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Comparison of odour active compounds detected by gas-chromatography–olfactometry between hand-squeezed juices from different orange varieties

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Abstract

Twenty-two overall odour active compounds were detected by gas chromatography–olfactometry in hand-squeezed juices of Moro, Tarocco, Washington navel and Valencia late oranges. The blood juices (Moro and Tarocco) had an higher total odour intensity than the blond ones (Washington navel and Valencia late) and were characterized by 14 and 15 different odorants, while the blond ones by 12. Moreover, methyl butanoate and ethyl octanoate were the odorants perceived in both the blood juices but not in the blond ones. Linalool was perceived only in the blond juices even if with a low frequency of detection. The aroma profile of the orange juice samples shows the same distribution, of the descriptors grouped as fruity, citrus, herbaceous, spicy and floral, for the Moro and Tarocco juices, while the aroma profiles of Washington navel and Valencia late were very different when compared with the blood varieties and each other, but in accord to those reported in the literature.

Keywords: Orange juice aroma; Olfactometry; Blood orange juice; Odour compounds

1. Introduction

Orange juice is the most widespread juice produced and consumed in the world. The fresh orange juice flavour due to the complex combination of several odours components that include alcohols, aldehydes, esters, ketones and hydrocarbons has been extensively investigated (Nispero-Carriedo & Shaw, 1990; Moshonas & Shaw, 1989, 1994, 1995). Maccarone, Campisi, Fallico, Rapisarda, and Sgarlata (1998) investigated aroma compounds from 72 orange juice samples derived from the most widespread Italian cultivars, they found a different distribution of flavour constituents between the blond and the blood varieties. Blood orange juice is a peculiar Italian product different from the blond one for the colour due to the presence of anthocyanins (Chandler, 1958; Maccarone, Maccarrone, Perrini, & Rapisarda, 1983; Maccarone, Rapisarda, Fanella, Arena, & Mondello, 1998), for the highest content of ascorbic acid (Rapisarda & Intelisano, 1996), for the different distribution of hydroxycinnamic acids (Arena, Fallico, Fanella, Maccarone, & Rapisarda, 1998; Rapisarda, Carollo, Fallico, Tomaselli, & Maccarone, 1998) and lipids (Arena, Campisi, Fallico, & Maccarone, 1998), and for the highest total antioxidant activity (Arena, Fallico, & Maccarone, 2001).

The gas-chromatography–olfactometry (GC/O) is a technique that uses the human nose as detector to distinguish the single volatile compounds eluted from the capillary column of a GC, as odorant or non odorant. This technique can be used to detect odour compounds present in very small amounts with a concentration above the threshold. Several studies were conducted to investigate the orange juice flavour by GC/O technique:

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Hinterholzer and Schieberle (1998) identified the most odour active volatiles in fresh and hand-extracted juice of Valencia late by odour dilution techniques; Tønder, Petersen, Poll, and Olsen (1998) compared the GC odour profile of fresh and stored orange juices; Bazemore, Goodner, and Rouseff (1999) studied the effect of the heating on volatile compounds of orange juices; Buettner and Schieberle (2001) evaluated the differences of odorants between hand-squeezed juices derived from Valencia late and Navel; Rega, Fournier, and Guichard (2003) compared the sensory quality of SPME flavour extracts of orange juice obtained by two different fibers at various equilibrium and exposure times. All these researches were conducted on blond orange juices, no data exist on the blood ones.

In the present study the application of GC/O, by a frequency of detection method, allowed identification of the odour active compounds present in a hand-squeezed juices from Moro, Tarocco, Washington navel and Valencia late orange. This allowed the comparison of odour profile between the blood juices (Moro and Tarocco) and the blond ones (Washington navel and Valencia late).

2. Materials and methods

2.1. Orange juices

Each juice was prepared from organic fruits picked from 5 different plants of each variety (Moro, Tarocco, Washington navel and Valencia late) in ripening periods, in the Palazzelli experimental farm managed by Istituto Sperimentale per l'Agrumicoltura (Acireale, Italy). Orange fruits were washed, wiped and the juice was extracted using a domestic squeezer, taking care to preserve the peels intact.

2.2. SPME conditions

Volatiles from orange juice headspace were extracted according to Rega et al. (2003) (G31 extract) with little modifications. A SPME fiber 50/30 μm DVB/CAR/PDMS (Supelco) previously conditioned was used. SPME extraction was performed for GC/O and GC/MS analysis on 3 ml of stirred juice (40 °C) contained in 7 ml vial sealed with a PTFE-lined screw cap. The equilibrium time was 30 min and then the SPME fiber was exposed to the headspace of the sample for 5 min. Before each extraction the fiber was held at 250 °C for 5 min and then at room temperature for 2 min.

2.3. Gas-chromatography/olfactometry analysis

The analytes adsorbed onto the coating of SPME were desorbed at 250 °C for 5 min in the injector of the gas-chromatograph. A GC SHIMADZU GR-17 AAF equipped with a flame ionization detector (FID) and a sniffing port was used. The operating conditions were the following: Supelcowax 10 capillary column (Supelco, 30 m, 0.32 mm i.d., 0.5 μm film thickness); Helium used as carrier gas in constant flow with a linear velocity of 35 cm/s.; splitless; the oven temperature was at 70 °C for 7 min, from 70 to 220 °C at 4 °C/min, then held 20 min at 220 °C. The GC effluent was equally split (1:1) through a Y (VSOS–SGE–Alltech Italia s.r.l) between FID (280 °C) and sniffing port equipped with a humidified air make up (30 ml/min).

The olfactometric evaluations, performed by detection frequency method according to Charles et al. (2000), was carried out by a group of six judges (female, average age 26 years) selected among people already experienced in Gas-chromatography/olfactometry (GC/O). All were non-smokers with no history of olfactory dysfunction and were in good health during the experiments. The orange juices of each variety were analyzed 18 times: every judge repeated the analysis (30 min) of each variety of orange juice three times during the same day with a pause between each olfactometric session of at least 1 h.

2.4. Gas-chromatography–mass spectrometry analysis

A Gas-chromatography–mass spectrometry (GC/MS) SHIMADZU QP 5050 A was used to identify the volatile compounds. A capillary column having similar characteristic of GC/O analysis was used. The oven temperature was the same used for GC/O analysis. Injector was kept at 220 °C; Helium was used as carrier gas in constant flow mode with a linear velocity of 24.5 cm/s. The transfer line was kept at 200 °C. Mass spectra in the electronic impact mode were generated at 70 eV and they were collected from m/z 35 to 400. A solution of hydrocarbons (C_8–C_{26}) was injected daily in the same conditions to calculate the Linear Retention Index (LRI). The volatile identification was done on the basis of LRI and by the comparison of mass spectra with MS data of reference compound and by the odour note.

3. Results and discussion

Table 1 reports the area distribution, calculated on GC/FID data, of 22 odour active compounds present in orange juices of different varieties. Limonene was the predominant compounds in all orange juices and its percentage changes from about 97 to 90% in Washington navel and Tarocco, respectively. β-myrcene, methyl butanoate, α-pinene and ethyl hexanoate were the most representative among the compounds in all varieties, except in Tarocco. These four compounds, together with limonene, explain about 99% of total distribution. The distribution of odour active compounds in Tarocco orange juice was different: methyl butanoate

Table 1
Area distribution (% – GC/FID data) of odour active compounds present in the juices of different variety of oranges

No.	LRI	Compounds[a]	Moro	Tarocco	Washington navel	Valencia late
1	993	Methyl butanoate	1.528	3.783	0.578	0.850
2	995	n.i.[b]	–	0.136	n.d.[c]	n.d.[c]
3	1030	α-Pinene	0.669	0.282	0.242	0.442
4	1049	Ethyl butanoate	0.317	1.227	0.192	0.106
5	1063	Ethyl 2-methyl butanoate	0.018	0.047	0.009	0.002
6	1099	Hexanal	–	0.510	0.221	0.017
7	1118	β-Pinene	0.018	0.043	0.010	0.019
8	1153	Z-3-hexenal	n.d.[c]	0.044	–	n.d.[c]
9	1175	β-Myrcene	4.828	2.395	0.801	3.651
10	1235	Limonene	91.91	90.12	97.36	94.35
11	1246	Ethyl hexanoate	0.477	0.674	0.389	0.377
12	1319	α-Terpinolene/octanal	0.005	0.293	0.016	0.018
13	1366	Hexanol	0.023	0.206	0.040	0.009
14	1411	Nonanal	0.007	0.018	0.024	0.004
15	1448	Ethyl octanoate	0.032	0.049	0.012	0.037
16	1473	n.i.[b]	0.050	0.012	0.018	0.072
17	1501	Decanal	n.d.[c]	0.016	0.015	n.d.[c]
18	1560	Linalool	0.032	n.d.[c]	0.003	0.006
19	1640	No peak	–	–	–	–
20	1704	n.i.[b]	0.056	0.133	0.070	0.031
21	1867	n.i.[b]	0.026	0.013	–	0.011
22	1887	n.i.[b]	–	–	–	–

[a] Identified by comparing LRI, mass spectra and odour note.
[b] Not identified.
[c] Not detectable.

was the second component with the highest percentage between the four varieties, followed by β-myrcene, ethyl butanoate, ethyl hexanoate, α-terpinolene/octanal, α-pinene, hexanal and the unidentified compound n. 2. In Tarocco orange juice nine odour active compounds explain about 99% of total distribution.

The unidentified compounds n. 2 was absent in the Moro orange juices and not well separated from methyl butanoate in Washington navel and in Valencia late juices, thus it was impossible to integrate. Z-3-hexenal was identified in Tarocco, decanal was identified in Washington navel and Tarocco but the area of these peaks was not integrable in Valencia late and Moro orange juices; the linalool peak was not detectable in Tarocco orange juice.

Table 2 reports the frequency of detection of each odour active compounds perceived in orange juice samples. The best perceived odorants (frequency of detection ⩾14/18) for each variety were: in Moro ethyl butanoate (pineapple), ethyl 2-methyl butanoate (fruity), β-myrcene (balsamic), methyl butanoate (strawberry), an unidentified compound with retention time 14.3 (rose, smoky) (n. 19 – no peak by GC–FID or GC–MS but detected 15/18 times), α-pinene (pine), limonene (lemon, mint); in Tarocco ethyl butanoate, methyl butanoate, and ethyl hexanoate (orange); in Washington navel ethyl butanoate; in Valencia late ethyl butanoate and ethyl 2-methyl butanoate.

The unidentified compound n. 19 was perceived in all samples with the highest frequency of detection in the Moro juice, thus, this compound was present in very small amount and have correspondingly a low odour threshold.

The blood juice, Moro and Tarocco, were characterized by 14 and 15 different odorants, respectively, while the blond ones, Washington navel and Valencia late, by 12. Moreover blood varieties had an higher total odour intensity (146 Moro, 123 Tarocco) than the blond ones (82) indicating a more rich smell. The compounds perceived in both the blood juice varieties but not perceived in the blond ones were methyl butanoate and ethyl octanoate, while only linalool was perceived in both blond varieties (even if with only 7 total frequency of detection) but not in the blood varieties.

Tønder et al. (1998), studying the GC odour profiling of freshly and stored orange juice found two sesquiterpenes, octanal, acetic acid, ethyl butanoate, β-pinene, linalool/octanol, 2-pentanone, citral and limonene as the most odour active compounds in an orange juice made from a concentrate. Citral, linalool, vanillin, ethyl 2-methyl butanoate, ethyl butanoate and limonene and other not identified components were reported by Marin, Acree, Hotchkiss, and Nagy (1992) among the 15 most odour active compounds founds in a hand-squeezed orange juices.

The descriptors reported in Table 2 were grouped as fruity, spicy, herbaceous, citrus, floral and the corresponding frequency of detection were added to have the odour profile of orange juices of four varieties (Fig. 1). The fruity note formed the main odour note of the overall

Table 2
Frequency of detection of the odour active compounds detected in orange juices from different varieties

No.	Compounds	Descriptors	Variety[a]			
			M	T	W	V
1	Methyl butanoate	Strawberry	16	14	0	0
2	n.i.	Garlic	0	10	4	0
3	α-Pinene	Pine	14	11	10	9
4	Ethyl butanoate	Pineapple	18	15	15	16
5	Ethyl 2-methyl butanoate	Fruity	17	12	4	14
6	Hexanal	Cut grass	0	3	4	0
7	β-Pinene	Pungent	3	0	0	0
8	Z-3-hexenal	Grass	4	5	0	5
9	β-Myrcene	Balsamic	17	10	7	7
10	Limonene	Lemon, mint	14	6	10	10
11	Ethyl hexanoate	Orange, pungent	11	14	10	5
12	α-Terpinolene/octanal	Grass, woody	0	3	3	0
13	Hexanol	Grass, floral	0	0	0	3
14	Nonanal	Melon, floral	8	5	0	3
15	Ethyl octanoate	Fruity	3	6	0	0
16	n.i.	Orange, spicy	0	4	0	0
17	Decanal	Lemon, pungent	0	0	0	3
18	Linalool	Floral, spicy	0	0	4	3
19	No peak	Rose, smoky	15	5	6	4
20	n.i.	Orange, pungent	0	0	5	0
21	n.i.	Fresh, lemon	3	0	0	0
22	n.i.	Fruity, floral	3	0	0	0
	Total		146	123	82	82

[a] M, Moro; T, Tarocco; W, Washington navel; V, Valencia late.

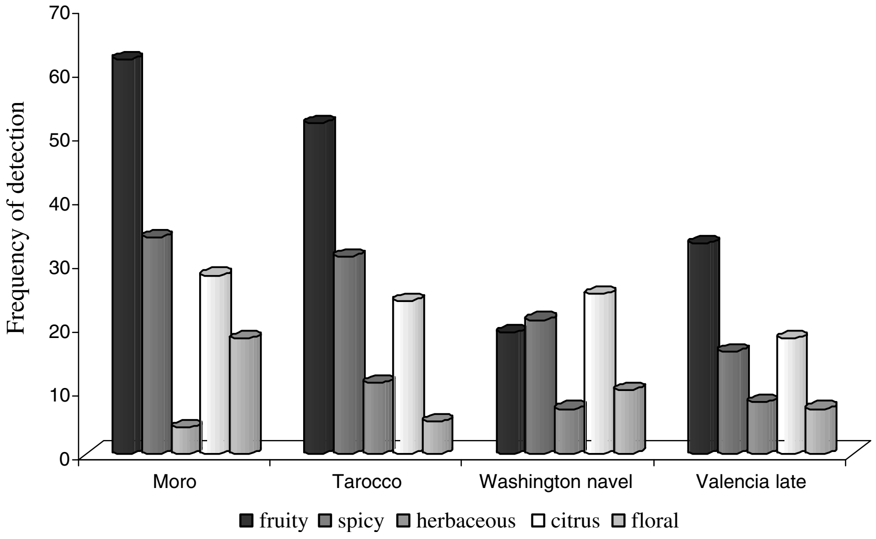

Fig. 1. Odour profile of hand-squeezed juices from Moro, Tarocco, Washington navel and Valencia late oranges.

aroma of Moro and Tarocco and Valencia late orange juices, even if the frequency of detection was higher in the blood juices compared to the Valencia late. Moro and Tarocco had a similar distribution of the descriptors characterized by the highest fruity odour followed by a spicy and citrusy odour perceived. The profile of Washington navel was completely different from the other juices, the citrus odour was the main perceived note followed by spicy and fruity. These differences in Washington navel profile were due to low odour contribute of ethyl 2-methyl butanoate (fruity) and to the odour contribute of the unidentified component n.20 (citrus) perceived only in this variety. Buettner and Schieberle (2001) found a flavour profile, by a sensory experiments, of a hand-squeezed juice of Valencia late and Navel similar to our GC/O profile of these varieties confirming our results. Low and similar in all orange juices samples was the contribution of floral and herbaceous note.

4. Conclusion

The GC/O analysis of juice from two blood and two blond orange varieties showed noteworthy differences. Richest and intensively perceived juices were the blood ones, where methyl butanoate was the major characterizing odour active compound. Moreover the aroma profile of blood juice from Moro and Tarocco oranges were similarly characterized by the high fruity odour note followed by a spicy and citrus odour. This results extend the knowledgements on blood orange juice and once again confirm previously works (Arena et al. 1998; Arena, Fallico, et al. 1998; Arena et al. 2001; Maccarone et al., 1983, 1998; Rapisarda and Intelisano, 1996; Rapisarda et al., 1998) that indicate: the blood orange juice is a different product respect to the blond one and not only for the colour.

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