Citrus aurantium L.: Cultivar impact on sensory profile

Diana De Santis Writing - original draft * desdiana@unitus.it, Maria Teresa Frangipane Writing - original draft

DIBAF, Department for Innovation in Biological, Agro-Food and Forest Systems, University of Tuscia, Via San Camillo De Lellis snc, 01100, Viterbo, Italy

*Corresponding author.

Abstract

The purpose of this study was to identify the sensory attributes for the evaluation of bitter orange (*Citrus aurantium* L.) sensory profile, in three different cultivars (*Canaliculata,* C1; *Crispifolia,* C2; *Salicifolia* C3), so that it was possible to highlight their sensory characteristics, through the application of the official procedure. Our results revealed that the odor of C1 was mainly characterized by the citrus flavor, while the aroma of C3 was governed primarily by specific flowers. C2 had a lower aromatic intensity and, overall, a more balanced flavor enriched with cooked fruit that characterized it. Greater knowledge of the peculiar sensorial properties of each cultivar could enhance and make their food use more appropriate.

Keywords: *Citrus aurantium* L.; Sensory analysis; Cultivars; Bitter orange

Introduction

The genus *Citrus* of the family Rutaceae includes several important fruits such as oranges, tangerines, limes, lemons, bitter orange, and grapefruits. Citrus fruits are one of the most important fruits tree crop, with worldwide agricultural production of over 100 million tons per year (Marìn et al., 2007; USDA, 2019). It is cultivated mainly in the regions where the climate and the soil favor its development, for this reason, Italy is one of the major citrus fruit producers and exporters (Daovy, 2009). In a recent study (Cano-Lamadrid et al., 2018), the high potential of citrus fruits for the food processing industry is also demonstrated.

The Citrus genus includes various fruits with similar but well-defined features. Among these, *Citrus aurantium* L. (CA), also known as sour or bitter orange (Mabberley, 2004), although resembling the orange, differs by several characters. Its pulp is acidic, and the albedo is more bitter. The fruits are mainly used for dessert, they have important economic value for their essential oils (Deterre et al., 2014). In fact, they are used as aroma flavor in many food products, including alcoholic and non-alcoholic beverages, marmalades, gelatins, sweets, soft drinks, ice creams, dairy products, oils, candies, and cakes (Nguyen et al., 2009; Karoui et al., 2010). Essential oil relative to the peel and leaves has a very characteristic pleasant odor (Deterre et al., 2012; Moufida and Marzouk, 2003) and it has very important antimicrobial and antioxidant activities (Trabelsi et al., 2014; Essadik et al., 2015). The flowers are used for the preparation of infusions, the essential oil extracted from the flowers themselves is generally used in the preparation of perfumes and cosmetic products (Ellouze et al., 2011).

The Food and Drug Administration (FDA) has approved bitter orange (in small amounts) as a flavouring agent (Khazaei et al., 2012).

The juice of the fruit is used in salads for sour taste instead of lemon juice, and the peel is used in marmalade production (Ersus and Cam, 2007).

In some parts of the world, bitter orange is a fundamental ingredient of regional cuisine. In Mexico, sour orange is consumed raw with salt and hot pepper. In southern India, fresh fruit is also frequently used in *pachadis*, a yogurt-based side dish. In Afghanistan sour oranges are very popular, furthermore, the young fruits are commonly used as food ingredients in China (Zhang et al., 2007).

Many people prefer them to lemons and sour orange juice is squeezed on food the same way lemon is used in the Mediterranean (Lim, 2012).

Historically, sour orange had been used in traditional Chinese medicine for clinical applications, including indigestion, dysentery and as an expectorant (Blumenthal, 2004; Stohs et al., 2007). The dried bark is used for disorders of the urinary tract (~~Karthikejan and Karthikejan, 2014~~Karthikeyan and Karthikeyan, 2014).

Gattuso et al. (2007) report on the composition of CA that is very different from sweet orange, but it is quite like that of grapefruit, being rich in naringin, neohesperidin and neoeriocitrin. These flavanones are responsible for the bitter test (Malik et al., 2014).

The extract of the immature fruit or peel of bitter orange and its principal protoalkaloidal constituent, p-synephrine, are used widely in weight management and weight loss as well as in sports performance products (Stohs et al., 2011; Suryawanshi, 2011; Stohs, 2017).

Much attention has been paid to the health benefits of dietary phenolics that have antioxidant activities stronger than those of vitamin C. In fact, the health benefits of *Citrus* fruit have mainly been attributed to the presence of these bioactive compounds (Ghasemi et al., 2009; Moulehi et al., 2012; Karimi et al., 2012; Lagha-Benamrouche and Madani, 2013; Suntar et al., 2018).

Jabri karoui and Marzouk (2013) reported that CA peel and juice antioxidant activity was high enough for the plant to be used as a potential resource of natural antioxidants for the food, moreover, bitter orange peel appears as a promising source of functional ingredient (Rafiq et al., 2018).

It is therefore clear that the aroma is an important characteristic of the product, for this reason, the sensory study, aimed to identify the aromatic profile, could be a key parameter for the recognition and subsequent commercial exploitation.

However, it cannot be ignored that the Citrus genus is taxonomically very confused due to the existence of numerous hybrids selected by humans and fixed in lines by the frequent vegetative propagations. The most reliable theories consider CA, as a result of the first cross between tangerine (C. *reticulata* Blanco) and pomelo (C. *maxima* (Burm.) Merr.), from which Oranges and Grapefruits probably originated (Mabberley, 2004).

From this derives a very high number of cultivars, for the CA, each with morphological characteristics clearly visible and different from the others, as reported by Lombardo et al., (2012) that classifies genetically, through molecular markers, at least 9 different, although, from previous taxonomic classifications, at least the double has been cited (SysTax, 2014; Calabrese, 1973).

These cultivars represent an interesting topic of study in many areas of scientific research, both for their complexity, which makes them attractive for botanical collections, and the composition of extracts and essential oils, as well as for their sensorial peculiarities.

However, very few studies have linked these aspects, such as a recent work (Deterre et al., 2014) which refers to the chemical and sensory differences ten samples of CA, just considering the geographical origin.

Taking this into account, our study aims to identify the attributes for the evaluation of the sensory profile of the sour orange to highlight the differences between some cultivars.

The knowledge of the sensory profile for CA of different cultivars can be used to facilitate communication between product developers, marketing professionals, suppliers and customers.

Furthermore, the identification of the sensory descriptors can be employed for the evaluation of the bitter orange available on the market and could be applied in product enhancement and quality control.

Materials and methods

Plant material

The fruits organically grown, were collected from private orchards located in northern Lazio, a region of central Italy and cataloged in cultivars comparing the peculiar morphological traits of fruits and leaves with those described in the taxonomic classifications of the Citrus aurantium L. species (Syst-Tax, 2014; Locatelli, 2005; Lombardo et al., 2012).

14 fruits, for each of the three cultivars identified (Canaliculata, C1; Crispifolia, C2; Salicifolia, C3), were picked and transported to the sensory laboratory of the Tuscia University of Viterbo (Italy) on the same day.

Citrus aurantium cv. Canaliculata is a cultivar, of medium vigor, cultivated in Tuscany since the sixteenth century. The fruits, which ripen in December, reach 120–150 g of weight, they are orange and slightly flattened at the poles.

Citrus aurantium cv. Crispifolia is an ancient and very vigorous cultivar, already known in Tuscany (Italy) in the seventeenth century. The fruits, which reach maturity in December, are medium-sized and slightly flattened at the poles with a rather rough peel.

Citrus aurantium cv. Salicifolia is one cultivated vigorous cultivar, known and widespread in Italy from the 18th century. The fruits, which mature in December, reaching 150 g in weight, are bright orange color when ripe (Locatelli, 2005).

Each sample, weighing about 150 g, was chosen based on established criteria: the selected fruits were ripe and had no signs of injury or infection, stored at 15 °C and analyzed within five days after the harvest. The citrus peel, composed of flavedo and albedo, were removed from the fresh pulp of the three CVs of citrus studied. The assessors evaluated the aroma on seven small peel strips, deprived of albedo, conditioned in polyethylene bags, freshly prepared.

Sensory analysis

Development descriptors

The sensory analysis was carried out on about 5 g of bitter orange peel (exclusively flavedo), cut into 7 small strips (2 cm wide, 2.5 cm long) from each cultivar, placed in a disposable 50 ml plastic cup without odors, at room temperature, coded with 3-digit numbers.

All assessors were provided with mineral water, inviting them to drink, after each sample tested. Drinking water, although not strictly necessary to clean the palate between tests, being exclusively olfactory, was useful, in our opinion, to standardize the procedure.

The tests were performed on the samples by a sensory panel composed of fourteen judges (eight women and six men) aged between 25 and 55 and specialized in descriptive analysis with 5 years of experience.

The profiling of the CA was performed in a sensory evaluation laboratory that conforms with the international standards (UNI ISO 8589:2007-R2017), according to the UNI EN ISO13299 (2016). The judges were asked to evaluate only by sniffing the three samples obtained by randomizing the fruits collected, to identify the sensory attributes that best describe each cultivar.

All the descriptors were collected into a list that was then reduced by eliminating the inappropriate terms and by grouping synonymous and descriptors which unequivocally referred to the same characteristic. The identified terms were then discussed by the assessors to be sure that they could share the correct meaning of each descriptor.

Finally, the assessors evaluated three cultivars of the bitter oranges using the descriptors, according to a linear intensity scale from 0 to 9, where (0) was absence and (9) was high intensity.

The results were collected and calculated using the geometric mean (M), according to the UNI ISO11035, 1994:

$$M = \sqrt{F*I}$$

"**F**" is the ratio between the number of times a descriptor is mentioned, and the maximum number of times this descriptor could be mentioned;

"**I**" is the ratio between the intensity given a descriptor by the panel and the maximum possible intensity for the same descriptor.

The profile sheet, with the list of attributes with a geometric mean value (M) greater than 30% (Table 1), was then used by the judges to evaluate the three different samples. Besides, a line was left in each section to allow judges to add any perceived descriptors not present.

alt-text: Table 1

Table 1

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Definition, standards and weight percentage of the attributes selected in a first stage by panel.

	Attributes	Definition	Standards	Weight%
1	**AROMATIC INTENSITY**	Aroma associated with global odor	Orange peel = 6	**78.27**
2	**FLORAL**	Aromatic blend of a combination of flowers	NS [a]	**66.38**
3	**Specific flowers (SF)**	Aroma associated with specific flower	Mixture of fresh flower petals in covered plastic glass = 9	**46.59**
4	**orange blossom**	Aroma associated with orange blossom	Orange blossom = 9	**35.40**
5	magnolia	Aroma associated with magnolia flower	Magnolia flower = 9	3.86
6	rose	Soft floral fragrance associated with rose flower	Rose petal = 9	8.11
7	violet	Aroma associated with violet flower	Violet flower = 9	5.04
8	**jasmine**	Aroma associated with jasmine flower	Jasmine flower = 9	**31.18**
9	Various flowers	Aroma associated with non-specified flowers	Linalool 0,1 mg in 1L water = 7	25.63

10	Honey	Aroma associated with honey	Acacia honey = 9	12.79
11	**FRUITY**	An aroma combination associated with a variety of different fruits.	NS[a]	**65.24**
12	**Citrus fruits (CF)**	An aroma combination associated with citrus fruits such as orange, lemon, lime, grapefruit and pomelo	Fresh-squeezed orange, lemon, lime and grapefruit in equal parts ~~parts~~ = 9	**60.48**
13	**orange**	Aroma associated with orange	Fresh orange = 9	**48.29**
14	**lemon**	Aroma associated with lemon	Fresh lemon = 9	**35.13**
15	**tangerine**	Aroma associated with tangerine	Fresh tangerine = 9	**33.70**
16	lime	Aroma associated with lime	Fresh lime = 9	18.27
17	bergamot	Aroma associated with bergamot	Fresh bergamot = 9	2.24
18	ginger	Aroma associated with ginger	Fresh ginger = 9	11.36
19	**grapefruit**	Aroma associated with grapefruit	Fresh grapefruit = 9	**36.64**
20	**Light fruits with stone (LF)**	A sweet, floral, aromatic blend, reminiscent of variety of ripe fruits such as apricots, peaches	Ripe peach and apricot mix = 9	**38.15**
21	peach	Aroma associated with peach	Fresh peach = 9	21.48
22	apricot	Aroma associated with apricot	Fresh apricot = 9	15.64
23	**Cooked fruits (COF)**	Aroma associated with the process of heating/cooking fruits	NS[a]	**42.12**
24	**jam**	Aroma associated with jam	Peach, apricot and plum jam in equal parts = 9	**35.18**
25	canned pineapple	Aroma associated with canned pineapple	Water diluted canned pineapple juice 1:1 = 5	10.37
26	**marmalade**	Aroma associated with marmalade	Marmalade (orange jam) = 9	**34.95**
27	fruit in syrup	Aroma associated with fruit picked at the peak of ripeness and packed in sugar syrup	Mixed fruit in syrup = 9	11.66
28	**Exotic fruits (EF)**	Aroma associated with pineapple, mango, banana	Fresh cutted pineapple, mango, banana in equal parts = 9	**37.73**
29	**pineapple**	Aroma associated with fresh pineapple	Fresh cutted pineapple = 9	**37.13**
30	**VEGETABLE**	Aroma associated with vegetables	NS[a]	**39.32**
31	**Fresh vegetable (FV)**	Fresh aromatics associated with green vegetables	Fresh parsley water (10 g of chopped fresh parsley soaked in 300 ml of deionized water, for 15 'and then filtered) = 9	**32.11**
32	**Dry vegetable**	The dry, slightly dusty aromatics with the	Natural Lecithin closed in a plastic cup = 9	**35.47**

	(DV)	absence of green; associated dry grain stems		
33	**SPICY**	Aroma associated with spices, wood and pastry	NS [a]	**33.80**
34	**Spices (SP)**	Aroma associated with spices blend	3 g of anise, cinnamon, cloves in equal parts, crushed and diluted in 25 ml of water = 9	**32.84**
35	Wood	Aroma associated with wood chips	Wood chips closed in a plastic bag = 9	24.95
36	**Pastry (PA)**	Aroma associated with cake and candied fruits	Freshly baked sponge cake = 9	**35.89**
37	vanilla	Aroma associated with vanilla	Put 0.5 g of Vanillin in 250 mL of water and cover.	14.37
38	cake	Aroma associated with freshly baked cake	Freshly baked pound cake = 9	13.83
39	**candied**	Aroma associated with candied fruits	Shredded mixed candied fruits = 9	**31.50**

Table Footnotes

[a] NS No standard provide.

The reference standards for the identified attributes, of the sensory profile of our samples, were mainly natural products supplied to the judges in the training phase and corresponding to the maximum intensity (9) of the evaluation scale. For all other descriptors have been given precise standards and respectively as *Spices*: 3 g of anise, cinnamon, cloves in equal parts, crushed and diluted in 25 ml of water, as *Wood*: wood chips closed in a plastic bag, as *Pastry*: freshly baked sponge cake and as *Candied*: shredded mixed candied fruits.

Descriptive analysis

The samples were evaluated using the list of selected attributes (Table 1), on a linear scale of 10 points from 0 to 9, where 0 corresponded to the absence of perception and 9 to the maximum intensity.

A disposable plastic cup with seven small peel strips for each of the three randomized cultivars, prepared as described above, were provided to the judges, in two daily sessions, for a total of 2 replicas. The sensory study (two sessions with reference standards and test on the samples), was conducted overall in 3 days.

Statistical analysis

For data processing, we have chosen to use attribute classes (sub-families and specifics) separately, to avoid redundancy in discrimination, without losing the information provided by specific attributes.

Two-way Analyses of Variance (ANOVA) was performed on the data to verify the differences between the cultivars for each variable and the means were compared using Fisher's less significant differences (LSD) at

P = 0.05. Principal component analysis (PCA) was used to analyze sensory data collected from tests and to study the importance of attributes in discriminating CA cultivars.

PCA was performed and figures were drawn using Unscrambler ® Camo Software AS v.9.7 (Oslo, Norway).

An ANOVA test was applied to more specific descriptors to assess significance. Significantly different attributes are labeled with an asterisk in the spider plot used to graphically represent the characteristics of the samples.

Results and discussion

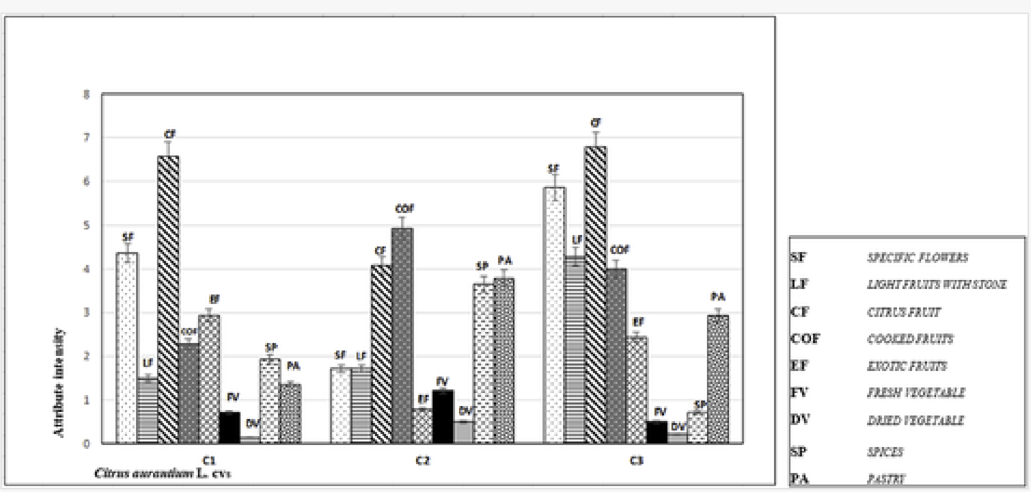
Sensory analysis

From 39 selected attributes, 15 were excluded by applying the ISO 11035:1994 (M<30%). The final list was composed of 24 odorous attributes grouped into families, subfamilies and specific attributes: Aromatic intensity; Floral: Specific flowers (orange blossom, jasmine); Fruity: Citrus fruits (orange, lemon, tangerine, grapefruit), Light fruits, Cooked fruits (jam, marmalade), Exotic fruits (pineapple); Vegetable: Fresh vegetable, Dry vegetable; Spicy: Spices, Pastry (candied).

The results of the sensory evaluation reported in Fig. 1, show that the flavor of sour orange mainly consisted of fruity, floral, vegetable and spicy notes. In Fig. 2, the different profiles of the three cultivars analyzed were represented in detail through specific descriptors.

alt-text: Fig. 1

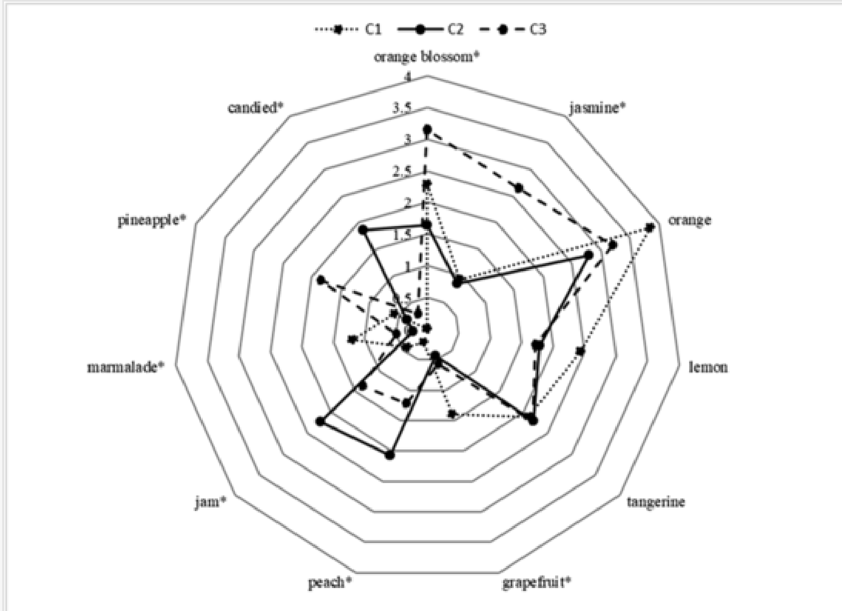
Fig. 1



Intensity of sensory profile attributes for each *Citrus aurantium* L. cultivars.
Legend: C1-*Canaliculata;* C2-*Crispifolia*; C3-*Salicifolia*.

alt-text: Fig. 2

Fig. 2



Spider plot of flavor specific attributes for each *Citrus aurantium* L. cultivars (*significant differences $p < 0.05$).
Legend: C1-*Canaliculata;* C2-*Crispifolia*; C3-*Salicifolia*.

The high concentrations of limonene in the aroma of Citrus aurantium L., found by many authors through GC-MS studies (Deterre et al., 2014; Trabelsi et al., 2014; Zhang et al., 2017; Radan et al., 2018) confirm the smell of *citrus*-like fruits in all our samples.

A *fruity* perception, with a more pronounced *citrus (CF)* character, was detected in the C1 cultivar (*Canaliculata*), not attenuated by the strong presence of other aromatic notes, while the floral character (SF) was noticeable in C3 (*Salicifolia*) and the sensory profile of the C2 (Crispifolia) sample showed predominant notes of cooked fruit and spices (Fig. 1).

In C1 the character of *citrus fruits*, which prevails over all other attributes, was represented above all by *orange* but also *lemon*, *grapefruit, tangerine,* in harmony with other fruits, sometimes *cooked* as *marmalade* (Fig. 2).

Instead, the sensory profile of the C2 sample appears more balanced, but with a lower content of *floral* and *citrus* odors, compared to C1. In fact, the *citrus fruit* character is accompanied by evident notes of *cooked fruits* (*jam), pastry (candy*), *exotic and spicy* notes (*pineapple and spicies*), harmonized by moderate notes of *light fruits with stone* (*peach*) and *specific flower (orange blossom)*.

In C3 the *floral* note with prominent *specific flowers (orange blossom, jasmine)* is evident. The freshness of the floral note is supported by a marked *citrus* aroma. In particular, the *citrus* notes, are mitigated by *light fruits with stone* and *cooked fruits* of medium intensity. The smell of *exotic fruit*, more precisely *pineapple*, was unexpectedly perceived (Fig. 2).

Overall, we can highlight that the judges assigned a higher aromatic intensity (7.6 ± 0.6) at C1 compared to C2 (5.5 ± 0.7), while the C3 cultivar showed an intermediate value (6.6 ± 0.7).

As mentioned above, blank lines have been inserted in the profile sheet, to allow judges to add any attributes to better specify the aroma.

This explains the recovery of the "peach" descriptor, which was reported by 64% of the judges, despite having obtained an M-*value below 30%.*

Panel performance

The summary results of ANOVA (Table 2) showed that the panel was highly discriminating ($P < 0.01$) towards the product (*Product effect*) and that it was consensual ($P > 0.5$) on the use of the scale (*Judge effect*). The judge-product interaction (*Interaction effect*) showed significant differences ($P < 0.05$) only for the attribute *Exotic fruit* (EF), for which the judges could have used different scales in the evaluation.

alt-text: Table 2

Table 2

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F-values and probability (p) from two-way ANOVA with interaction (Product x Judge).

Attribute	Product effect		Judge effect		Interaction effect (Product x Judge)	
	F	P	F	P	F	P
SF	148.00	0	0.15	0.83	0.10	0.92
CF	12.60	0.0001	0.39	0.54	0.29	0.75
LF	53.38	0	0.05	0.84	0.15	0.86
COF	95.60	0	0.17	0.69	0.68	0.51
EF	70.80	0	0.24	0.63	3.47	0.04
FV	31.62	0	0.41	0.52	0.14	0.87
DV	10.14	0.0005	0.06	0.80	0.02	0.97
SP	90.53	0	0.01	0.90	0.67	0.51
PA	103.80	0	3.84	0.54	1.88	0.16

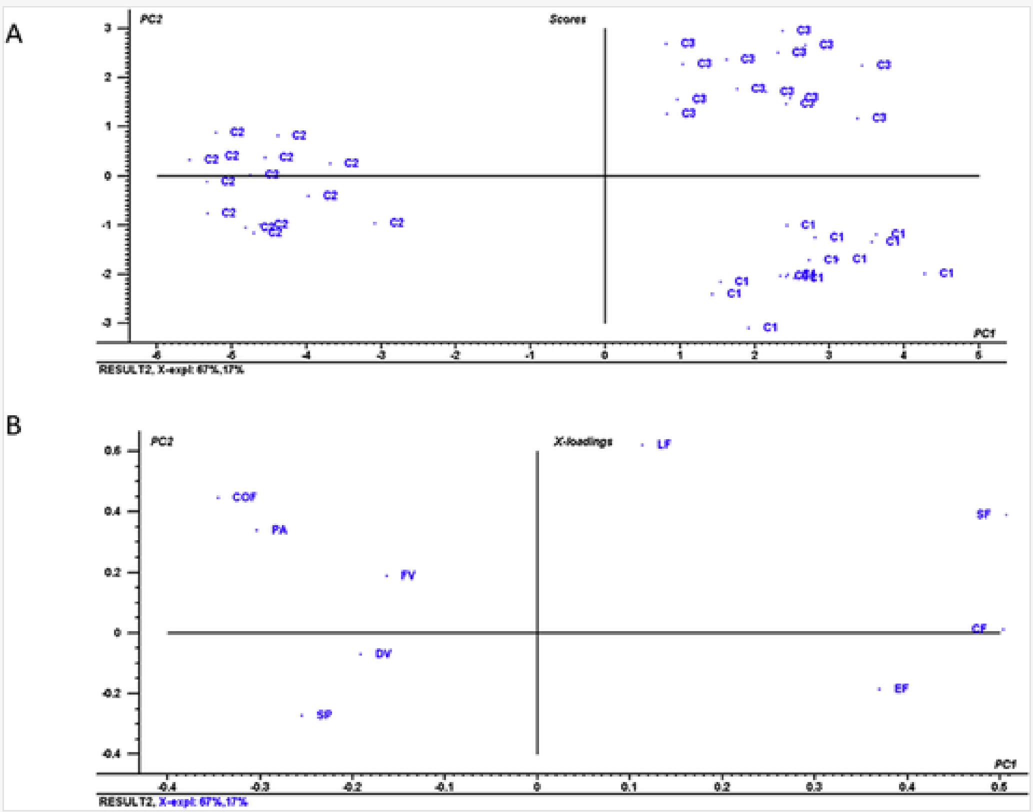
Principal component analysis

The principal component analysis (PCA), frequently used in the clustering of the samples, was applied to our sensory data (sub-families attributes, Table 1), to assess which attributes most discriminated the three cultivars (C1:*Canaliculata*; C2: *Crispifolia*; C3: *Salicifolia*).

The first two PC dimensions explained 84% of the variability of the experimental data of this study (Fig. 3).

alt-text: Fig. 3

Fig. 3



Score (A) and loading (B) plots of the PC analysis.

The loadings plot in Table 3 indicates that the first of the two components, which explains 67% of the variance, is more influenced by high value of *Specific flowers* (SF), *Citrus fruits* (CF) and *Exotic fruit* (EF) and low values of *Cooked fruits* (COF) and *Pastry* (PA) sensory attributes.

alt-text: Table 3

Table 3

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Factor loadings for the two first principal components and communalities.

	PC 1 (67% EV)	PC 2 (17% EV)	COMMUNALITIES
SF	**0.507**	**0.391**	**0.4099**
CF	**0.503**	0.013	**0.2532**
LF	0.113	**0.620**	**0.3972**
COF	**−0.346**	**0.448**	**0.3204**
EF	**0.370**	−0.184	0.1708
FV	−0.164	0.189	0.0626
DV	−0.191	−0.068	0.0411
PA	**−0.304**	**0.339**	**0.2073**
SP	−0.255	−0.270	0.1379

The distribution along the second component (17% explained variance) is conditioned by higher values of *Light fruit* (LF), *Cooked fruits* (COF), *Specific flowers* (SF) and *Pastry* (PA) sensory attributes. The variables that showed a greater discriminating power, to describe CA cv_s aromatic differences, according to the calculated communalities (Table 3 and Fig. 3 B), seem to be mainly SF, LF, COF, followed by CF and PA.

The score plot (Fig. 3A) shows the optimal separation between CA cultivars, confirming the differences that affect the olfactory sensory profile, evaluated through descriptive analysis.

From the distribution of samples according to PCA, the three CVs studied are strongly characterized by specific profiles. In particular, the cv Salicifolia (C3 sample), is characterized by fresh notes of flowers and light fruits, in cv. Canaliculate (C1sample), citrus and exotic fruit aromatic notes prevail, while for cv. Crispifolia (C2 sample), the odor profile is more complex and linked to cooked fruit, pastry, vegetal, and slightly spicy notes.

Conclusion

Numerous studies on the Citrus genus have highlighted its great complexity. An interesting and recent review (Gonzales-Mas et al., 2019), on the volatile compounds identified in the most studied essential oils of 10 Citrus spp., highlights the differences and the similarities of Citrus aurantium L., compared to other species.

Over to the volatile compounds common to the genus Citrus, as monoterpenoid compounds, in CA, higher levels of linalyl acetate, geranyl acetate, eucalyptol, and sesquiterpene germacrene D were found.

Moreover, CA is also distinguished by the presence of some monoterpenes, hydrocarbons such as α-ocimene and oxygenated compounds such as α-terpinen-4-ol acetate exclusively described in this species.

Comparing the fragrances of the volatile compounds of the bitter orange aroma, as well as the GC/O data, reported by various authors (Essadik et al., 2015; Cano-Lamadrid et al., 2018; González-Mas et al., 2019), we find a strong correspondence with the results of our work, in the general description of the CA aroma, although all data available in the literature on this species do not specify the cv analyzed. From our data, it emerges instead that among the cultivars of bitter orange, there are notable aromatic differences.

Besides, as is well known, the aroma plays a key role in gastronomy, and our work can help to choose the cultivar, with the most suitable profile and aromatic intensity, for a specific recipe.

The awareness of the aromatic specificities of the different bitter orange cultivars would favor a more widespread use and therefore cultivation, and could, therefore, contribute to the conservation of an important part of agricultural biodiversity.

CRediT authorship contribution statement

Diana De Santis: Writing - original draft. **Maria Teresa Frangipane:** Writing - original draft.

Appendix A Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijgfs.2020.100203.

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The corrections made in this section will be reviewed and approved by journal production editor.

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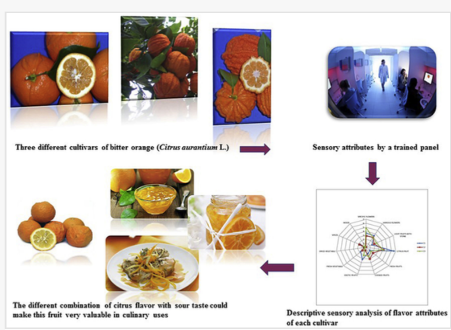
Graphical abstract

alt-text: Image 1



Appendix A Supplementary data

The following is the Supplementary data to this article:

Multimedia Component 1

Multimedia component 1

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