Rheological Properties and Sugar Composition of Locust Bean Gum from Different Carob Varieties (Ceratonia siliqua L.)

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Hydroxycinnamic Acids as Markers of Italian Blood Orange Juices

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Extraction, resolution, and determination of the *trans*-hydroxycinnamic acids (caffeic, *p*-coumaric, ferulic, and sinapic) were performed in 82 orange juices derived from the most important blood and blond varieties grown in Italy. Soluble solids, acidity, and anthocyanins were also determined. Hydroxycinnamic acids were more abundant in blood orange than in blond juices. Ferulic acid was the major component in all cases, but the distribution of the four acids was typical in each variety. Discriminant analysis of the experimental results showed that these acids could be used as markers of blood and blond varieties. The statistical model was used to recognize some mixtures of blood and blond juices.

Keywords: *Discriminant analysis; hydroxycinnamic acids; orange juices; varietal markers*

INTRODUCTION

Citrus fruits contain esters and glycosides of *trans*-hydroxycinnamic acids (Risch and Herrmann, 1988; Fernandez de Simon et al., 1992) which are prevalently derivatives from ferulic acid (Wheaton and Stewart, 1965; Reshke and Herrmann, 1981; Risch et al., 1987). Peleg et al. (1991) determined the distribution of bound and free caffeic, *p*-coumaric, ferulic, and sinapic acids in the Shamuti orange fruit and confirmed ferulic acid predominance over the other hydroxycinnamic acids. Ferulic acid has been extensively studied as a precursor of *p*-vinylguaiacol, the most detrimental off-flavor that forms in orange juice during storage (Tatum et al., 1975; Naim et al., 1992, 1994). Ferulic and *p*-coumaric acids and the corresponding *p*-vinylphenols have been also determined in some processed Italian blood orange juices (Fallico et al., 1996). Recently, the role of hydroxycinnamic acid compounds as antioxidants and free radical scavengers has been pointed out (Chen and Ho, 1997).

In the past few years the European market demand for Italian blood orange juices has increased despite their higher cost. The increased consumer preference is due to the presence of anthocyanins, to the higher L-ascorbic acid content, and to a fresher acidulous taste (Sturiale, 1995). Less expensive blond juices may be added to highly colored blood juices to supply a product labeled as 100% blood orange juice. We report here the distribution of caffeic, *p*-coumaric, ferulic, and sinapic acids in 82 orange juices derived from the most important varieties grown in Italy (44 blood juices from Tarocco, Sanguinello, and Moro oranges and 38 blond juices from Naveline, Ovale calabrese, and Valencia late oranges) with the aim of characterizing blood and blond juices by multivariate pattern recognition. This statistical procedure was performed on several foods and beverages to discriminate their geographical origin and/or authenticity for inspection purposes. In particular, flavanone glycosides discriminated the different citrus fruit juices (Rouseff, 1988), while amino acids discriminated the juices of Spanish oranges (Aristoy et al., 1989) and grape, apple, and pineapple juices (Dizy et al., 1992). Flavanone glycosides also discriminated orange juices of different varieties (Mouly et al., 1994) and the ones adulterated by sour orange or grapefruit juices (Marini and Balestrieri, 1995).

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

variety (no. of juices)	harvest period	code	cases	numbering
Tarocco (10)	Feb 1995	T1	5	01−05
	March 1995	T2	5	06−10
Sanguinello (15)	Feb 1995	S1	5	11−15
	March 1995	S2	5	16−20
	April 1995	S3	5	21−25
Moro (19)	Jan 1995	M1	5	26−30
	Feb 1995	M2	5	31−35
	March 1995	M3	5	36−40
	April 1995	M4	4	41−44
Naveline (8)	Oct 1994	N1	5	45−49
	Nov 1994	N2	3	50−52
Ovale calabrese (15)	March 1995	O1	5	53−57
	April 1995	O2	5	58−62
	June 1995	O3	5	63−67
Valencia late (15)	March 1995	V1	5	68−72
	April 1995	V2	5	73−77
	June 1995	V3	5	78−82

MATERIALS AND METHODS

Preparation of Samples and Standards. Orange juices were obtained from fruits harvested at the Palazzelli experimental farm of the Istituto Sperimentale per l'Agrumicoltura (Acireale) in the territory of Lentini (Syracuse). The fruits of each cultivar were systematically sampled from five different plants in different harvest periods, from October 1994 to June 1995. Numbering and codification of the samples are reported in Table 1. The juices were prepared using a domestic squeezer, bottled in 500-mL bottles with a screw plug, and stored at −18 °C for analysis. No food preservatives were added. Soluble solids (°Brix) and total acidity (as anhydrous citric acid) were determined according to standard methods

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Table 2. Average Concentrations and Standard Deviations of Soluble Solids, Acidity, Anthocyanins, and *trans*-Hydroxycinnamic Acids in the Orange Juices of Different Varieties[a]

code[b]	soluble solids (g%)	acidity (g%)	anthocyanins (mg/L)	caffeic acid (mg/L)	*p*-coumaric acid (mg/L)	ferulic acid (mg/L)	sinapic acid (mg/L)	total hydroxycinnamic acids (mg/L)
T1	11.1 (0.7)	1.8 (0.1)	70.5 (21.5)	6.2 (1.3)	17.8 (2.5)	34.9 (3.0)	13.6 (2.4)	72.5 (9.2)
T2	11.6 (0.6)	1.9 (0.2)	80.0 (26.9)	7.9 (2.0)	28.5 (0.4)	41.8 (3.2)	16.7 (3.6)	94.9 (13.2)
mean	11.4 (0.7)cd	1.9 (0.2)c		7.1 (1.8)c	23.2 (6.6)b	38.3 (4.7)b	15.1 (3.3)cd	83.7 (16.4)
S1	11.6 (0.5)	1.9 (0.1)	57.0 (22.7)	8.4 (3.0)	26.2 (5.3)	46.3 (5.7)	17.3 (3.1)	98.2 (17.1)
S2	11.7 (0.2)	1.7 (0.0)	51.5 (10.5)	7.4 (1.5)	22.8 (5.0)	43.0 (5.1)	15.7 (2.3)	88.9 (13.9)
S3	12.0 (0.4)	1.4 (0.1)	49.4 (21.8)	6.7 (1.5)	25.8 (3.3)	43.4 (5.2)	17.5 (2.6)	93.4 (12.6)
mean	11.8 (0.4)d	1.7 (0.2)c		7.5 (2.1)c	25.0 (4.5)b	44.2 (5.2)c	16.9 (2.6)d	93.6 (14.4)
M1	10.7 (0.4)	2.2 (0.2)	146.9 (46.0)	6.9 (1.2)	30.1 (5.1)	36.0 (3.0)	11.8 (2.9)	84.8 (12.2)
M2	11.5 (0.3)	1.7 (0.2)	296.7 (49.9)	12.7 (1.1)	40.9 (7.8)	47.7 (9.5)	18.7 (2.1)	120.0 (20.5)
M3	11.0 (0.2)	1.4 (0.1)	313.9 (46.7)	15.1 (2.7)	40.7 (5.8)	51.7 (9.8)	22.2 (5.5)	129.7 (23.8)
M4	11.4 (0.5)	1.3 (0.1)	253.6 (52.0)	14.6 (1.8)	44.6 (9.2)	63.7 (10.8)	35.9 (6.6)	158.8 (28.4)
mean	11.2 (0.5)bc	1.7 (0.4)c		12.2 (3.8)d	38.8 (8.5)c	49.0 (12.6)d	21.4 (9.6)e	121.5 (34.5)
N1	10.8 (0.7)	1.9 (0.3)		4.9 (0.5)	10.8 (0.9)	38.3 (2.5)	9.1 (0.5)	63.1 (4.4)
N2	12.1 (0.2)	1.3 (0.1)		5.2 (0.5)	14.4 (2.3)	37.9 (3.2)	12.0 (1.5)	69.5 (7.5)
mean	11.3 (0.9)bc	1.7 (0.4)c		5.1 (0.5)b	12.1 (2.3)a	38.2 (2.6)b	10.2 (1.8)ab	65.5 (7.1)
O1	9.7 (0.5)	1.1 (0.1)		3.7 (0.3)	28.9 (5.3)	30.7 (2.1)	11.0 (1.5)	74.3 (9.2)
O2	9.6 (0.5)	0.9 (0.1)		3.6 (1.1)	27.2 (5.8)	32.0 (3.5)	13.1 (1.3)	75.9 (11.7)
O3	9.6 (0.1)	0.9 (0.0)		2.4 (0.6)	16.5 (4.5)	30.7 (1.5)	15.5 (0.6)	65.1 (7.2)
mean	9.6 (0.4)a	1.0 (0.2)a		3.2 (0.9)ab	24.2 (7.5)b	31.2 (2.4)a	13.2 (2.2)bc	71.8 (12.3)
V1	8.8 (0.5)	1.9 (0.2)		2.0 (0.2)	8.0 (2.2)	40.0 (4.8)	7.8 (23.)	57.8 (9.5)
V2	10.2 (0.2)	1.4 (0.1)		2.1 (0.3)	8.2 (1.4)	39.5 (1.0)	9.5 (1.1)	59.3 (3.8)
V3	9.9 (0.5)	1.1 (0.1)		2.1 (0.4)	7.9 (1.8)	33.5 (2.9)	9.6 (2.1)	53.1 (7.2)
mean	9.7 (0.7)a	1.5 (0.2)b		2.1 (0.3)a	8.0 (1.7)a	37.7 (4.3)b	9.0 (2.0)a	56.7 (8.3)

[a] Means in the same column followed by a common letter are not significantly different ($P < 0.05$). [b] See Table 1.

(Tateo, 1969). Anthocyanins in the blood orange juices were determined according to the method of Rapisarda et al. (1994) and expressed as cyanidin 3-glucoside. The *trans*-hydroxycinnamic acids used as standards were analytical grade commercial products (Sigma-Aldrich, Milan, Italy). Solvents and water were of HPLC grade.

Extraction of Hydroxycinnamic Acids. A new extraction procedure was carried out. The juice was centrifuged at 5000 rpm for 20 min. Ten milliliters of clear juice was added to 10 mL of 2 N NaOH and stored at room temperature in the dark. Complete hydrolysis of the bound forms of hydroxycinnamic acids occurred in 4 h, as confirmed by longer experiments. The solution was then acidified with 2 N HCl to pH ~2 and passed through a Varian Mega Bond Elut C_{18}, 1000 mg, cartridge, previously conditioned with water (0.01% HCl). Hydroxycinnamic acids were adsorbed in the C_{18} solid matrix, washed with water, and then eluted with 1% HCl methanol. The alcoholic solution was evaporated under vacuum and the residue diluted with 5 mL of the HPLC mobile phase (see later). The reliability of the method was checked by some recovery trials of appropriate mixtures of standard hydroxycinnamic acids.

HPLC Determination. The hydroxycinnamic acids containing solution (20 μL) was injected into a liquid chromatograph (Waters model 600E) equipped with a W-484 UV detector, a W-746 Data Module Integrator, and a Hypersil ODS 5-μm column (25 cm, 4.6 mm i.d., Policonsult Scientifica, Milan, Italy). Chromatograms were recorded with a 300-nm UV light, and isocratic elution was performed with a solvent mixture of 18% tetrahydrofuran and 82% water−acetic acid (98−2) at a flow rate of 1 mL/min (Rouseff et al., 1992). The peak assignments were based on the retention times of the standard acids and the chromatograms in the mixture. The concentration of each acid was calculated from the experimental peak area by analytical interpolation in standard calibration lines. The results of the linear regressions (slope, standard deviation, correlation coefficient, significance level) were as follows: caffeic acid, 220243, 993, 0.9999, 0.001; *p*-coumaric acid, 394499, 2567, 0.9999, 0.001; ferulic acid, 233797, 2130, 0.9990, 0.001; sinapic acid, 99022, 186, 0.9999, 0.001. Extraction and HPLC analysis of hydroxycinnamic acids in the 82 juices were carried out in duplicate.

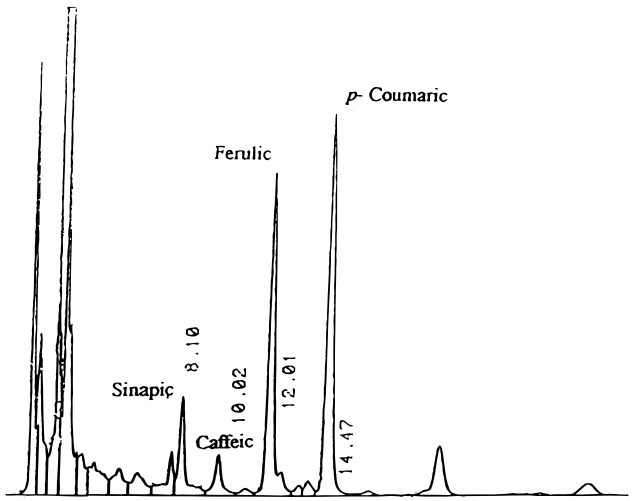

Figure 1. Typical HPLC of hydroxycinnamic acids extracted from an orange juice.

Statistics. Experimental data were processed by analysis of variance (ANOVA): specific differences in the mean values of variables were determined by least significant differences at a 95% confidence level. Multivariate analyses were performed by principal component and linear discriminant analysis, using the Statgraphic plus software for Windows (Manugistic Inc., Rockville, MD).

Table 3. Correlation Matrix of the Analytical Data of the Orange Juice Samples[a]

variable	acidity	caffeic acid	*p*-coumaric acid	ferulic acid	sinapic acid	total hydroxy-cinnamic acids	anthocyanins
soluble solids	0.3786**	0.6047**	0.4244**	0.5153**	0.4552**	0.5408**	0.3970**
acidity		0.2209*	0.0073	0.2174*	−0.0828	0.0876	0.1913
caffeic acid			0.8184**	0.7368**	0.7821**	0.9131**	0.8887**
p-coumaric acid				0.5580**	0.7605**	0.9002**	0.8089**
ferulic acid					0.7603**	0.8438**	0.6468**
sinapic acid						0.9144**	0.6901**
total hydroxycinnamic acids							0.8426**

[a] Significance level: *, 0.05; **, 0.01.

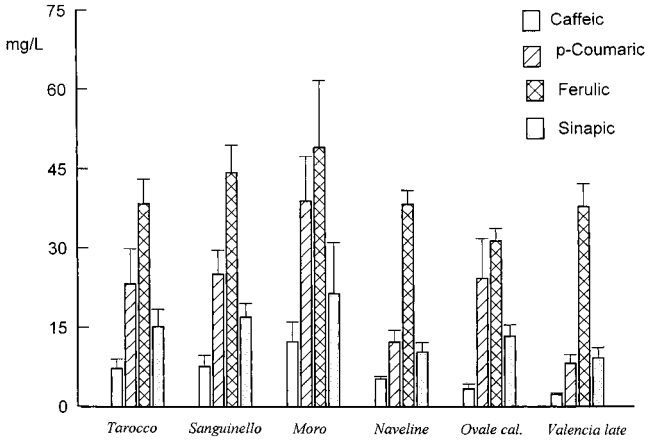

Figure 2. Mean concentrations of hydroxycinnamic acids, and standard deviations, in some varieties of Italian orange juices.

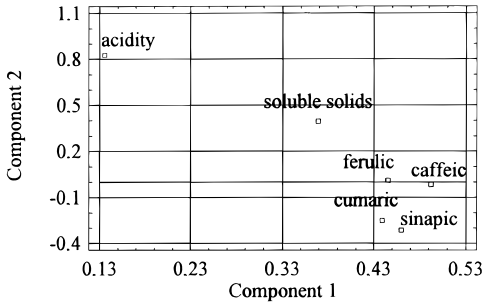

Figure 3. Plot of principal component weights.

RESULTS AND DISCUSSION

Figure 1 reports a typical HPLC analysis of the hydroxycinnamic acids extracted from a juice after alkaline hydrolysis and acidification. Peaks at 8.1, 10.0, 12.0, and 14.5 min correspond to sinapic, caffeic, ferulic, and *p*-coumaric acids, respectively. Table 2 reports the content of each hydroxycinnamic acid in the 82 univarietal juices summarized for each harvest period, together with the standard deviation. Ferulic acid is the major component in all cases, followed by *p*-coumaric, sinapic, and caffeic acid. Total concentration of the four hydroxycinnamic acids increases with ripening in Tarocco and Moro cultivars, but a similar trend is not evident in the others. The blood orange juices, especially those of the Moro cultivar, contain more hydroxycinnamic acids than the blond ones. Figure 2 shows the mean content of each acid. Cultivars Tarocco and Sanguinello have similar values. Calculation of the relative percentages pointed out some significant differences: Moro shows the lowest mean percentage of ferulic (40.5%) and the highest of caffeic acid (9.9%), whereas Valencia late shows the highest percentages of ferulic (66.3%) and the lowest of caffeic acid (3.7%). The percentage of sinapic acid is almost constant in all cultivars (16−19%).

Statistical comparison by Anova (Table 2) indicates that a single variable is not able to differentiate the six varieties; however, soluble solids discriminate cultivars Ovale and Valencia from other varieties, while acidity differentiates Ovale from Valencia. Moreover, hydroxycinnamic acids discriminate Moro from all varieties, caffeic distinguishes Tarocco and Sanguinello from the blond varieties, and ferulic differentiates Ovale and Sanguinello.

The ratio between the sum of the concentrations of ferulic and sinapic acids and that of caffeic and *p*-coumaric acids may be a simple parameter for differentiating blood and blond varieties. In fact, it assumes a mean value of 1.7 ± 0.4 for the blood juices, varying from 1.23 for M2 to 2.02 for T1 samples. The ratios of cultivars Naveline and Valencia late blond juices range from 2.54 for N2 to 4.75 for V2 samples, whereas Ovale calabrese juices show a ratio between 1.28 and 2.44, similar to those of the blood juices. However, the real concentration of caffeic acid in Ovale calabrese juices is significatively lower than in blood orange juices.

The correlation coefficients of linear regressions between all of the combinations of two experimental variables were calculated (Table 3). Soluble solids were fairly correlated with the other variables, whereas acidity showed poor correlations. Hydroxycinnamic acids were highly correlated with each other and also with anthocyanins. The latter correlations were expected because hydroxycinnamic acids are the precursors of the anthocyanins (Harborne, 1967; Heller and Forkmann, 1988). The great amount of these acids in the blood juices may be one of the pigmentation controlling factors. Moreover, the dominant aglycon in the anthocyanins of blood orange juices, i.e. cyanidin (Maccarone et al., 1983, 1985a), has the same hydroxylation pattern as caffeic acid; therefore, the increase in anthocyanin concentrations from Tarocco and Sanguinello to Moro may be related to the corresponding rise in caffeic acid concentrations. Blond juices do not contain anthocyanins and have only low caffeic acid content. Moreover, this acid behaves like a stabilizing compound of the orange juice anthocyanins (Maccarone et al., 1985b) by formation of hydrophobic complexes (Maccarone and Passerini, 1990; Maccarone et al., 1992). These findings explain the highly significant correlation between caffeic acid and anthocyanins ($r = 0.8887$, $P < 0.01$).

Principal component analysis identifies two components that cumulatively explain 80.81% of the total variance (Figure 3). In the first component (60.77% EV) the most important variables are caffeic and ferulic acids, while in the second component (20.04% EV) the highest weight is due to the acidity.

The hydroxycinnamic acid distribution patterns can be used to differentiate between blood and blond varieties by linear discriminant analysis. This statistical

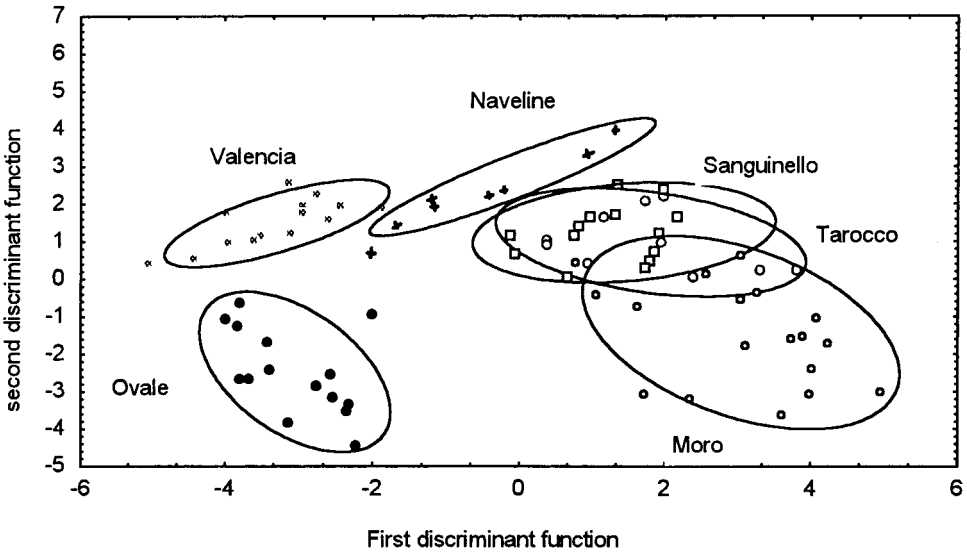

Figure 4. Plot of first two discriminant functions for 82 authentic univarietal orange juices.

Table 4. Discriminant Analysis: Statistical Results

DA	cases	groups	discriminant functions	eigenvalue	% variance	canonic correlation	Wilk's λ	χ^2	P
1	82	2	1	3.0383	100	0.8674	0.2476	107.4776	0.0000
2	82	6	1	7.2769	62.51	0.9377	0.01309	325.1798	0.0000
			2	3.4385	29.54	0.8802	0.1084	166.6691	0.0000
3	44	3	1	3.8182	94.61	0.8902	0.1704	68.1209	0.0000
			2	0.2177	5.39	0.4228	0.8212	7.5833	0.1807
4	38	3	1	12.1211	67.82	0.9611	0.0113	145.7254	0.0000
			2	5.7506	32.18	0.9230	0.1481	62.0631	0.0000
5	94	3	1	2.8477	95.48	0.8603	0.2290	130.4401	0.0000
			2	0.1348	4.52	0.3446	0.8812	11.1891	0.0478
6	94	7	1	6.2353	60.89	0.9283	0.01523	361.9339	0.0000
			2	2.9899	29.20	0.8656	0.1102	190.7524	0.0000

Table 5. Discriminant Analysis: Standardized Coefficients of Discriminant Function

DA	cases	DF	caffeic acid	*p*-coumaric acid	ferulic acid	sinapic acid	soluble solids	acidity
1	82	1	0.3772	0.3755	−0.4072	0.5199	0.4685	0.7890
2	82	1	0.7727	−0.0046	−0.3743	0.2955	0.5472	0.8456
		2	−0.0273	−1.0701	0.5880	−0.2082	0.5627	0.2061
3	44	1	−0.6399	−1.0337	−0.2277	0.5844	0.9446	−0.3301
		2	0.3831	−0.4660	−0.8927	1.0277	−0.3118	0.7753
4	38	1	0.5981	−1.2549	0.5534	−0.7603	0.5048	−0.0423
		2	1.1647	−0.2415	−0.7973	0.6258	0.0766	0.4481
5	94	1	0.3710	0.3912	−0.4336	0.5281	0.4479	0.7924
		2	−0.9336	0.5602	−0.2835	−0.0937	−0.0408	0.7927
6	94	1	0.7353	0.1767	−0.4878	0.3519	0.4210	0.7856
		2	0.0383	−0.9963	0.5123	−0.1545	0.5505	0.3234

technique identifies some linear functions of the original variables on which most information is retained, thus reducing the dimensions of the system. Analogies and/or differences can be recognized by plotting the samples onto the space of the first two most informative functions. The data set included the concentration of each hydroxycinnamic acid, soluble solids, and acidities of the 82 juices. Anthocyanins were not included since the color of the blood juices could be considered a misleading factor. Table 4 reports the statistical results concerning the robustness of discriminant analyses, while Tables 5 and 6 report the standardized coefficients of discriminant functions (DF) and the results of classifications, respectively.

In the first discriminant analysis the juices were separated in two groups: 44 blood (53.66%) and 38 blond (46.34%). The blood juices were clearly differentiated from the blond ones: 77 of 82 juices (93.9%) were correctly classified. The five outlier juices were one Sanguinello and four Naveline (juices 13, 47, 48, 49, and 52). Discriminant analysis by stepwise selection indicated that three variables (acidity, soluble solids, and *p*-coumaric acid) were the real predictors of blond and blood orange juices; in fact, 76 of 82 juices were correctly classified.

In the second discriminant analysis the juices were separated in six groups corresponding to the six varieties. The first discriminant function accounts for 62.51% of the total variance, while the second function accounts for 29.94% (Table 4). Overall, 72 juices were correctly classified (87.8%); in particular, three ungrouped Tarocco were classified as Sanguinello, and two ungrouped Moro were classified as Tarocco for the low content of hydroxycinnamic acids due to unripened fruits. Figure 4 shows the distribution of the 82 juices onto the space defined by the first two functions. Apart from some overlap in the regions of Tarocco, Sanguinello, and Moro, all six cultivars appeared to be well separated. Subsequently, the blond and blood juices were separately considered by grouping them in their specific varieties,

Table 6. Discriminant Analysis: Classification of Results

DA	1		2						3			4			5			6						
varieties	blood	blond	1	2	3	4	5	6	1	2	3	4	5	6	blood	blond	mixtures	1	2	3	4	5	6	mixtures
cases	44	38	10	15	19	8	15	15	10	15	19	8	15	15	44	38	12	10	15	19	8	15	15	12
blood juices	43	1													43	1								
1, Tarocco			7	3	–	–	–	–	6	4	–	–	–	–	–	–	–	7	3	–	–	–	–	–
2, Sanguinello			1	12	1	1	–	–	2	13	–	–	–	–	–	–	–	1	12	1	1	–	–	–
3, Moro			2	–	17	–	–	–	1	–	18	–	–	–	–	–	–	4	–	15	–	–	–	–
blond juices	4	34													4	34								
4, Naveline			–	1	–	6	1	–	–	–	–	8	–	–	–	–	–	–	1	–	6	1	–	–
5, Valencia late			–	–	–	–	15	–	–	–	–	–	15	–	–	–	–	–	–	–	–	15	–	–
6, Ovale calabrese			–	–	–	–	–	15	–	–	–	–	–	15	–	–	–	–	–	–	–	–	15	–
mixtures			–	–	–	–	–	–	–	–	–	–	–	–	2	3	7	–	2	–	–	–	–	10
% cases	97.7	89.5	70.0	80.0	89.5	75.0	100	100	60.0	86.7	94.7	100	100	100	97.7	89.5	58.3	70.0	80.0	78.9	75.0	100	100	83.3
% total	93.9		87.8						84.1			100			89.4			85.1						

Table 7. Calculated Data for 12 Mixtures of Univarietal Orange Juices

no.	mixture[a] (composition %)	soluble solids (g%)	acidity (g%)	caffeic acid (mg/L)	*p*-coumaric acid (mg/L)	ferulic acid (mg/L)	sinapic acid (mg/L)	ratio	anthocyanins (mg/L)
83	M4−V3 (50−50)	10.65	1.24	8.37	26.25	48.57	22.76	8.61	126.8
84	M3−N2 (50−50)	11.57	1.34	10.19	27.53	44.82	17.11	8.92	157.0
85	M3−N2−V3 (33.3−33.3−33.3)	11.01	1.27	7.50	20.98	41.04	14.61	8.57	104.6
86	M3−O2 (30−70)	10.30	1.13	9.37	33.93	41.86	17.65	9.51	157.0
87	M3−N2 (30−70)	11.79	1.32	8.21	22.28	42.06	15.08	8.92	94.2
88	M4−N2 (50−50)	11.77	1.32	9.92	29.51	50.79	23.97	8.94	126.8
89	M2−O3 (50−50)	10.56	1.28	7.56	28.70	39.21	17.12	8.99	148.3
90	M3−S3−O3 (33.3−33.3−33.3)	10.86	1.23	8.08	27.65	41.93	18.41	9.05	121.1
91	M3−O2−V3 (33.3−33.3−33.3)	10.17	1.13	6.96	25.25	39.07	14.92	9.14	104.6
92	M2−O3 (40−60)	10.37	1.19	6.52	26.26	37.51	16.80	9.43	118.7
93	M2−O3 (30−70)	10.18	1.11	5.49	23.83	35.81	16.47	9.87	89.0
94	M3−O3 (30−70)	10.02	1.02	6.21	23.76	37.00	17.50	10.22	94.2

[a] See Table 1.

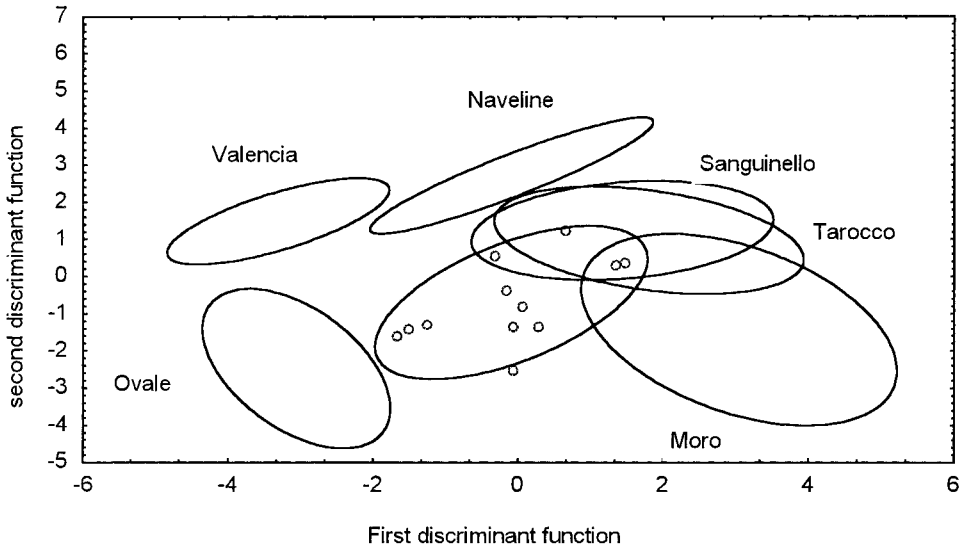

Figure 5. Plot of the two first discriminant functions for 12 simulated orange juices.

and classification was satisfactory (Table 6). In this case, the stepwise selection procedure indicates *p*-coumaric acid and soluble solids as the most predictive variables for the blood juices (81.8%), while the four hydroxycinnamic acids were able to correctly classify 100% of the blond juices.

Notwithstanding the limited data set (juices were obtained from fruits harvested from a single farm during one season), the statistical model was used to classify 12 computer-simulated mixtures made with some univarietal blood and blond juices (Table 7). The choices of the parent juices and relative proportions were made by taking into account that the soluble solids/acidity ratio and anthocyanin content should range within the limits of the commercially produced blood juices, i.e., ratio 8.5−10.5 and anthocyanins 80−150 mg/L. The data set of the mixtures (Table 7) was processed both as a third population of juices together with the blood and blond juices (DA 5) and as a seventh group in comparison with the six univarietal groups (DA 6). In the former analysis the 12 mixtures were classified blood (16.67%), blond (25%), and mixture (58.33%). Cumulatively, 83.33% of the simulated juices were not recognized as authentic red juices notwithstanding their being red in color. The two juices classified as blood were Moro−Naveline mixtures (no. 84 and 88). In the latter discriminant analysis (no. 6), the insertion of the 12 simulated juices in the varietal model confirms that mixtures prevalently do not belong to any variety of the statistical model. Most of the mixtures were projected outside the border regions of blond and blood varieties (Figure 5). Only two mixtures (no. 87 and 88), both arising from Moro and Naveline juices, were classified as Sanguinello, because it occurs in the region between Moro and Naveline. Consequently, if the mixtures are made with only blood juices, they should remain inside the "blood" area, around the centroids of Tarocco, Sanguinello, and Moro, whereas the mixtures of blood and blond juices fall out of the univarietal borders, around the lines joining the corresponding group centroids.

In conclusion, the results pointed out the effectiveness of hydroxycinnamic acids as varietal markers of Italian blood orange juices. However, the statistical model should be generalized. Other juices from fruits collected in different geographic areas over several years should be considered. In fact, the mean level of hydroxycinnamic acids and their interrelationships (e.g., due to various factors, such as pedoclimatic conditions and plant nutrition) may be different.

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