Essential Oil of Cami, a New Citrus Hybrid†

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The chemical composition of the essential oil of a new Citrus hybrid, named Cami, obtained by crossbreeding the Mapo tangelo (*Citrus deliciosa* Ten. x *C. paradisi* Macf.) as male parent and the 50-15A-6 hybrid (*C. clementina* Hort. ex Tan. x *C. deliciosa* Ten.) as female parent, was analyzed by GC/MS and compared with those of its parents. In total, 51 components were fully characterized and grouped in six classes (monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpenes, aliphatic aldehydes, aliphatic alcohols, esters) for an easier comparison of all oils. Variations during the fruit development of the percentage of the six classes are also described. A statistical treatment by linear discriminant analysis (LDA) of the compositional data from GC analyses was also carried out. The peel essential oil of the new Citrus hybrid Cami shows compositional features resembling those of Mapo tangelo and Avana mandarin, but in an original and unique way, thus making it an interesting new Citrus essential oil. Furthermore, the new fruit on account of its excellent characteristics is already in production.

Keywords: *Citrus spp.; hybridization; new Citrus hybrid; essential oil composition; seasonal variation; statistical analysis*

Citrus cultivation is probably one of the most important commercial and industrial agronomical activities of the world, and in southern Italy it represents an important and strategical economic resource with strong social implications.

The worldwide production of fresh fruits and of all derivatives, such as juices and essential oils, is constantly increasing (F.A.O., 1995); therefore, it is important to ensure the high quality of fruits, suitable for the fresh-fruit market and for industrial processing, in order to hold out against the strong competition. Moreover, this is in accordance with the consumer's requirements, which are ever more addressed toward high-quality products with well-standardized nutritional content.

With this in mind, the joint research of our groups concerns the genetic improvements of high quality cultivars and the production of new citrus fruits, namely hybrids (Starrantino, 1980, 1992). In particular, our interest, in these last years, has been focused on the study of the chemical composition of new Citrus hybrids (Rapisarda *et al.*, 1990, 1995; Ruberto *et al.*, 1993, 1994, 1997) with the aim of phytochemical characterization and, possibly, the evaluation of these new fruits for their introduction into the fresh market and into the industrial chain of trasformation.

This study is a part of a large hybridization project whose aims are, on one hand, the production of seedless fruits with optimal size, easily removable peel, new and original organoleptic characteristics, and possibly with precocious or late ripening and, on the other hand, to obtain plants with improved resistance against diseases and environmental stress and high productivity (Parisi *et al.*, 1993; Ruberto *et al.*, 1993, 1994; Salerno and Cutuli, 1977).

We wish to report here the results of the analysis of the peel essential oils of Cami, a new mandarin fruit, in comparison with those of its parents.

MATERIALS AND METHODS

Hybridization. Cami mandarin comes from a crossbreeding, started in 1973, between the monoembryonic hybrid 50-15A-6 [Comune clementine (*Citrus clementina* Hort. ex Tan.) x Avana mandarin (*C. deliciosa* Ten.)], used as female parent, and the Mapo tangelo [(*C. deliciosa* Ten.) x Duncan grapefruit (*C. paradisi* Macf.)], used as male parent. Seeds obtained from the fruits of this cross were sowed in January 1974, and in 1977 from the apical part of seedlings some scions were cut and grafted on Troyer citrange. In 1980, plants were transferred to the experimental field Palazzelli (Lentini), and in 1985 the first fruits were obtained.

Plant Material. Fruits of Cami mandarin, Comune clementine, Avana mandarin, 50-15A-6 hybrid, Mapo tangelo, and Duncan grapefruit were collected three times during the season 1994/95 depending on their different ripening development. Fifteen to twenty fruits coming from the head of plants according to the cardinal points were collected at each picking. All the trees are cultivated in the experimental field Palazzelli of the Citrus Experimental Institute (Istituto Sperimentale per l'Agrumicoltura), Lentini, Sicily.

Isolation and Analysis of Essential Oil. Fresh rind tissue (flavedo, 10 g) of each sample was subjected to simultaneous steam distillation−extraction (SDE) for 3 h with a modified Likens−Nickerson apparatus using a 1:1 mixture of pentane:diethyl ether as the solvent (Godefroot *et al.*, 1981). Essential oil yields were in the average values (Di Giacomo and Mincione, 1994).

The essential oil solutions were immediately analyzed on a Hewlett-Packard gas chromatograph, Model 5890, equipped with a flame ionization detector and coupled to an electronic integrator. Analytical conditions: HP-1 dimethylpolysiloxane capillary column (25 m × 0.2 mm), helium as carrier gas.

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Table 1. Chemical Composition of *Citrus* Essential Oils[a]

compd	Comune clementine	Avana mandarin	50-15A-6 hybrid	Cami hybrid	Mapo hybrid	Duncan grapefruit
monoterpene hydrocarbons	**97.59**	**97.68**	**97.60**	**96.85**	**97.12**	**96.80**
2 α-thujene	t	0.66	t	0.65	0.54	t
3 α-pinene[b]	0.47	1.79	0.49	1.78	1.56	0.42
4 camphene[b]	t	0.01	t	t	t	t
5 sabinene[b]	0.38	0.22	0.37	0.49	0.16	1.20
6 β-pinene[b]	0.03	1.36	0.03	1.36	1.14	0.02
8 myrcene[b]	1.83	1.73	1.76	1.70	1.76	1.76
9 α-phellandrene[b]	0.03	0.06	0.03	0.07	0.06	0.08
10 δ-3-carene[b]	0.02		0.04		t	
11α-terpinene[b]	0.01	0.40	0.01	0.36	0.35	0.01
12 β-phellandrene[b]		0.39		0.20	0.31	
13 *p*-cymene[b]						
14 limonene[b]	94.77	73.58	94.83	72.57	75.78	93.05
15 (*Z*)-β-ocimene	t	t	t	0.01	t	t
16 (*E*)-β-ocimene	0.02	0.02	0.01	0.28	0.12	0.24
17 γ-terpinene[b]	0.02	16.68	0.02	16.60	14.62	0.02
20 terpinolene[b]	0.01	0.78	0.01	0.78	0.72	t
oxygenated monoterpenes	**1.18**	**1.06**	**1.19**	**1.28**	**1.48**	**1.71**
19 linalool oxide						0.66
22 linalool[b]	0.81	0.19	0.77	0.41	0.45	0.45
23 isopulegol						0.04
24 citronellal[b]	0.07	0.04	0.05	0.04	0.02	
26 terpinen-4-ol[b]	0.05	0.20	0.04	0.29	0.24	0.09
27 α-terpineol[b]	0.12	0.38	0.11	0.25	0.48	0.20
29 carveol[b]	0.01					0.10
30 nerol[b]	0.02	0.04	t	0.03	0.04	0.03
31 neral[b]	t	t	0.04	t	0.06	0.06
32 geraniol[b]	0.04	0.03	0.05	0.02	0.03	t
33 geranial[b]	0.06	0.07	0.09	0.02	0.10	0.08
35 thymol[b]		0.11	0.04	0.22	0.06	
sesquiterpenes	**0.17**	**0.27**	**0.11**	**0.38**	**0.30**	**0.94**
41 α-copaene[b]	0.02					0.06
44 β-caryophyllene[b]	t	0.06		0.06	0.26	0.31
46 α-humulene[b]	t	0.03		0.01	0.02	0.03
47 β-bisabolene	0.01					
48 farnesene		0.04	0.02	0.06	0.02	
49 β-sinensal	0.03					
50 α-sinensal	0.11	0.14	0.09	0.25		
51 nootkatone	t					0.54
aliphatic aldehydes	**0.78**	**0.20**	**0.50**	**0.31**	**0.31**	**0.52**
7 octanal[b]	0.37	0.08	0.24	0.12	0.20	0.23
21 nonanal[b]		0.01	t	t	0.02	0.07
28 decanal[b]	0.33	0.11	0.26	0.19	0.09	0.22
42 dodecanal[b]	0.06					t
45 2-dodecenal[c]	0.02					t
aliphatic alcohols	**0.05**	**0.02**	**0.07**	**0.02**	**0.06**	**0.12**
1 hex-1-en-3-ol	t					
18 octanol[b]	0.05	0.02	0.07	0.02	0.06	0.07
25 nonanol[b]	t	t				0.02
34 decanol[b]						0.03
esters	**t**			**t**	**0.05**	**0.12**
36 citronellyl acetate				t	0.01	
37 terpinyl acetate						0.04
38 neryl acetate	t				0.04	t
39 geranyl acetate					t	0.08
43 decyl acetate						t
40 methyl *N*-methylanthranilate		0.48	0.02	0.28	0.11	

[a] The numbering refers to elution order; values (area percent) refer to the last picking of each fruit and represent averages of three determinations (t = trace < 0.01%). [b] Co-injection with authentic sample. [c] Correct isomer not identified.

Injection in split mode (1:50), injection volume 1 μL, injector and detector temperature 250 and 270 °C, respectively. The oven temperature was held at 60 °C for 6 min and then programmed from 60 to 270 °C at 3 °C/min.

GC/MS analyses were carried out on the same chromatograph equipped with a Hewlett-Packard MS computerized system, Model 5971A, ionization voltage 70 eV, electron multiplier 1700 V, ion source temperature 180 °C, GC conditions same as above.

Identification of components was based on GC retention times (Jennings and Shibamoto, 1980), computer matching with NBS library, comparison of the fragmentation patterns with those reported in the literature (Adams, 1995; Eight Peak Index, 1974; Jennings and Shibamoto, 1980) and whenever possible, coinjection with authentic samples.

Pure standards were purchased from Aldrich Chemical Co., Extrasynthese, France, and Fluka Chemie AG, Switzerland.

LDA and variance analysis were made by processing all data with the aid of the SPSS statistical package (SPSS, 1996). Mean separations were achieved according to Duncan's multiple range test.

RESULTS AND DISCUSSION

Cami mandarin is a hybrid from the crossbreeding between the monoembrionic hybrid named 50-15A-6

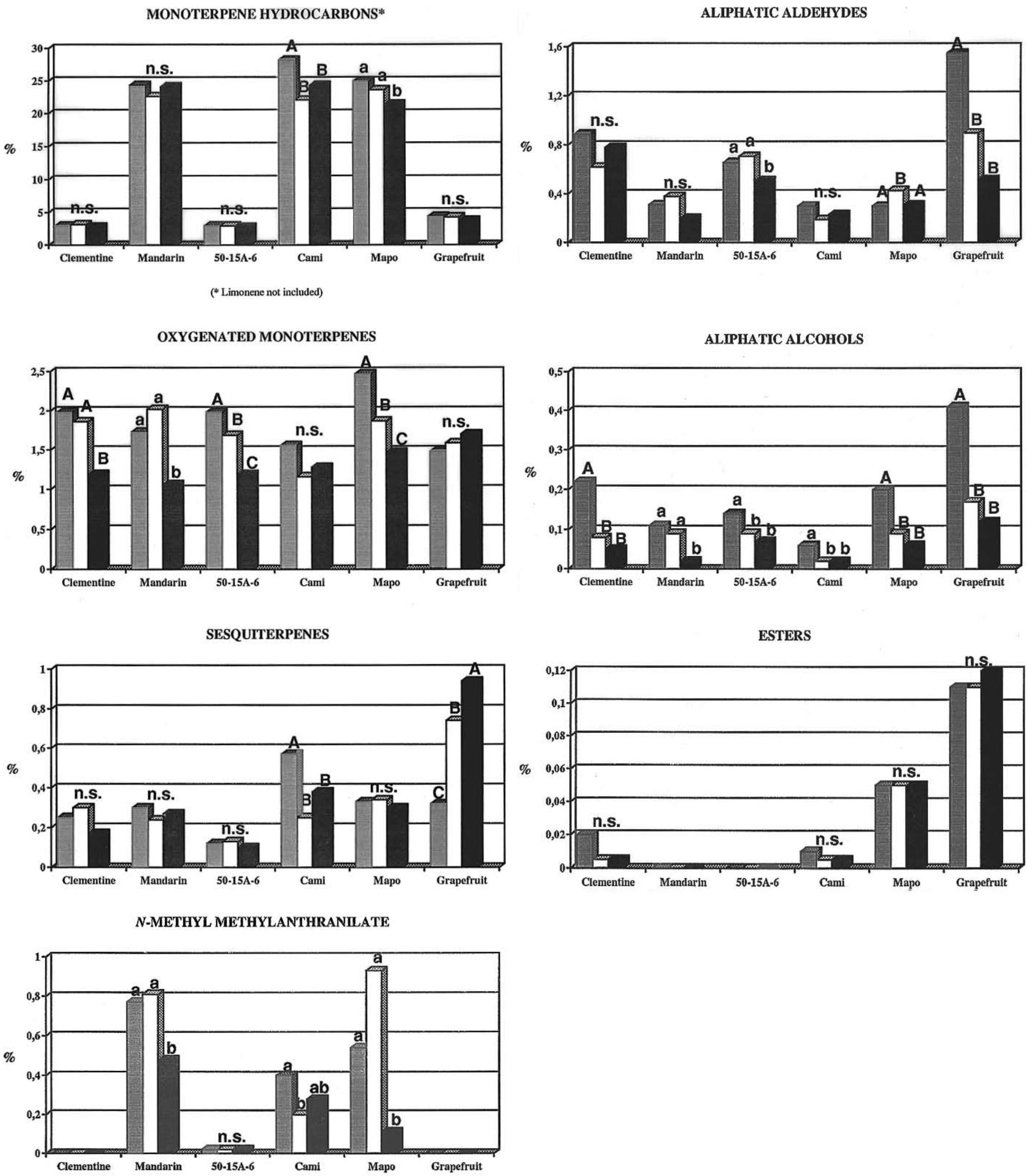

Figure 1. Variation during fruit maturation of the percentage composition of the classes of minor components of the essential oils in clementine, mandarin, 50-15A-6 hybrid, Cami, Mapo, and grapefruit (shaded box = first coll.; open box = second coll.; solid box = third coll.; n.s. = nonsignificant, lowercase letters = significant at $P \leq 0.05$, capital letters = significant at $P \leq 0.01$).

used as the female parent and the Mapo tangelo as the male parent.

The choice of 50-15A-6 hybrid was due mainly to the its high productivity and fair quality of fruits, even if in general not good enough for a commercial diffusion, and also for the monoembryonic character of the seeds (Russo and Starrantino, 1972). The Mapo tangelo was chosen for its high productivity and precocious ripening and for the excellent organoleptic characteristics and the optimal size of fruits (Russo, 1972; Dugo *et al.* 1990).

The Cami comes from a selection of 139 plants obtained during the hybridization program, and the final fruit has an optimal size with peel and pulp very close to that of clementine, while the taste and the aroma are very original, resembling Mapo tangelo and Avana mandarin. On account of these favorable characteristics, the Cami is already in production in several areas of Sicily and Calabria (Starrantino, 1992).

Table 1 shows the chemical composition of the peel essential oils of Cami in comparison with that of all

Table 2. Discriminant Analysis

discriminant function	eigenvalue	rel percentage	canonical correlation
1	343.7649	84.36	0.998 549
2	34.1840	8.39	0.985 687
3	18.3255	4.49	0.973 784
4	7.9691	1.95	0.942 606
5	3.2310	0.79	0.873 869

functions derived	Wilks λ	χ^2	DF	sig. level
0	0.000 000	608.0418	120	0.000 000
1	0.000 039	386.0130	92	0.000 000
2	0.001 364	250.7106	66	0.000 000
3	0.026 352	138.1763	42	0.000 000
4	0.236 353	54.8123	20	0.000 044

species involved in its production. On the whole, the composition of the essential oil of Cami mandarin is close to that of Mapo tangelo, but the contribution of mandarin parents is essential for its original aroma.

Concerning the qualitative and quantitative composition of the monoterpene hydrocarbon fraction, Cami oil can be considered similar to mandarin oil, as for tangelo Mapo oil. The composition of the oxygenated monoterpene fraction is qualitatively similar to that of both parents (Mapo and 50-15A-6 hybrids); however, the thymol content of Cami oil is decidedly higher than that of both parents. The total amount of sesquiterpenes in Cami oil is similar to that of Mapo oil; the qualitative composition of this fraction shows the trace of both parent species, but every parent shows a composition of this fraction very different from the other. The composition of aliphatic aldehyde and alcohol fractions is similar for the three species, while esters are practically absent in Cami oil, as in its female parent oil (50-15A-6).

Finally, particular mention has to be ascribed to methyl *N*-methylanthranilate, a specific component of Avana mandarin, whose percentage in Cami is higher than in both parents, the 50-15A-6 and Mapo hybrids.

Broadening the compositional comparison of the essential oil of Cami with those of all species involved in this crossbreeding, the peculiar mandarin character of the new hybrid is confirmed. This can be testified not only from the higher similarity with its male parent

Table 3. Standardized Determinant Function Coefficient

compd	function 1	function 2	function 3
α-thujene	−2.3301	−0.8486	2.3367
α-pinene	−0.0635	0.2876	0.7028
camphene	0.2716	−0.3351	−0.6647
sabinene	−0.8589	−0.8730	1.6098
β-pinene	1.1977	0.4296	−3.4873
octanal	0.1543	−0.7886	−0.2819
myrcene	0.3027	0.0793	−0.4871
α-phellandrene	0.2007	0.6427	0.8940
α-terpinene	−0.3897	−0.3825	−1.1461
limonene	−0.9033	−1.1480	−0.3530
(*E*)-β-ocimene	1.0343	0.4314	0.6416
γ-terpinene	−3.2114	−1.2795	−0.1889
octanol	−0.0476	0.8180	0.4849
terpinolene	1.8297	0.5637	0.0361
linalool	0.7176	−0.5068	−0.2107
terpinen-4-ol	1.2805	1.1683	0.4130
α-terpineol	−0.3620	−0.4366	−0.3102
decanal	0.5840	−0.7974	0.6317
nerol	−1.1748	−1.4195	1.2938
neral	1.2567	1.2446	−0.6254
geraniol	−1.0025	−0.5991	−0.1166
geranial	−1.1678	0.0427	−1.0371
β-caryophyllene	0.1257	0.7310	0.1316
α-humulene	0.3011	0.0614	−0.7207
eigenvalue	343.7649	34.1840	18.3255
cum. prop.	0.8436	0.9275	0.9724

Mapo but also for the presence of some characteristic compounds, such as thymol, α-sinensal, and methyl *N*-methylanthranilate, which enhance the mandarin-like nature of Cami. This is of little surprise since Avana mandarin was involved in the production of both parents of Cami.

A study of the seasonal variation of the composition of each essential oil has also been carried out. The histograms of Figure 1 illustrate the variation during fruit development of the percentage of the minor constituents grouped in classes. As has been observed in similar cases (Ruberto *et al.*, 1993), an easy interpretation of the seasonal variations is not always possible because of the fruit development and their "degree" of ripening. The Duncan grapefruit is the sole species that shows a regular behavior. In fact, monoterpene hydrocarbons, aliphatic aldehydes, and alcohols decrease during the development, while oxygenated monoterpenes, sesquiterpenes, and esters have an opposite be-

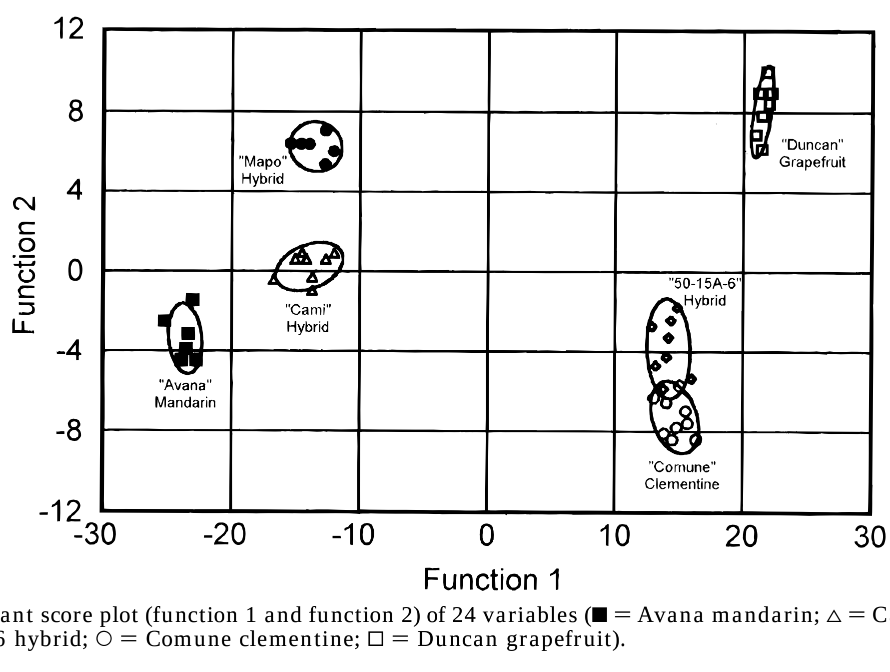

Figure 2. Discriminant score plot (function 1 and function 2) of 24 variables (■ = Avana mandarin; △ = Cami hybrid; ● = Mapo hybrid; ◇ = 50-15A-6 hybrid; ○ = Comune clementine; □ = Duncan grapefruit).

havior. All the other species analyzed here show a decrease in all classes of compounds between the first and the last picking, but such variations are quite irregular. However, from the comparison of data of Figure 1 it is clear that the observed percentage variations are more similar for Cami and Avana mandarin than between Cami and its parents.

With the aim of obtaining a best differentiation of all the species involved in the production of Cami mandarin, and therefore their best characterization, the components of each essential oil were investigated by means of multivariate analysis, applying, in particular, an explorative linear discriminant analysis (LDA). In fact, throughout the application of this statistical methodology it is possible to classify a new entity, *i.e.*, the new hybrid, in the context of already defined groups, identifiable with all the species involved in its production. Twenty-four components of the essential oils relative to the three collections of each sample were chosen as original variables of the statistical analysis.

Table 2 shows the discriminant functions, which are the result of the statistical analysis, whose role is to discriminate in a optimal way all the species here considered. In particular, the eigenvalue associated to the first function contributed 84.36% of the variance of original data, the eigenvalue associated to the second function contributed 8.39% of the variance, and the eigenvalue associated to the third one contributed 4.49% of the variance. Therefore, the combination of the first and the second function gives almost 93% of the variance of the system. Table 3 reports the statistical weight and therefore the importance of each variable, namely the 24 components of the essential oils; γ-terpinene, α-thujene, terpinolene, terpinen-4-ol, and neral are the main components for the first function and nerol, neral, γ-terpinene, and terpinen-4-ol for the second one.

The graphic representation of the 24 variables in the two components (functions 1 and 2) is shown in Figure 2. It is clear that mandarin, clementine, and grapefruit are clearly differentiated and are situated at the vertices of an ideal triangle. The 50-15A-6 hybrid and clementine are partially laid one upon the other, accounting for a greater likeness of the hybrid oil with that of its female parent, clementine, than the male parent, mandarin. The Mapo tangelo differentiates from both parents, mandarin and grapefruit, being closer to mandarin. Furthermore, the graph allows us to discriminate Mapo, grapefruit, and clementine, according to the first function (function 1), which contributes *ca.* 84% of the total variance, whereas the second function (function 2), which contributes *ca.* 8.4%, gives a clear discrimination between Mapo and mandarin, but not between Mapo and grapefruit. Finally, concerning the Cami mandarin, it is placed between the parents (50-15A-6 hybrid and Mapo) and mandarin. Also, in this case, the first function, the most important, gives the highest contribution to the differentiation, clearly separating the Cami from the female parent (50-15A-6 hybrid), while the second function shows the diversity with both parents, being more marked with the male parent Mapo. Therefore, the new hybrid Cami, concerning its essential oil, is quite distant from grapefruit and clementine and also different from one of the parents, the 50-15A-6 hybrid, but posseses many features of Mapo, the male parent, and mandarin.

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