidant activity may vary significantly depending on harvesting periods, environmental conditions, and agronomic techniques [10,32]. Furthermore, it was observed that the antioxidant capacity of Fino 49 and Verna significantly exceeded the values reported by other authors [32,42]. It is important to consider that the effectiveness of antioxidant compounds mainly depends on their chemical structure, as this determines their reactivity towards free radicals and another reactive oxygen species (ROS), thereby influencing antioxidant activity [45]. Additionally, the effectiveness of antioxidant molecules is also influenced by their concentration [46]. The ABTS⁺ radical scavenging method has been reported to be useful for studying the antioxidant mechanisms of several lipophilic and hydrophilic compounds [17]. However, DPPH[•] assay has been widely used to measure the antioxidant activity of several compounds belonging to the hydroxycinnamic acid category [46].

4.4. Marker placement: morphological traits and biochemical parameters of lemon juices performed by principal component analysis (PCA)

All the physico-chemical characteristics were involved in distinguishing the unique characteristics of the studied genotype. Principal

component analysis (PCA) allowed the identification of the most significant traits for each cultivar (Fig. 4A and B). Specifically, four clusters were identified, and the two principal components explained 57.8 % of the total variability. PC1 was strongly and positively associated with compounds F3 and F4, while it showed a negative correlation with fruit weight and the number of seeds. These are relevant aspects for the consumers [31]. PC2 was positively influenced by F15 and ascorbic acid, while it was negatively correlated with F10 and F12 (Fig. 4A). In detail, the distribution of genotypes in the PCA biplot (Fig. 4B) distinguished four main groups. Analysis of variance (ANOVA) confirmed that the differences between these clusters were significantly explained mainly by the variables F8, F10, F5, F12, F1, and F4 ($p < 0.001$), which therefore represent the main discriminating factors. Variables such as F3, C3, N.S., and F14 showed significant but less marked differences ($p < 0.01$), while M.A., N., F2, and F11 contributed only marginally to the separation of the groups ($p < 0.05$). Overall, F8 emerged as the variable with the greatest discriminating power, followed by F10, F5, and F12, which together explained most of the differences observed between the clusters. These results indicate that the variability among lemon cultivars is strongly associated with specific secondary metabolites and morphological and physical characteristics. This analysis allowed us to highlight the differences between the different genotypes and the aptitudes of each cultivar; furthermore, metabolic differences can be used as a potential biochemical marker for varietal distinction and the valorization of local genotypes.

5. Conclusions

The results showed that each variety had different peculiarities and metabolites highly characterized some genotypes; the phytochemical profiling could therefore be a strategy for *C. limon* discrimination and valorization.

Overall, F. Adamo, F. Scandurra, F. Cerza and F. Continella and could be considered as seedless fruit and therefore may be appreciated as fresh fruit; Lemox and Fino 49 showed the highest fruit weight, while the highest juice percentage was recorded in F. Adamo, outlining its major aptitude for processed juice.

As a whole, the results obtained lay the foundation for the selection of the most commercially valuable lemon cultivars in terms of market preferences and nutritional value.

CRedit authorship contribution statement

Giulia Modica: Writing – original draft, Investigation, Formal analysis. **Luana Pulvirenti:** Writing – original draft, Investigation, Formal analysis. **Alessandra Gentile:** Writing – review & editing, Supervision, Funding acquisition. **Giuseppe Ruberto:** Writing – review &

editing, Supervision. **Laura Siracusa:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Data curation. **Alberto Continella:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Conceptualization.

Funding

This research was funded by the Ministero dell'agricoltura, della sovranità alimentare e delle foreste (MASAF), project title: Difesa degli Agrumeti Italiani dal Malsecco – AGRIVITA (CUP: C83C23000650006), and by the Project “Innovazione per la valorizzazione della limonicoltura siciliana - SICILEMON” – cod. CUP: G89H20000460009, Sottomisura 16.1 del PSR Sicilia 2014–2022.

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.

Acknowledgment

This paper is dedicated to the memory of Professor Giovanni Continella, specialist in lemon crop management, enthusiastic agricultural researcher and a guide for generations of students and young researchers.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jafr.2026.102658>.

Data availability

Data will be made available on request.

References

- M. Amenta, G. Ballistreri, S. Fabroni, F.V. Romeo, A. Spina, P. Rapisarda, Qualitative and nutraceutical aspects of lemon fruits grown on the mountainsides of the Mount Etna: a first step for a protected designation of origin or protected geographical indication application of the brand name ‘Limone dell’Etna’, *Food Res. Int.* 74 (2015) 250–259.
- G.H. Barry, M. Caruso, J.F.G. Gmitter, in: M. Talon, M. Caruso, F.G. Gmitter Jr (Eds.), *Commercial Scion Varieties in the Genus Citrus*, Elsevier Inc, Duxford, United Kingdom, 2020, pp. 83–104. Available at: <https://www.sciencedirect.com/book/9780128121634/the-genus-citrus>.
- <https://www.fao.org/faostat/en/>.
- <https://www.istat.it/>.
- G. Modica, T. Strano, E. Napoli, S. Seminara, M. Aguilar-Hernández, P. Legua, A. Gentile, G. Ruberto, A. Continella, Qualitative traits and peel essential oil profiles of 24 Italian and international lemon varieties, *Food Biosci.* 59 (2024) 103881.
- C. Oliveri, G. Modica, P. Bella, G. Dimaria, G. Cirvilleri, A. Continella, V. Catara, Preliminary evaluation of a zinc-copper-citric acid biocomplex for the control of *Plenodomus tracheiphilus* causal agent of citrus mal secco disease, *Acta Hort.* 1354 (2022) 231–236.
- C.C. Ngugi, E. Oyoo-Okoth, M. Muchiri, Effects of dietary levels of essential oil (EO) extract from bitter lemon (*Citrus limon*) fruit peels on growth, biochemical, haemato-immunological parameters and disease resistance in Juvenile *Laboe victorinus* fingerlings challenged with *Aeromonas hydrophila*, *Aquac. Res.* 48 (2017) 2253–2265, <https://doi.org/10.1111/are.13062>.
- E. González-Molina, R. Domínguez-Perles, D.A. Moreno, C. García-Viguera, Natural bioactive compounds of *Citrus limon* for food and health, *J. Pharm. Biomed. Anal.* 51 (2010) 327–345.
- O. Benavente-García, J. Castillo, Update on uses and properties of Citrus flavonoids: new findings in anticancer, cardiovascular, and anti-inflammatory activity, *J. Agric. Food Chem.* 56 (2008) 6185–6205, <https://doi.org/10.1021/jf8006568>.
- A. Di Matteo, G. Di Rauso, A.C. Simeone, M.A. Rao, C. Di Vaio, Morphological characteristics, ascorbic acid and antioxidant activity during fruit ripening of four lemon (*Citrus limon* L. Burm. F.) cultivars, *Sci. Hort.* 276 (2021) 109741, <https://doi.org/10.1016/j.scienta.2020.109741>.
- V. Narukulla, Y. Lahane, P. Uge, S. Pandey, K. Fiske, K. Kawale, P.T. K. Jagannadham, V. Ziogas, Production of Triploid Seedless Sweet Orange [*Citrus sinensis* (L.) Osbeck] cv. Mosambi: A Success Story, *Agronomy* 14 (2024) 829.
- M.G. Aguilar-Hernández, D. Núñez-Gómez, M.Á. Forner-Giner, F. Hernández, J. J. Pastor-Pérez, P. Legua, Quality parameters of Spanish lemons with commercial interest, *Foods* 10 (2021) 62.
- L. Siracusa, G. Ruberto, Plant polyphenol profiles as a tool for traceability and valuable support to biodiversity, in: *Polyphenols in Plants*, Academic Press, 2014, pp. 15–33.
- K. Robards, M. Antolovich, Analytical chemistry of fruit bioflavonoids. A review, *Analyst* 122 (2) (1997) 11–34.
- C. Pannitteri, A. Continella, L.L. Cicero, A. Gentile, S. La Malfa, E. Sperlinga, E. M. Napoli, T. Strano, G. Ruberto, L. Siracusa, Influence of postharvest treatments on qualitative and chemical parameters of Tarocco blood orange fruits to be used for fresh chilled juice, *Food Chem.* 230 (2017) 441–447.
- G. Modica, C. Pannitteri, M. Di Guardo, S. La Malfa, A. Gentile, G. Ruberto, L. Pulvirenti, L. Parafati, A. Continella, L. Siracusa, Influence of rootstock genotype on individual metabolic responses and antioxidant potential of blood orange cv. Tarocco Scirè, *J. Food Compos. Anal.* 105 (2022) 104246 <https://doi.org/10.1016/j.jfca.2021.104246>.
- P. Legua, G. Modica, I. Porras, A. Conesa, A. Continella, Bioactive compounds, antioxidant activity and fruit quality evaluation of eleven blood orange cultivars, *J. Sci. Food Agric.* 102 (2022) 2960–2971.
- A. Liaw, M. Wiener, Classification and regression by randomForest, *R. News* 2 (2002) 18–22.
- T. Galili, A. O’Callaghan, J. Sidi, C. Sievert, Heatmaply: an R package for creating interactive cluster heatmaps for online publishing, *Bioinformatics* (2017), <https://doi.org/10.1093/bioinformatics/btx657>.
- H. Wickham, M. Averick, J. Bryan, W. Chang, L.D.A. McGowan, R. François, G. Grolemond, A. Hayes, L. Henry, J. Hester, M. Kuhn, T. Lin Pedersen, E. Miller, S. Milton Bache, K. Müller, J. Ooms, D. Robinson, D. Paige Seide, V. Spinu, K. Takahashi, D. Vaughan, C. Wilke, K. Woo, H. Yutani, Welcome to the tidyverse, *JOSS* 4 (43) (2019) 1686.
- R. Tundis, M.R. Loizzo, M. Bonesi, F. Menichini, Citrus flavonoids: Health-promoting effects and potential applications in the food industry: an overview, *Food Chem.* 160 (2014) 371–388.
- G. Gattuso, D. Barreca, C. Gargiulli, U. Leuzzi, C. Caristi, Flavonoid composition of citrus juices, *Molecules* 12 (2007) 1641–1673.
- D. Barreca, E. Bellocchio, C. Caristi, U. Leuzzi, G. Gattuso, Flavonoid composition and antioxidant activity of juices from Chinotto (*Citrus myrtifolia* Raf.) fruits at different ripening stages, *Food Chem.* 120 (2010) 661–666.
- Y. Nogata, K. Sakamoto, H. Shiratsuchi, T. Ishii, M. Yano, H. Ohta, Flavonoid composition of fruit tissues of citrus species, *Biotechnol. Biochem.* 70 (2006) 178–192.
- D. Del Rio, A.J. Stewart, N. Pellegrini, A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress, *Nutr. Metabol. Cardiovasc. Dis.* 14 (4) (2004) 199–209.
- H. Zhang, H. Zhang, Y. Hu, Y. Wang, Comparative metabolomics of different citrus species and genotypes for juice quality and flavonoid biosynthesis, *J. Agric. Food Chem.* 69 (3) (2021) 953–963.
- M. Scordino, L. Sabatino, P. Traulo, Analytical characterization of lemon juice: determination of hesperidin and other flavanones, *Food Chem.* 135 (3) (2012) 1953–1959.
- I. Samanigo, J.M. Carrillo, M. Cano-Lamadrid, F. Hernández, Flavonoid-based chemotaxonomic classification and antioxidant properties of new *Citrus limon* varieties, *Molecules* 27 (8) (2022) 2571.
- Z. Zou, W. Xi, Y. Hu, C. Nie, Z. Zhou, Flavonoids and antioxidant activity in major citrus varieties from China, *Food Chem.* 245 (2020) 1158–1164.
- E.M. García-Castello, A.D. Rodríguez-Lopez, B. Díaz-Reinoso, Valorization of citrus by-products: extraction of bioactive compounds and their applications, *FBP* 117 (2019) 367–379.
- C.C. Angel, H.C.J. Andrés, J. Salinas-Ruiz, F. Osorio-Acosta, F. Hernández-Rosas, Preferences for lemon consumption by Mexicans and its relationship with consumer needs, emotions, and attitudes, *IJFST (Int. J. Food Sci. Technol.)* 58 (2) (2023) 646–655.
- M.G. Aguilar-Hernández, P. Sánchez-Bravo, F. Hernández, Á.A. Carbonell-Barrachina, J.J. Pastor-Pérez, P. Legua, Determination of the volatile profile of lemon peel oils as affected by rootstock, *Foods* 9 (2) (2020) 241.
- J.J. Martínez-Nicolas, D. Núñez-Gómez, V. Lidón, R. Martínez-Font, P. Melgarejo, F. Hernández, P. Legua, Physico-chemical attributes of lemon fruits as affected by growing substrate and rootstock, *Foods* 11 (16) (2022) 2487.
- S.W. Zhang, G.X. Huang, F. Ding, X.H. He, J.C. Pan, Mechanism of seedlessness in a new lemon cultivar ‘Xiangshui’ [*Citrus limon* (L.) Burm. F.], *Sex. Plant Reprod.* 25 (2012) 337–345.
- C. Di Vaio, G. Graziani, A. Gaspari, G. Scaglione, S. Nocerino, A. Ritieni, Essential oils content and antioxidant properties of peel ethanol extract in 18 lemon cultivars, *Sci. Hort.* 126 (1) (2010) 50–55.
- C. Catalano, G. Las Casas, A. Giuffrida, F. Ferlito, M. Di Guardo, A. Continella, S. Bennici, S. La Malfa, A. Gentile, G. Distefano, Reproductive biology factors hampering lemon [*Citrus limon* (L.) burm. F.] genetic improvement, *Agriculture* 12 (12) (2022) 2020, <https://doi.org/10.3390/agriculture12122020>.
- A. Dubey, R. Sharma, Effect of rootstocks on tree growth, yield, quality and leaf mineral composition of lemon (*Citrus limon* (L.) Burm.), *Sci. Hort.* 200 (2016) 131–136.
- A. Salvo, M. Bruno, G.L. La Torre, R. Vadalà, A.F. Mottese, E. Saija, V. Mangano, K. E. Casale, N. Cicero, G. Dugo, Interdonato lemon from Nizza di Sicilia (Italy):

- chemical composition of hexane extract of lemon peel and histochemical investigation, *Nat. Prod. Res.* 30 (13) (2016) 1517–1525.
- [39] P. Zhang, Z. Zhou, Postharvest ethephon degreening improves fruit color, flavor quality and increases antioxidant capacity in 'eureka' lemon (*Citrus limon* (L.) Burm. f.), *Sci. Hort.* 248 (2019) 70–80.
- [40] H. Yu, C. Zhang, C. Lu, Y. Wang, C. Ge, G. Huang, H. Wang, The lemon genome and DNA methylome unveil epigenetic regulation of citric acid biosynthesis during fruit development, *Hortic. Res.* 11 (3) (2024).
- [41] E. González-Molina, D.A. Moreno, C. García-Viguera, Genotype and harvest time influence the phytochemical quality of Fino lemon juice (*Citrus limon* (L.) Burm. F.) for industrial use, *J. Agric. Food Chem.* 56 (5) (2008) 1669–1675.
- [42] E. González-Molina, D.A. Moreno, C. García-Viguera, A new drink rich in healthy bioactives combining lemon and pomegranate juices, *Food Chem.* 115 (4) (2009) 1364–1372.
- [43] N. Martí, P. Mena, J.A. Cánovas, V. Micol, D. Saura, Vitamin C and the role of citrus juices as functional food, *Nat. Prod. Commun.* 4 (5) (2009) 1934578X0900400506.
- [44] Z. Zou, X. Wapeng, H. Yan, N. Chao, Z. Zhiqin, Antioxidant activity of citrus fruits, *Food Chem.* 196 (2016) 885–896.
- [45] G. Modica, L. Pulvirenti, T. Strano, S. La Malfa, A. Gentile, C. Drago, A. Continella, L. Siracusa, Metabolite content and antioxidant potential of blood oranges as affected by rootstock, *Food Chem.* 478 (2025) 143634.
- [46] I.G. Munteanu, C. Apetrei, Analytical methods used in determining antioxidant activity: a review, *Int. J. Mol. Sci.* 22 (7) (2021) 3380.