Flavor Components of Italian Orange Juices

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Nineteen aromatic components were identified by GC/MS and quantified by GC-FID in 72 orange juices derived from the most widespread blond and blood cultivars grown in Itay. Naveline and Washington navel juices were characterized by a relatively high content of *trans*-2-hexenol; Valencia late was characterized by myrcene and Ovale calabrese by myrcene and linalool. Sanguinello and Moro juices were distinguished by valencene, whereas no single flavor differentiated Tarocco juices. Statistical treatment of the data by principal component and linear discriminant analyses pointed out the effectiveness of a reduced number of variables to discriminate most of the blond and blood juices, differentiating among their specific varieties. The most predictive flavors (myrcene and valencene among terpenes, hexenols and linalool among alcohols) are known to contribute powerful citrusy and fruity-green top notes, respectively.

Keywords: *Flavor components; orange juices; multivariate analysis*

INTRODUCTION

Orange juice is the most popular fruit beverage worldwide, and its great demand is a result of its nutritional and sensory properties. Its fresh and delicate aroma is due to a complex combination of several odorous components that have an interdependent quantitative relationship (Moshonas and Shaw, 1995). The main contributors are some liposoluble terpenes and terpenoids located in the flavedo sacs (peel oil), which move into the juice during extraction; other lipophilic or water soluble components are present in the vesicles of the endocarp (Nagy and Shaw, 1990).

Orange juice flavors have been extensively investigated. Radford et al. (1974) studied the distribution of volatile compounds between pulp and serum; Rouseff and Nagy (1987) correlated chemical and sensory data for identifying quality factors in Florida orange juice; Moshonas and Shaw (1989) monitored a gradual decrease in several flavors and an increase in undesiderable components during storage; Shaw et al. (1993) classified commercial orange juice types by pattern recognition of volatile constituents; Moshonas and Shaw (1994) determined 46 flavor components in fresh orange juices extracted from varieties growing in Florida and California; Baldwin et al. (1995) studied the effect of coatings on Valencia oranges by measuring the content of some aroma compounds during a prolonged stored of fruits. Moreover, Barbieri et al. (1996) investigated the volatile components of some Italian orange juices and pointed out that the ratio between terpenes (except limonene) and sesquiterpenes is significantly higher in Valencia juice than in blood juices.

Recently, we carried out research into the effects of thermal treatment on the constituents of processed blood orange juices, including flavor components (Maccarone et al., 1996) and hydroxycinnamic acids as off-flavor precursors (Fallico et al., 1996). The present study reports on the distribution of 19 aromatic components in 72 different juices derived from fruits of the most widespread blond and blood cultivars grown in Italy (Naveline, Washington navel, Valencia late, Ovale calabrese, Tarocco, Sanguinello, and Moro) and is aimed at characterizing blood and blond juices. Since 19 components were quantified by a single analytical determination, the multivariate analysis appears to be very suitable to identify the most predictive variables and to recognize intervarietal differences.

Table 1. Variety, Code, and Numbering of the Orange Juice Samples

no.	variety	color[a]	harvest period	code	cases	numbering
1	Naveline	B	Jan 1997	N1	1	1
		B	Feb 1997	N2	3	2–4
2	Washington navel	B	March 1997	W1	4	5–8
		B	April 1997	W2	5	9–13
3	Valencia late	B	March 1997	V1	4	14–17
		B	April 1997	V2	5	18–22
		B	May 1997	V3	4	23–26
4	Ovale calabrese	B	April 1997	O1	4	27–30
		B	May 1997	O2	3	31–33
5	Tarocco	R	Jan 1997	T1	5	34–38
		R	Feb 1997	T2	5	39–43
		R	March 1997	T3	4	44–47
6	Sanguinello	R	March 1997	S1	4	48–51
		R	May 1997	S2	5	52–56
7	Moro	R	Jan 1997	M1	2	57–58
		R	Feb 1997	M2	5	59–63
		R	March 1997	M3	4	64–67
		R	April 1997	M4	5	68–72

[a] B, blond juices; R, red juices.

MATERIALS AND METHODS

Each juice was prepared from 10 fruits systematically picked from 5 different plants of each variety in various ripening periods, from January to May 1997, in the Palazzelli

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S0021-8561(97)00949-7 CCC: $15.00
Published on Web 05/28/1998

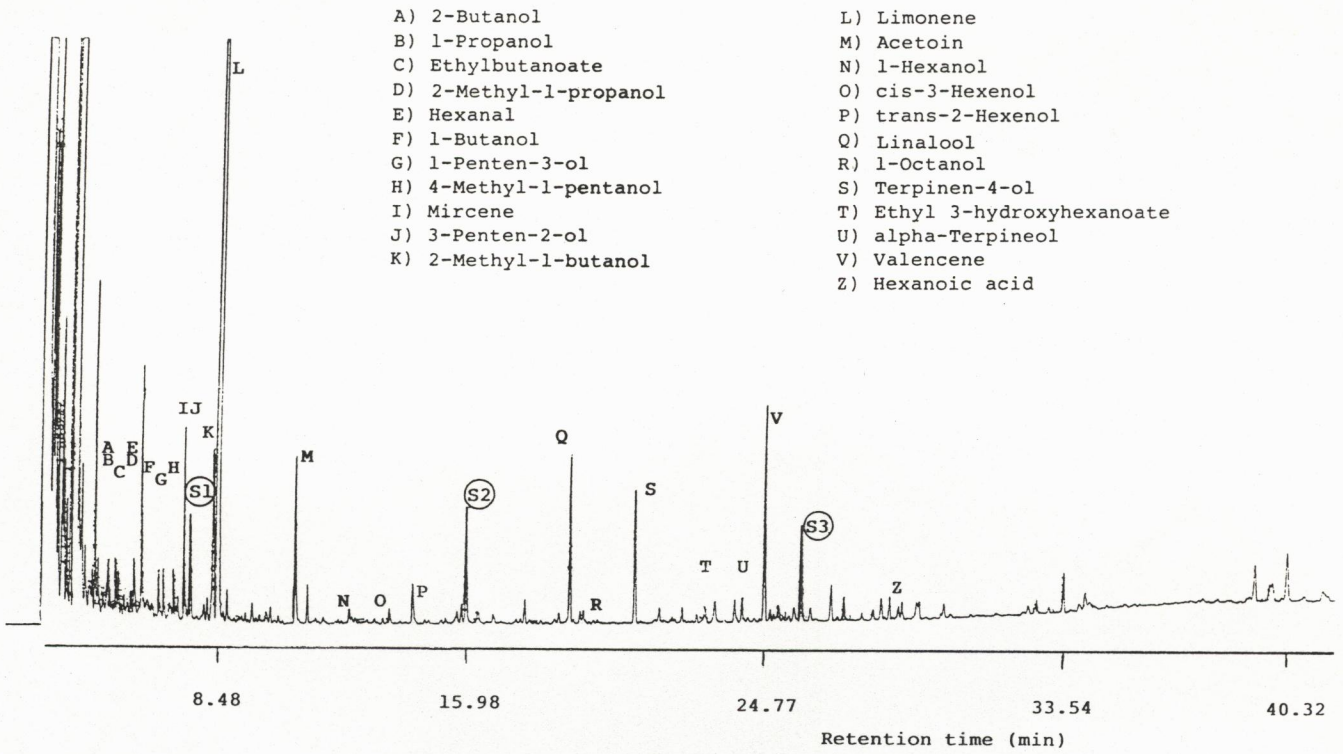

Figure 1. Gas chromatogram of aromatic components extracted from an orange juice.

experimental farm managed by Istituto Sperimentale per l'Agrumicoltura (Acireale, Italy). Table 1 reports codes and numbering of the 72 orange juices belonging to 4 blond and 3 blood varieties. The juices were extracted using a domestic squeezer, taking care to preserve the peels intact and to clean the squeezer.

Two milliliters of pentane/dichloromethane solution (2/1 in volume) containing three gas chromatographic standards (1-pentylethanoate, 12.02 mg/L; 1-heptanol, 11.80 mg/L; β-citronellol, 11.08 mg/L) was added as internal standard to 200 mL of juice. After addition of ~100 mL of pentane/dichloromethane solution, the aromatic components were extracted from the mixture by a continuous liquid–liquid apparatus for 20 h at 40 °C (Maccarone et al., 1996). The organic layer was then separated and concentrated under vacuum at 20 °C, and the volume was made up to 2 mL with pentane/dichloromethane solution.

The flavor components were separated and identified by GC/MS apparatus by comparing retention times and mass spectra with those of standards and then quantified by the internal standard method, using the FID response factors previously measured by standard flavors. Figure 1 shows a typical chromatogram of aromatic components extracted from an orange juice: peaks A–M were quantified by S1 (1-pentylethanoate), N–S by S2 (1-heptanol), and T–Z by S3 (β-citronellol). The peaks at retention times >30 min were also identified by mass spectra as heavy hydrocarbons or lipids. Blank experiments revealed that some peaks were artifacts of the extraction and concentration procedures. The GC/MS-FID system (Varian, model Saturn 3) was equipped with two identical columns connected to mass and FID detectors, respectively. The analysis conditions were the following: columns, Crompack WCOT CP Wax 52 CB (50 m × 0.25 mm, df = 0.2); temperatures, initial isotherm of 55 °C for 5 min, gradient of 3 °C/min from 55° to 220 °C, final isotherm of 220 °C for 5 min, injector at 230 °C, detector at 250 °C; carrier, helium at a head pressure of 22 psi (flow = 1.2 mL/min). Samples of 0.1 μL were injected. Concentrations of aromatic components were expressed in milligrams per liter referred to juice. The lowest detectable concentration was 0.004 mg/L. GC analysis of each extract was carried out in triplicate, and the coefficient of variation in quantitation of peaks does

Statistical comparison of mean concentrations was performed by ANOVA to reveal significant differences for single variable. Multivariate analyses (principal components and linear discriminant analyses) were applied using Statgraphics Plus software for Windows (Manugistic Inc., Rockville, MD). Linear discriminant analysis was applied according to two procedures: the former using all variables as predictors, the latter using the most important predictor variables by a stepwise selection algorithm.

RESULTS AND DISCUSSION

GC/MS analysis of the flavor components extracted from orange juices allowed identification of 23 compounds, and 19 of them were quantitatively determined by GC-FID (Figure 1): 3 terpenes (limonene, myrcene, and valencene), 3 terpenols (linalool, terpinen-4-ol, and α-terpineol), 6 saturated and 4 unsaturated aliphatic alcohols, 2 carbonylic compounds, and 1 carboxylic acid. The remaining 4 components (1-propanol, 2-butanol, ethylbutanoate, and ethyl 3-hydroxyhexanoate) were not quantified because the corresponding peaks either were not well separated from adjacent peaks or were present in traces or absent in some juices.

Table 2 reports the mean concentrations of the 19 components in the 72 orange juices grouped by variety. The content of some components increases with ripening: for example, valencene regularly increases from 0.004 mg/L in V1 to 0.056 mg/L in V3 in the Valencia late juices and from 0.20 mg/L in M1 to 0.69 mg/L in M4 in Moro juices. Mean concentration of all components is 15.74 mg/L, passing from a minimum value of 2.9 mg/L in Tarocco to 40 mg/L in Ovale calabrese. This large variation can be partly ascribed to different amounts of peel oil transferred into the juice during squeezing and partly to different amounts of peculiar components of the endocarp (juice oil and water soluble components). The absence of octanal, nonanal, decanal,

Table 2. Mean Concentrations of Flavor Components (Milligrams per Liter) in 72 Orange Juices of Different Varieties[a]

component	variety							mean
	Naveline	Washington navel	Valencia late	Ovale calabrese	Tarocco	Sanguinello	Moro	
(1) limonene	7.05 (3.76)^{ab}	10.08 (4.93)^{b}	17.27 (8.55)^{c}	38.38 (13.14)^{d}	1.9 (0.84)^{a}	14.4 (4.89)^{bc}	16.06 (6.24)^{c}	14.24 (11.68)
(2) myrcene	0.091 (0.074)^{abc}	0.098 (0.042)^{b}	0.20 (0.068)^{d}	0.38 (0.13)^{e}	0.024 (0.015)^{a}	0.14 (0.038)^{bc}	0.15 (0.055)^{c}	0.15 (0.112)
(3) valencene	0.072 (0.025)^{bc}	0.17 (0.082)^{c}	0.032 (0.035)^{a}	0.11 (0.11)^{bc}	0.023 (0.020)^{a}	0.53 (0.26)^{d}	0.43 (0.22)^{d}	0.21 (0.240)
(4) linalool	0.036 (0.028)^{ab}	0.061 (0.041)^{abc}	0.056 (0.020)^{ab}	0.29 (0.19)^{d}	0.010 (0.006)^{a}	0.071 (0.024)^{bc}	0.11 (0.055)^{c}	0.083 (0.100)
(5) terpinen-4-ol	0.080 (0.006)^{a}	0.090 (0.036)^{a}	0.14 (0.091)^{a}	0.11 (0.034)^{a}	0.12 (0.038)^{a}	0.25 (0.067)^{b}	0.22 (0.078)^{b}	0.16 (0.085)
(6) α-terpineol	0.011 (0.002)^{ab}	0.026 (0.014)^{bc}	0.010 (0.006)^{a}	0.012 (0.009)^{ab}	0.004 (0.000)^{a}	0.041 (0.032)^{d}	0.030 (0.012)^{cd}	0.019 (0.019)
(7) 1-butanol	0.058 (0.020)^{b}	0.047 (0.010)^{ab}	0.047 (0.008)^{ab}	0.038 (0.011)^{a}	0.047 (0.013)^{ab}	0.044 (0.008)^{ab}	0.054 (0.018)^{b}	0.048 (0.014)
(8) 2-methyl-1-propanol	0.038 (0.05)^{abc}	0.060 (0.024)^{c}	0.062 (0.047)^{c}	0.032 (0.024)^{ab}	0.043 (0.009)^{abc}	0.025 (0.014)^{a}	0.053 (0.029)^{bc}	0.047 (0.029)
(9) 2-methyl-1-butanol	0.33 (0.13)^{a}	0.29 (0.084)^{a}	0.37 (0.087)^{a}	0.40 (0.25)^{a}	0.31 (0.069)^{a}	0.34 (0.081)^{a}	0.35 (0.12)^{a}	0.34 (0.118)
(10) 4-methyl-2-pentanol	0.027 (0.019)^{a}	0.037 (0.005)^{abc}	0.044 (0.014)^{bc}	0.036 (0.012)^{abc}	0.030 (0.019)^{a}	0.045 (0.013)^{c}	0.033 (0.015)^{ab}	0.037 (0.015)
(11) 1-penten-3-ol	0.18 (0.088)^{c}	0.13 (0.032)ab	0.11 (0.032)^{ab}	0.086 (0.031)^{a}	0.13 (0.042)^{bc}	0.098 (0.038)^{ab}	0.13 (0.038)^{b}	0.12 (0.047)
(12) 3-penten-2-ol	0.010 (0.007)^{ab}	0.010 (0.004)^{ab}	0.011 (0.005)^{b}	0.10 (0.006)^{ab}	0.006 (0.004)^{a}	0.007 (0.004)^{ab}	0.007 (0.004)^{a}	0.009 (0.005)
(13) *trans*-2-hexenol	0.14 (0.076)^{b}	0.11 (0.071)^{b}	0.042 (0.009)^{a}	0.034 (0.018)^{a}	0.047 (0.017)^{a}	0.060 (0.018)^{a}	0.039 (0.010)^{a}	0.058 (0.043)
(14) *cis*-3-hexenol	0.071 (0.066)^{c}	0.055 (0.045)^{bc}	0.009 (0.005)^{a}	0.032 (0.025)^{ab}	0.020 (0.021)^{a}	0.015 (0.011)^{a}	0.023 (0.016)^{a}	0.026 (0.030)
(15) hexanol	0.021 (0.014)^{bc}	0.024 (0.025)^{c}	0.005 (0.001)^{a}	0.009 (0.006)^{ab}	0.005 (0.003)^{a}	0.010 (0.007)^{ab}	0.006 (0.003)^{a}	0.009 (0.012)
(16) octanol	0.012 (0.008)^{abcd}	0.026 (0.026)^{d}	0.008 (0.006)^{ab}	0.017 (0.019)^{bcd}	0.004 (0.000)^{a}	0.009 (0.006)^{abc}	0.020 (0.016)^{cd}	0.013 (0.015)
(17) acetoin	0.049 (0.018)^{ab}	0.063 (0.034)^{b}	0.074 (0.030)^{bc}	0.025 (0.009)^{a}	0.077 (0.055)^{bc}	0.068 (0.029)^{bc}	0.099 (0.039)^{c}	0.072 (0.041)
(18) hexanal	0.10 (0.038)^{c}	0.079 (0.027)^{bc}	0.086 (0.041)^{bc}	0.055 (0.031)^{ab}	0.091 (0.048)^{c}	0.044 (0.015)^{a}	0.068 (0.020)^{abc}	0.075 (0.037)
(19) hexanoic acid	0.035 (0.017)^{d}	0.025 (0.008)^{cd}	0.017 (0.006)^{ab}	0.016 (0.006)^{ab}	0.022 (0.008)^{bc}	0.025 (0.011)^{cd}	0.013 (0.006)^{a}	0.020 (0.010)
total concentration	8.23	11.48	18.59	40.07	2.91	16.22	17.90	15.74

[a] Standard deviation in parentheses. Means in the same row followed by a common letter are not significantly different ($P < 0.05$).

Figure 2. Plot of principal component weights. Key for numbers is that of Table 2.

processed orange juices, are exclusive constituents of peel oil. Nevertheless, a small amount of limonene, which acts as a carrier of myrcene, inevitably moves from peel to juice. Therefore, limonene is the predominant component, even if it is present in much smaller amount than in processed juices (Nisperos-Carriedo and Shaw, 1990; Moshonas and Shaw, 1994). The major components (except limonene) are 2-methyl-1-butanol (0.34 mg/L), valencene (0.21 mg/L), terpinen-4-ol (0.16 mg/L), myrcene (0,15 mg/L), 1-penten-3-ol (0.12 mg/L), linalool (0.083 mg/L), and hexanal (0.075 mg/L). These eight components form ~98% of flavors; in particular, 2-methyl-1-butanol predominates in blond varieties, whereas valencene prevails in the blood varieties Sanguinello and Moro. Moreover, the latter varieties are characterized by the same sequence of flavors, in the order valencene, 2-methyl-1-butanol, terpinen-4-ol, myrcene, 1-penten-3-ol, and linalool, whereas the distribution of flavors in Tarocco is almost similar to that of the blond varieties. The flavor distribution of oranges growing in Italy cannot be correctly compared with that of oranges growing elsewhere (Moshonas and Shaw, 1994) because extractions of the aromatic components were performed according to different methodologies.

Statistical comparison of the data in Table 2 indicates that some components could be used to differentiate varieties. In fact, concentrations of valencene in Sanguinello and Moro and of *trans*-2-hexenol in Naveline and Washington navel are significantly higher than in other varieties, whereas no single flavor differentiates Tarocco. The high content of valencene in Sanguinello in comparison with Valencia was also observed by Barbieri et al. (1996). Other variables do not seem to present significant intervarietal differences; in particular, the content of 2-methyl-1-butanol is practically constant. High concentrations of limonene and myrcene in Ovale calabrese and Valencia late are almost certainly not because of the variety but because of the relatively high peel oil level (high limonene values, also) in the samples. Myrcene is almost always found in orange peel oil and juice at a level of 1−2% of the limonene present (Di Giacomo and Mincione, 1994; Moshonas and Shaw, 1994), just as Table 2 reports. Therefore, these differences are related to the amount of peel oil introduced into the juice and not because of varietal differences.

Previous observations were confirmed by the principal component analysis that identified two components that cumulatively explain 37.5% of total variance (Figure 2). The first component (horizontal) includes hexenols and hexanol (compounds **13**−**15**) as the most significant

Table 3. Classification of Orange Juices by Linear Discriminant Analysis

	discriminant anal.:[a]	1		2							3				4		
	varieties:	blond	blood	1	2	3	4	5	6	7	1	2	3	4	5	6	7
	cases:	33	39	4	9	13	7	14	9	16	4	9	13	7	14	9	16
								Classification A[b]									
blond juices		30	3														
1, Naveline				4							4						
2, Washington navel					7			2				9					
3, Valencia late						13							13				
4, Ovale calabrese							7							7			
blood juices		2	37														
5, Tarocco								14							14		
6, Sanguinello									9							9	
7, Moro								1		15							16
% cases correctly classified for varieties		90.91	94.87	75	77.78	100	100	100	77.78	93.75	100	100	100	100	100	100	100
% total cases correctly classified		93.06					91.67						100			100	
								Classification B[c]									
predictor variables		myrcene, valencene, terpinen-4-ol, *trans*-2-hexenol, 3-penten-2-ol		myrcene, valencene, *trans*-2-hexenol, *cis*-3-hexenol							myrcene, valencene, linalool, *trans*-2-hexenol, α-terpineol				myrcene, linalool, hexanoic acid, hexanol		
% cases correctly classified for varieties		84.85	94.87	75	22.22	92.31	85.71	100	66.67	87.5	75	100	100	85.71	100	77.78	87.5
% total cases correctly classified		90.28					79.17						93.94			89.74	

[a] Discriminant analyses: (1) 72 juices separated in 2 groups (blond and blood varieties); (2) 72 juices separated in 7 groups (7 varieties); (3) 33 juices separated in 4 groups (blond varieties); (4) 39 juices separated in 3 groups (blood varieties). [b] Classification A: all flavors were used as predictor variables. [c] Classification B: the most predictive variables were selected by a stepwise procedure.

variables. These constituents are known to contribute powerful "fruity-green" top notes (Shaw, 1991). The second component (vertical) includes limonene and myrcene (compounds **1** and **2**), which are responsible for "citrusy" aroma (Shaw, 1991). The contributions of valencene and linalool (compounds **3** and **4**) are shared in both horizontal and vertical components.

The correlation coefficients between concentrations of the most important flavors point out that limonene and myrcene are linearly correlated in all juices ($R = 0.869$), whereas limonene and valencene appear to be strongly scattered ($R = 0.189$). These results can be explained if the origin of the components is considered: limonene and myrcene are both constituents of peel oil, whereas valencene originates prevalently from the lipidic fraction of endocarp (Shaw, 1991). A fair correlation is also observed between *trans*-2-hexenol and *cis*-3-hexenol, both originating in the aqueous fraction of endocarp ($R = 0.827$).

A multivariate pattern recognition of GC flavor profiles was then performed to differentiate blond and blood juices. The linear discriminant analysis was applied, and the results of classification are given in Table 3. In the first discriminant analysis all juices were separated in two groups corresponding to the blond and blood varieties. The classification using all flavors as predictor variables (classification A) was correct for 67 juices of 72 (93.06%). Only 3 blond and 2 blood juices were incorrectly classified. Stepwise selection (classification B) pointed out the most important variables (myrcene, valencene, terpinen-4-ol, and *trans*-2-hexenol). Only four components were sufficient to correctly classify 65 juices (90.28%). In the second discriminant analysis, the juices were separated in 7 groups corresponding to the varieties, and classification was correct for 66 juices (91.67%). Figure 3 shows the projection of samples on the space defined by the two more informative discriminant functions, which cumulatively explain 81.3% of total variance, and appear to be statistically robust (canonical correlations > 0.91; $P < 0.0000$); Ovale

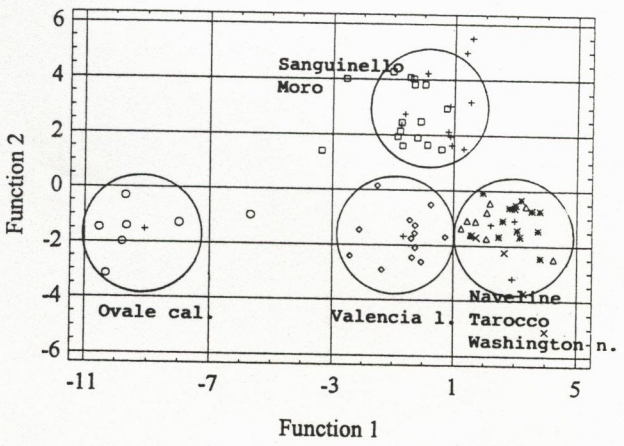

Figure 3. Plot of discriminant functions for blond and blood orange juices.

calabrese and Valencia late juices are clearly separated from those of other varieties, Sanguinello and Moro juices are located in a distinct region, wheras Naveline and Washington navel juices appear to be overlapped in the region occupied by Tarocco. Stepwise procedure selected five predictor variables (myrcene, valencene, *trans*-2-hexenol, *cis*-3-hexenol, and 3-penten-2-ol) that correctly classify 79.17% of juices. The blond and blood juices were considered separately by grouping them in their corresponding varieties (Table 3, discriminant analyses 3 and 4). The results of classification were satisfactory; in fact, both blond and blood varieties were completely differentiated (100%) using all flavors as predictor variables. Figures 4 and 5 show the two-dimensional representations of blood and blond varieties, respectively. In these cases high percentages of correct classification were also obtained on the basis of the most predictive flavors, that is, myrcene, valencene, linalool, *trans*-2-hexenol, and α-terpineol for blond juices and myrcene, linalool, hexanoic acid, and hexanol for blood juices.

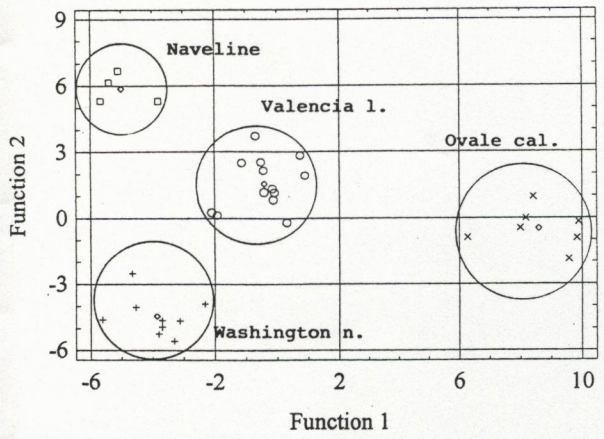

Figure 4. Plot of discriminant functions for blond orange juices.

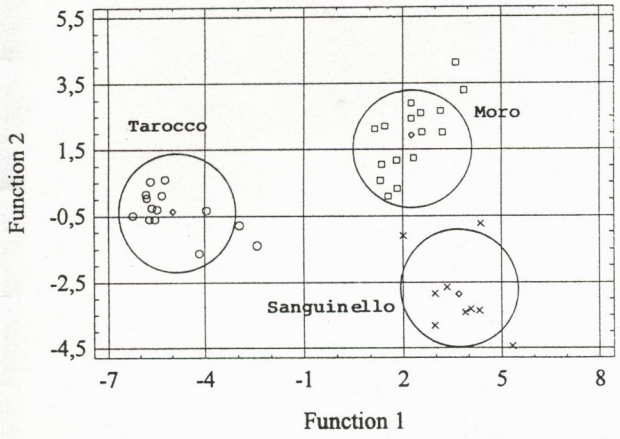

Figure 5. Plot of discriminant functions for blood orange juices.

Application of discriminant analysis using all variables as predictors was obviously more effective than stepwise selection in differentiating varieties; however, the latter procedure detected the most significant flavors, thus strongly reducing the analytical model in each case. The contributions of such components to the sensory differentiation between blond and blood juices cannot be assessed at present because the olfactory threshold values in juices are not known. Threshold values of several aromatic constituents of orange juices have been determined in aqueous solution (Ahmed et al., 1978a; Moshonas and Shaw, 1994), but these values probably are much lower than those predictable in juice because of interference with acids, sugars, and pectins (Ahmed et al., 1978b,c). Further studies are needed to verify whether differences in chemical composition correspond to real differences in sensory perceptions.

CONCLUSION

Distribution of flavor constituents in Italian blood orange juices is different from that in blond juices. Some constituents are also effective in discriminating among the specific varieties, notwithstanding the large variability of concentration depending on ripening periods and extraction technologies. Analogous differences have been already observed using multivariate pattern recognition of flavanone glycosides (Mouly et al., 1994) and hydroxycinnamic acids (Arena et al., 1997; Rapisarda et al., 1998). The variety-predictor flavors are few in comparison with the great number of volatile compounds: valencene among terpenes and *trans*-2-hexenol among alcohols clearly differentiate the blood varieties Sanguinello and Moro from blond Naveline and Washington navel. On the contrary, flavor distribution in the blood Tarocco appears to be similar to that of blond varieties. The multivariate pattern recognition of GC profiles of flavors could be used to differentiate blood from blond varieties.

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Received for review November 7, 1997. Accepted April 3, 1998.

JF970949D