

Fatty Acids of Italian Blood Orange Juices

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This paper reports the composition of fatty acids in two types of Italian blood orange juices: one is a not-from-concentrate (NFC) juice and the other is a reconstituted-from-concentrate (RFC) juice. Both juices have been purposely processed from the same stock of blood oranges. Fatty acids have been separated and identified as methyl esters by GC/MS, and 11 of them have been determined by GC-FID. Four acids (linoleic, palmitic, linolenic, and vaccenic) constitute ~88% of total acids. Unsaturated acids predominate (77%) over saturated (23%). Mean concentration of fatty acids is ~680 mg/L in both juices. Separation and quantification of the lipid classes point out that neutral acylglycerols and polar phosphatides are much more abundant than glycolipids. Significant differences of fatty acid distribution have been found in the isolated fractions, but each class exhibits similar profiles both in NFC and in RFC juices, indicating that no selective degradation of any acid occurs during the process of thermal evaporation. Linear discriminant analysis of fatty acid concentrations, relative to 90 Italian orange juices taken from the literature, allows blood and blond juices to be differentiated and oleic, palmitoleic, and linolenic acids to be selected as the most predictive variables. Moreover, palmitoleic, palmitic, and linolenic acids differentiate the blood juices (Tarocco, Sanguinello, and Moro) and linoleic, oleic, and palmitoleic acids the blond juices (Naveline, Ovale, and Valencia).

Keywords: *Fatty acids; lipid fractions; orange juice; multivariate analysis*

INTRODUCTION

Notwithstanding the negligible amounts of lipids in orange juice (<0.1%), oxidation of unsaturated fatty acids released from lipids during thermal treatment and storage causes significant alteration of flavor due to formation of unpleasant aroma compounds having low sensory perception thresholds, such as aldehydes, ketones, and carboxylic acids with a low number of carbon atoms (Galliard, 1975). For this reason several studies concerning lipid components of orange juices have been carried out. Nordby and Nagy (1969) first identified fatty acids in various citrus juices, including those of Valencia oranges. Nagy and Nordby (1970) performed chromatographic separation of neutral and polar lipids of orange juice and studied the effect of storage temperature on the formation of free fatty acids arising from primary hydrolysis of phospholipids. The fatty acid profile from triglycerides (Nagy and Nordby, 1974) and sterols (Nordby and Nagy, 1974) has been proposed as the chemotaxonomic marker to determine parentage of citrus fruit hybrids. Nordby and Nagy (1979) examined the lipid classes of three Florida orange cultivars during maturation. Stack et al. (1986) determined simultaneously fatty acids and sterols in a commercial frozen concentrate and in the juices obtained from four orange varieties. Nicolosi Asmundo et al. (1987) quantified fatty acid composition in 90 juices belonging to 6 Italian blood and blond cultivars during ripening. Recently Maccarone et al. (1996) evaluated the effect of thermal treatment on several constituents of blood orange juices, including fatty acids.

Anthocyanins are the actual markers of the Italian blood orange juices, but other components showed

significant differences of distribution with respect to blond juices. Hydroxycinnamic acids allowed blood and blond orange varieties to be distinguished (Rapisarda et al., 1998) and commercial blood juices to be authenticated (Arena et al., 1998). Intervarietal differentiation was also pointed out by multivariate pattern recognition involving amino acids (Aristoy et al., 1989), flavanone glycosides (Mouly et al., 1994), and flavor constituents (Maccarone et al., 1998).

The present study is aimed at determining fatty acids in the different lipid classes of Italian blood orange juices (acylglycerols, glycolipids, and phospholipids). The effect of thermal treatment on orange juice has been investigated by measuring the distribution of fatty acids in a not-from-concentrate (NFC) juice at 12 °Brix and in a reconstituted at 12 °Brix from a concentrate at 55 °Brix (RFC), both juices being obtained with the same stock of blood oranges, purposely processed for the present research. The paper also reports evidence for differentiation of blood and blond orange varieties by fatty acids. Multivariate pattern recognition was applied to data of 90 juices obtained from the most widespread blood and blond oranges grown in Italy (Nicolosi Asmundo et al., 1987) to identify the variety–predictor fatty acids.

MATERIALS AND METHODS

Extraction of lipids from processed juices was carried out using the procedure of Nagy and Nordby (1970) with some modification. Orange juice (200 mL) containing ~10% pulp was placed in a separator funnel and extracted with 300 mL of a mixture of trichloromethane and methanol (2:1, v:v). The organic phase was separated and filtered upon glass wool, and the aqueous phase was extracted twice using two aliquots of the solvent mixture. After filtration, the three extracts were combined and evaporated under vacuum at 30 °C. Trichlo-

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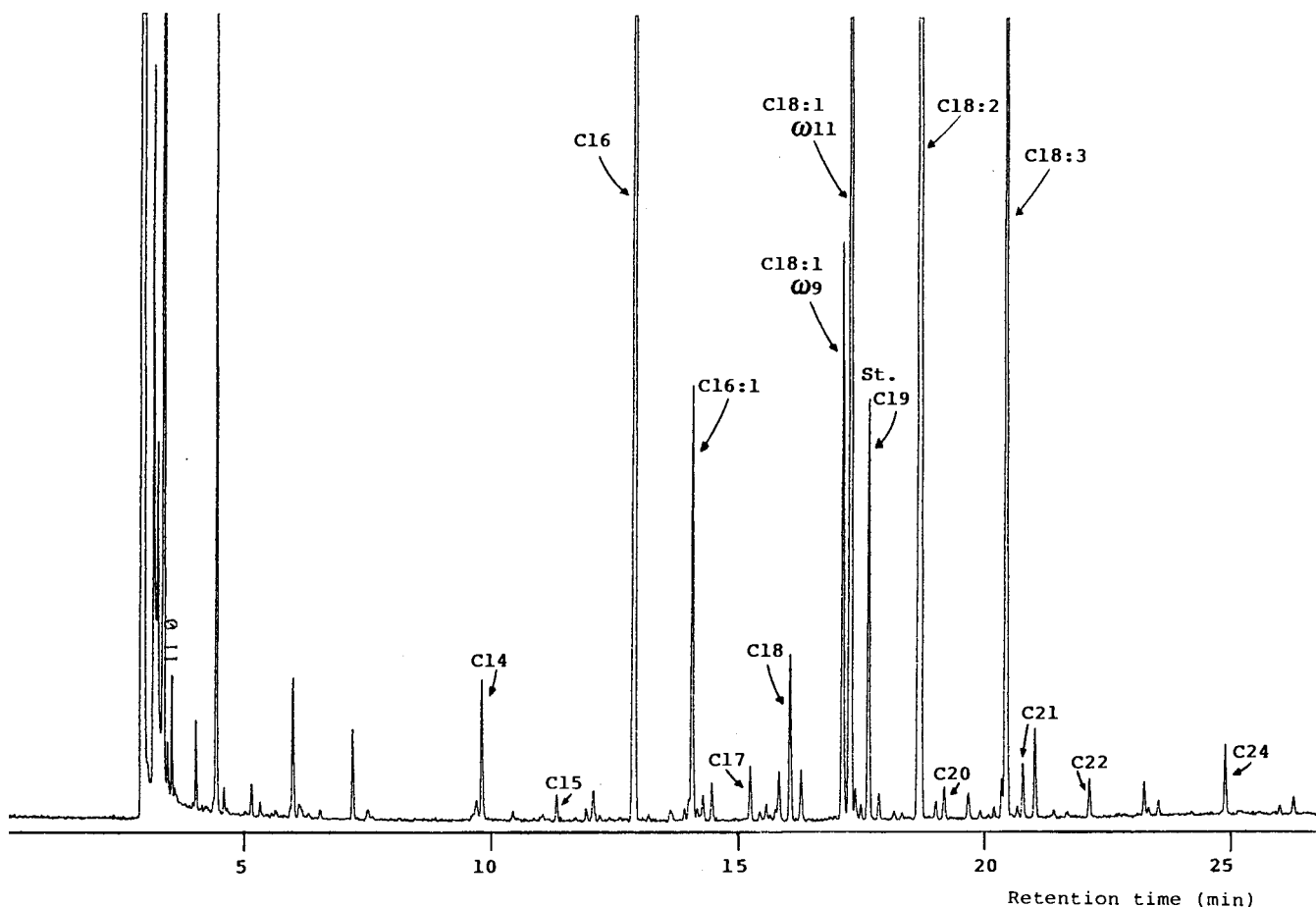


Figure 1. Gas chromatograms of fatty acid methyl esters of a blood orange juice.

romethane (50 mL) and water (50 mL) were added to the concentrated solution, and, after shaking in separator funnel, the organic phase was separated and dried on anhydrous sodium sulfate. The aqueous phase was in turn extracted twice with trichloromethane, and the organic layers were dried and combined with the first extract. Solution was completely evaporated and the residue dissolved in 10 mL of trichloromethane. An aliquot (5 mL) was *trans*-esterified and analyzed by GC. The remaining aliquot was placed into a chromatographic column (length = 50 cm; i.d. = 11 mm) containing silica gel (10 g) to perform fractionation of lipid classes. The column was previously conditioned by trichloromethane overnight. Neutral acylglycerols were first eluted using 250 mL of trichloromethane at a rate flow of 0.5 mL/min, glycolipids were eluted using 500 mL of propanone at a rate flow of 0.5 mL/min, and phospholipids were then eluted using 150 mL of methanol at a rate flow of 0.5 mL/min. The purity of each lipid fraction was checked by TLC on silica gel G following the procedure of Higgins and Peng (1976): acylglycerols were developed with trichloromethane and revealed by phosphomolybdic acid, glycolipids and phospholipids were developed with a mixture of trichloromethane/propanone/methanol/ethanoic acid/water (65:20:10:10:3) and revealed using naphthalen-1-ol and ninhydrin, respectively. *trans*-Esterification of fatty acids was carried out on the extracts evaporated to dryness at 30 °C by adding 7.5 mL of 1 M NaOH in methanol and boiling for 5 min. After addition of BF₃ in methanol (7.5 mL) and 1-heptanol solution (5 mL) containing a known quantity of nonadecanoic acid as internal standard, the solution was boiled for 5 min. After cooling, the fatty acid methyl esters in heptanol solution were analyzed. Identification of fatty acids was performed by GC/MS (Varian, Saturn 3) and quantification by GC-FID (Varian 3700). The analysis conditions were as follows: column, WCOT fused silica 10 (length = 50 m; i.d. = 0.25 mm) CPSIL 88; temperatures,

Table 1. Total Concentration of Fatty Acids (as Methyl Esters) in Processed Blood Orange Juices^a

fatty acid	NFC		RFC	
	mg/L	%	mg/L	%
myristic (C14)	7.74 (1.49)	1.09	8.19 (1.04)	1.25
palmitic (C16)	137 (21.8)	19.3	128 (19.5)	19.5
palmitoleic (C16:1)	31.0 (5.3)	4.38	29.4 (5.58)	4.49
stearic (C18)	9.59 (1.35)	1.35	10.0 (1.3)	1.53
oleic (C18:1 ω9)	28.7 (3.7)	4.05	27.4 (4.0)	4.18
vaccenic (C18:1 ω11)	116 (12.3)	16.4	107 (13.0)	16.3
linoleic (C18:2)	240 (23.4)	33.9	220 (28.1)	33.6
linolenic (C18:3)	131 (13.7)	18.5	118 (18.0)	18.0
arachidic (C20)	1.60 (0.24)	0.23	1.36 (0.23)	0.21
behenic (C22)	2.08 (0.34)	0.29	1.77 (0.50)	0.27
lignoceric (C24)	3.46 (0.37)	0.49	3.85 (1.80)	0.59
total	708 (84)	99.98	655 (93)	99.92

^a Mean values from four extractions separately worked up. Each extract was analyzed in triplicate. Standard deviation in parentheses.

initial isotherm of 140 °C for 3 min, gradient, 4 °C/min from 140 to 230 °C, final isotherm, 230 °C for 10 min; carrier, helium at a head pressure of 25 psi, flow = 1.3 mL/min, vent = 200 mL/min. Samples of 0.1 μL were injected. Concentrations of fatty acid methyl esters were calculated by the internal standard method, using FID response factors previously determined by standard methyl esters and expressed as milligrams per Liter referred to juice. Extractions were carried out in quadruplicate, and GC analysis of each extract was done in triplicate. The coefficient of variation in quantification of peaks does not exceed ±7%.

Statistical comparison of concentration data was performed by ANOVA to reveal significant differences for each fatty acid among varieties. Linear discriminant analysis was applied

Table 2. Concentrations and Percentages of Fatty Acids in the Isolated Lipid Fractions of Processed Blood Orange Juices^a

concn	acylglycerols		glycolipids		phospholipids		calcd total fatty acids	
	NFC	RFC	NFC	RFC	NFC	RFC	NFC	RFC
mg/L	391.7 (22.2)	324.4 (72.8)	64.6 (11.8)	54.7 (5.9)	297.2 (20.6)	295.7 (43.1)	753.5 (54.6)	674.8 (121.8)
%	52.0	48.1	8.6	8.1	39.4	43.8	100	100
percentages								
myristic	1.92	2.37	1.52	1.52	0.46	0.27	1.31	1.38
palmitic	13.1	12.4	18.4	19.3	25.2	24.2	18.3	18.1
palmitoleic	5.21	5.53	5.68	5.43	3.57	3.55	4.60	4.65
stearic	1.46	1.71	1.03	1.32	1.53	1.35	1.45	1.52
oleic	4.26	4.62	2.84	2.77	4.21	4.12	4.12	4.25
vaccenic	15.4	15.5	20.9	21.7	15.2	15.6	15.8	16.1
linoleic	36.3	34.9	20.1	19.0	35.2	35.9	34.5	34.1
linolenic	22.0	22.6	28.6	28.5	13.0	13.8	19.0	19.2
arachidic	0.17	0.14			0.34	0.25	0.22	0.18
behenic					0.48	0.47	0.19	0.21
lignoceric	0.20	0.25	1.01	0.43	0.77	0.52	0.49	0.38

^a Mean values from four extractions separately worked up. Each extract was analyzed in triplicate. Standard deviation in parentheses.

using Statgraphics Plus software for Windows by the stepwise selection procedure (Manugistic Inc., Rockville, MD).

RESULTS AND DISCUSSION

Fatty Acids of Processed Blood Orange Juices.

Figure 1 shows a typical gas chromatogram of fatty acids of a blood orange juice analyzed in the form of esters after *trans*-methylation of the lipid extract. Assignment of peaks was carried out by comparing retention times and mass spectra with those of standard methyl esters. Special attention was devoted to resolution and identification of the almost isochronous C18:1 isomers showing identical mass spectra: oleic (*cis*- ω -9), elaidinic (*trans*- ω -9), and vaccenic (*cis*- ω -11) acids were univocally recognized by gas chromatography of the lipid extract enriched with standards. Table 1 reports the concentrations and relative percentages of fatty acids of the NFC and RFC juices. The mean content from 12 analytical determinations is 708 mg/L in NFC juice and 655 mg/L in RFC juice, but standard deviations of the measurements indicate that such a difference is not significant. Therefore, the limits of precision of the analytical method do not permit the possible influence of the thermal treatment on the content of fatty acids in RFC juice. A partial acidic hydrolysis of glyceryl esters with formation of free fatty acids is highly probable (Nagy and Nordby, 1970), but it does not affect the total fatty acid content. The relative percentages of the single fatty acids are similar in both juices within the standard deviations. Eleven fatty acids have been quantified, and 4 of them (palmitic, vaccenic, linoleic, and linolenic) constitute ~88% of total acids. Unsaturated acids predominate (77%) over saturated (23%), the major component being linoleic (33%) followed by palmitic (19%) and linolenic acid (18%). Among the monounsaturated acids a noteworthy prevalence of vaccenic (16%) over palmitoleic (4%) and oleic (4%) is observed. Myristic, stearic, arachidic, behenic, and lignoceric acids altogether contribute <4%. Saturated C12, C15, C17, and C21 acids and unsaturated *trans*-stereoisomers are present in traces.

Quantification of fatty acids in the separated lipid fractions (Table 2) points out abundance of neutral acylglycerols (50%) and polar phosphatides (41%), whereas glycolipids contribute in minor amount (8%). The sum of fatty acid concentrations of each fraction strictly corresponds to total content previously deter-

Table 3. Values of the Unsaturated/Saturated Fatty Acid Ratio in the Isolated Lipid Fractions of Some Blond and Blood Orange Juices

juice	total	acyl-glycerols	glyco-lipids	phospho-lipids
blond juices ^a				
Hamlin	75/25	84/16	78/22	70/30
Pineapple	77/23	88/12	77/23	74/26
Valencia	77/23	89/11	78/22	74/26
commercial blood juices ^b				
NFC	77/23	83/17	78/22	71/29
RFC	76/24	83/17	77/23	73/27

^a Nagy and Nordby (1979). ^b This work.

mined. Recovery of lipid classes after chromatographic fractionation can be considered complete because the calculated total percentages of fatty acids (Table 2, last two columns) also correspond to those found in the nonfractionated extract. Lipid classes show the following characteristics: (i) linoleic acid is predominant in acylglycerols and phospholipids (~35%), whereas linolenic prevails in glycolipids (~28%); (ii) phospholipids are characterized by the largest content of palmitic (~25%) and the lowest content of linolenic (~13%); (iii) total concentration and distribution of fatty acids are similar in each lipid class for both NFC and RFC juices.

Although some authors (Nagy and Nordby, 1970; Vandercook et al., 1970) considered phospholipids as the most degradable lipids by oxidation during thermal treatments of orange juice, the quantitative profile of fatty acids of such fraction in RFC juice remained surprisingly the same as in the NFC juice. However, the presence of great amounts of antioxidants in the blood juices, such as L-ascorbic acid, anthocyanins, and hydroxycinnamic acids (Maccarone et al., 1996; Rapisarda et al., 1998), probably preserves polyunsaturated fatty acids from the autoxidation phenomena.

A comparison of these results with those of the literature points out several analogies together with some differences. Preliminarily, a remarkable variation in the content of fatty acids is observed: it ranges from about 650–700 mg/L in blood juices to 115–191 mg/L in American blond juices (Stack et al., 1986); intermediate levels from 200 to 450 mg/L are also reported (Nicolosi Asmundo et al., 1987; Maccarone et al., 1996). The differences can partly depend on the cultivar and on the stage of fruit maturity but mainly depend on the quantity of pulp present in the juice. In the present work lipid components were extracted from a juice

Table 4. Mean Values and Standard Deviations of Concentration of Fatty Acids (Milligrams per Liter) in Italian Orange Juices of Different Varieties^a

variety: juices: cases:	Tarocco 1–15 15	Sanguinello 16–31 16	Moro 32–50 19	Naveline 51–60 15	Ovale 66–75 10	Valencia 79–90 15	av
SS/A	9.45 (2.60) b,c	7.25 (1.13) a	6.51 (1.11) a	11.0 (3.8) c	8.01 (1.37) a,b	7.83 (2.00) a	8.26 (2.63)
palmitic	68.2 (5.8) c	63.7 (4.7) b	59.6 (5.0) a	55.7 (3.7) a	76.9 (7.0) d	58.2 (9.1) a	62.8 (8.7)
palmitoleic	16.0 (0.8) d	11.2 (0.8) a	11.8 (0.7) a	13.4 (2.0) b	14.8 (1.5) c	12.8 (2.1) b	13.2 (2.2)
oleic ^b	89.9 (15.2) c	75.2 (11.8) b	70.4 (10.3) b	68.5 (5.7) b	74.8 (7.9) b	54.6 (8.2) a	72.0 (14.6)
linoleic	131 (22) b,c	135 (27) c	128 (16) b,c	86.3 (14.3) a	171 (22) d	116 (28) b	125 (32)
linolenic	28.3 (7.6) a	40.9 (11.6) c	38.4 (12.6) c	35.9 (9.8) b,c	50.5 (5.9) d	32.0 (3.1) a,b	37.0 (11.2)
total fatty acids	333.1	326.4	308.2	259.8	392.0	273.2	310.5

^a Means in the same row followed by a different letter are significantly different at the 95% confidence level. ^b Oleic and vaccenic acids.

Table 5. Discriminating Fatty Acids among Orange Varieties

	Sanguinello	Moro	Naveline	Ovale	Valencia
Tarocco	palmitoleic	palmitoleic	palmitic linoleic	palmitic linoleic linolenic	oleic
Sanguinello			linoleic	palmitoleic linoleic linolenic	
Moro			linolenic	palmitoleic linolenic	
Naveline Ovale				linolenic	linolenic

containing 10% pulps. A clean juice, or a juice containing only a little pulp, necessarily yields minor amounts of fatty acids because lipid components are part of the solid particles of juice, being hydrophobic substances. Nevertheless, an effective comparison of blood and blond juices can be made using the relative percentages of fatty acids. Palmitic, palmitoleic, linoleic, and linolenic acids in Italian blood juices correspond to those of American blond juices apart from some quantitative difference for linoleic acid (Nagy and Nordby, 1979; Stack et al., 1986), whereas oleic acid is very different. The percentage of the latter acid probably includes the not separated C18:1 isomers (vaccenic and oleic acids). However, if vaccenic is added to oleic, both isomers amount to 20.5%, a percentage larger than 6–10% (Stack et al., 1986) and smaller than 29–33% (Nagy and Nordby, 1979) but similar to that found by Nicolosi Asmundo et al. (1987) for Italian orange juices.

The distribution of fatty acids in the isolated lipid fractions shows the same characteristics as in the blond juices (Nagy and Nordby, 1979): (i) palmitic acid increases, passing from acylglycerols to glycolipids and phospholipids; (ii) the lowest amounts of linoleic and linolenic acids are found in glycolipids and phospholipids, respectively. The analogies of fatty acid profiles in some blood and blond juices are summarized by the values of unsaturated/saturated acid ratio reported in Table 3.

Multivariate Pattern Recognition of Fatty Acids in Italian Orange Juices. The largest and most significant collection of data concerning fatty acids of Italian orange juices was reported by Nicolosi Asmundo et al. (1987). Preparation of samples, procedure of lipid extraction, and quantification of fatty acids were identical for 90 different juices obtained from the most widespread orange varieties: 50 blood, from Tarocco, Sanguinello, and Moro, and 40 blond, from Naveline, Ovale, and Valencia. These data appear to be very suitable to attempt differentiation between blood and blond varieties. Table 4 reports the mean concentrations of the most important fatty acids, together with

standard deviations; the soluble solids/acidity ratios (SS/A) of the orange juices are also reported as an index of maturity. The mean levels of each acid appear to be statistically different in some varieties. In fact, Tarocco is characterized by the highest content of oleic and palmitoleic acid, Naveline is distinguished from other varieties by the lowest level of linoleic acid, Ovale shows the highest concentrations of linoleic and linolenic acids, Valencia has the lowest content of oleic acid, and Sanguinello and Moro show comparable values for each fatty acid. Table 5 reports the distinctive fatty acids among varieties, obtained by one-way analysis of variance from the Box and Whisker plots. Tarocco and Ovale are clearly differentiated from all varieties, Naveline is undistinguishable only from Valencia, and no acid apparently discriminates among Sanguinello, Moro, and Valencia. The above characteristics appear to be promising for a varietal differentiation by multivariate pattern recognition.

Statistical treatment of data by linear discriminant analysis provided some linear combinations of the experimental variables, that is, discriminant functions, which have the form

$$C_1X_1 + C_2X_2 + C_3X_3 + \dots + C_nX_n$$

where $C_1, C_2, C_3, \dots, C_n$ were the standardized coefficients of the variables $X_1, X_2, X_3, \dots, X_n$. By plotting the values of the two more important discriminant functions for each juice in a two-dimensional space, it was possible to reveal analogies and differences among the juices. Moreover, the most variety–predictor variables were selected by applying the stepwise regression procedure by forward selection, using the method of successive approximations (Statgraphics Plus, 1995). Table 6 reports the statistical results concerning the robustness of discriminant analysis, and Table 7 reports the standardized coefficients of the most informative functions. From the relative magnitude of the coefficients it can be determined how the independent

Table 6. Linear Discriminant Analysis: Statistical Results

analysis	cases	groups	function	eigenvalue	variance %	canonical correl	Wilk's λ	χ^2	<i>P</i>
1	90	2	1	3.29	100	0.876	0.233	125.3	0.0000
2	90	6	1	10.1	58.3	0.954	0.0035	468.5	0.0000
			2	5.20	30.0	0.916	0.0394	268.5	0.0000
3	50	3	1	10.4	95.35	0.955	0.0578	129.7	0.0000
			2	0.51	4.65	0.581	0.6623	18.7	0.0003
4	40	3	1	14.8	85.4	0.968	0.0179	142.9	0.0000
			2	2.54	14.6	0.847	0.283	44.8	0.0000

Table 7. Linear Discriminant Analysis: *F*-to-Enter Value for Each Added Variable (*F*) and Standardized Coefficients of Discriminant Functions (*C*)^a

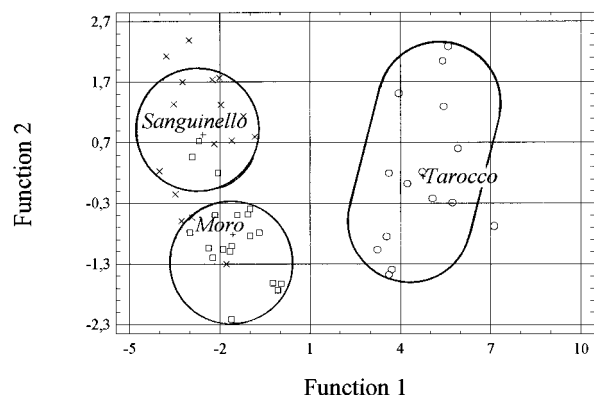
analysis	cases	groups	function	SS/A		C16		C16:1		C18:1		C18:2		C18:3	
				<i>F</i>	<i>C</i>	<i>F</i>	<i>C</i>	<i>F</i>	<i>C</i>	<i>F</i>	<i>C</i>	<i>F</i>	<i>C</i>	<i>F</i>	<i>C</i>
1	90	2	1	49.79	0.881			25.94	1.857	21.54	-2.365			58.01	1.239
2	90	6	1	32.67	0.678	6.53	0.0558	25.58	1.102	54.58	1.277	38.90	-1.622	13.06	-0.629
			2		1.26		-0.354		1.516		-2.386		1.186		0.635
3	50	3	1	4.05	0.539	7.39	-0.351	189.4	1.003					4.93	-0.422
			2		0.440		1.510		-0.625						-1.258
4	40	3	1			10.63	2.371	20.07	1.478	67.69	0.719	43.83	-4.526		
			2				1.443		-1.152		1.059		-0.687		

^a All coefficients have *P* < 0.05.

Table 8. Linear Discriminant Analysis: Classification of Results

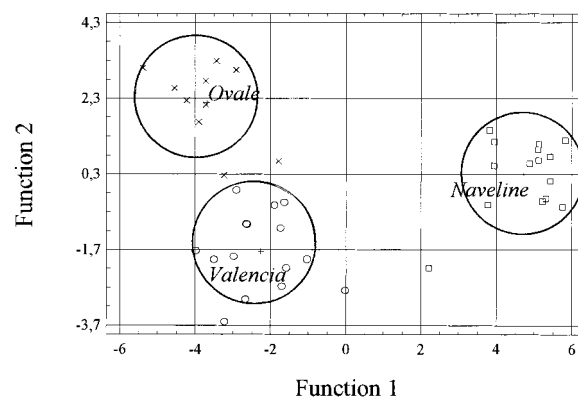
discriminant analysis	cases	groups	selected variables	cases correctly classified for variety ^a						total cases correctly classified		
				T	S	M	N	O	V	no.	%	
1	90	2	C18:1; C16:1; C18:3; SS/A		48			40			88	97.78
2	90	6	C16:1; C18:2; C18:1; SS/A; C18:3; C16	15	14	16	14	8	15		82	91.11
3	50	3	C16:1; C16; C18:3; SS/A	15	13	16					44	88.0
4	40	3	C18:2; C18:1; C16:1; C16				15	9	15		39	97.5

^a T, Tarocco; S, Sanguinello; M, Moro; N, Naveline; O, Ovale; V, Valencia.

**Figure 2.** Plot of discriminant functions for the 50 blood juices.

variables are being used to discriminate among the groups. Table 8 reports the results of classification of juices.

The first analysis concerns 90 juices separated in two groups on the basis of color, namely, blood and blond juices. The discriminant function selects four variables (C18:1, C16:1, C18:3, and SS/A) and correctly classifies 88 of 90 juices (97.78%). The concentrations of oleic (i.e., vaccenic and oleic acids), palmitoleic, and linolenic acids are the most important variables in differentiating blood and blond juices, because they have the highest standardized coefficients. The second analysis, which concerns 90 juices separated in 6 groups corresponding to the varieties, involves all variables to correctly classify 83 juices (91.11%), but a percentage of 88.89% is obtained using four selected acids (C16:1, C18:2, C18:1, and C18:3). In the third analysis the 50 blood juices

**Figure 3.** Plot of discriminant functions for the 40 blond juices.

are separated in 3 groups corresponding to Tarocco, Sanguinello, and Moro varieties, and in the fourth analysis the 40 blond juices are also separated in their corresponding groups (Naveline, Ovale, and Valencia). Results of the varietal differentiation are satisfactory in both cases. Figures 2 and 3 show blood and blond juices, respectively, on the space defined by values of the appropriate discriminant functions. Apart from some overlap for Sanguinello and Moro juices (Figure 2) the borders of each variety appear to be sufficiently definite. The most differentiating fatty acids are in the order palmitoleic, palmitic, and linolenic for blood juices and linoleic, oleic, and palmitoleic for blond juices.

The prediction capacity of the LDA procedure was estimated by performing a lot of discriminant analyses using the data set of a reduced number of juices, randomly selected for each variety, and calculating the

highest probability group for the remaining juices. In particular, we selected a model including 50% of juices (those denoted by odd number in Table 4) and added new rows to the current data file, filling in values for each of the independent variables of the juices denoted by even number but leaving the cell for variety blank. In all cases, the new juices (blood and blond varieties) were correctly classified in the actual groups with percentages >95%, thus confirming the robustness of the model.

Despite several analogies of fatty acid profiles in Italian blood and blond orange juices, the results of the LDA showed fatty acids to be very useful substances to discriminate between blood and blond juices and among their specific varieties. Hydroxycinnamic acids (Rapisarda et al., 1998) and flavor components (Maccarone et al., 1998) as fatty acids contribute to point out the specificity of Italian blood orange juices.

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