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Analytical Methods

Classification of Marsala wines according to their polyphenol, carbohydrate and heavy metal levels using canonical discriminant analysis

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ABSTRACT

Marsala is a popular Sicilian fortified aged wine with ancient tradition. Nowadays Marsala is exported all over the world and is considered one of the most important dessert wines. The aim of this study was to determine the concentration of carbohydrates, polyphenols and heavy metals in different types of Marsala wines and to achieve statistical classifications by stepwise forward canonical discriminant analysis (CDA). The obtained results provided evidence that different types of Marsala were correctly classified according to their phenolic and carbohydrate compositions. In particular, the residual sugars allowed a good discrimination among Marsalas having similar total sugar contents. CDA, performed using heavy metals as independent variables, showed that Superiore Ambra Secco and Vergine Marsalas were not discriminated, whereas a good separation among Fine Oro Dolce, Superiore Riserva and Fine Ambra Secco wines was obtained. Finally, an overall statistical model showed that the variables with the highest discriminant power were: tyrosol, caffeic acid, procyanidin B1, catechin, quercetin, kaempferol, lactose, rhamnose, zinc, copper and lead.

1. Introduction

Marsala wine has ancient origins, but only since 1773 has it been known all over the world, owing to the Englishman, John Woodhouse, who organized the first exportation of Marsala from Sicily to England. The English aristocracy appreciated its fruit-like taste (dry and even sweet), its amber-like and warm colour and its intense perfume. Nowadays, Marsala is exported all over the world and is considered one of the four most important dessert wines together with Madeira, sherry and Porto. It was the first Italian wine that received the CDO (controlled denomination of origin) recognition in 1969 (Italian Republic, 1969). It is exclusively produced in the province of Trapani (excluding the Egadi islands and the municipal district of Alcamo) and it is characterized by an average alcoholic content of around 18°. Marsala wine comes in three different colours: "Oro" (golden) and "Ambra" (amber) produced from the Grillo, Cataratto, Inzolia, Damaschino grapevine varieties, and "Rubino" (ruby) from Pingatello, Nerello Mascalese and Calabrese ones. All the vines used to produce Marsala wines, grow in the typical red Sicilian earth, particularly dry and sunny. Marsalas are also classified according to their contents of reducing sugars and age. The sweetest Marsalas are called "Dolce" (total sugars >100 g l^{-1}), followed by "Semi-secco" (total sugars from 40 to 100 g l^{-1}) and "Secco" (total sugars <40 g l^{-1}) which are the driest. Marsalas are matured in wooden barrels and ranked from the youngest to the oldest; the age grades are "Fine" (>1 year), "Superiore" (>2 years), "Superiore-Riserva" (>4 years), "Vergine" (>5 years) and "Stravecchio" (>10 years). During vinification, "Fine", "Superiore" and "Superiore-Riserva" Marsalas, are fortified with must, alcohol and wine (13% v.v. ethanol content), while "Vergine Soleras" Marsala is fortified only with alcohol and wine (Italian Republic, 1986).

The evaluation of "typical foods" has recently become one of the most important challenges for nutritionists and researchers in the field of food chemistry. Marsala wine is one of the most important typical Italian foods. Therefore, the characterization of macro and micro-constituent compositions of Marsala wines might be very interesting, from both enological and nutritional points of view (Di Stefano, 1985; Dugo et al., 2004). In a previous work (Dugo, La Pera, Pellicanò, Di Bella, & D'Imperio, 2005), we studied the influence of ageing period on the presence of inorganic anions and cations in different types of Marsala wines; the statistical elaboration of the results gave evidence that the age of the wine significantly influences the concentrations of inorganic elements in Marsala wines, which increased with prolonging of the maturation period.

The aim of the present study was to give further information about carbohydrates (rhamnose, xylose, fructose, glucose, saccharose, lactose and maltose), polyphenols (catechins, flavonoids,

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stilbenes, phenolic and cinnamic acids) and heavy metals (Cd, Cu, Pb and Zn) concentrations of different types of Marsala wines and to achieve their statistical classifications by stepwise forward canonical discriminant analysis (CDA) (Casavecchia, Magnisi, La Pera, Maisano, & Dugo, 2007).

2. Materials and methods

2.1. Samples

Sixteen samples of five different types of Marsala wines were studied; each sample was collected from a 25hl oak cask in a 750 ml dark glass bottle. Particularly, three samples of Marsala Superiore Ambra Secco, three of Marsala Fine Ambra Secco, four of Marsala Fine Oro Dolce, three of Marsala Superiore Riserva and three of Marsala Vergine Soleras were analysed. All the wines were produced from Inzolia variety in the Fici firm, in the C.D.O. zone of Marsala (Trapani, Sicily). The vines grew on a dry calcareous soil near the coast. In all the considered crop years (2000–2004), the grapes were harvested in the period 20 August–20 September; the newly cropped Vitis vinifera fruits were crushed, destemmed, and subjected to soft pressing in contact with the vinasses to achieve the extraction of the aromatic compounds. After the fermentation by selected yeasts, at controlled temperature (15 °C) in stainless-steel containers, all the wines, except Marsala Vergine, were spiked with cooked must, wine (13% alcoholic degree) and alcohol. Marsala Vergine wines were fortified only with wine and alcohol. After the fortifications, the wines were left to mature in oaken barrels. Three months after the end of the aging period, each sample was bottled and left to refine 2 months before uncorking and consuming. All the information concerning the studied samples is given in Table 1.

2.2. HPLC/MS analysis of polyphenols

The analysis of polyphenols was performed using a liquid chromatograph (Shimadzu, Milan, Italy) equipped with two LC10 AD pumps, an eluent mixing chamber, a manual injector with 20 μl loop (Rheodyne 7125), and a SPDM-10Avp diode array detector equipped with a semimicro-cell and operating at wavelengths between 200 and 600 nm. The system was coupled to a MS detector, Shimadzu 2010, equipped with an ESI interface. UV and MS data were acquired and processed using the operating system Windows NT 4.0 (La Torre, Saitta, Vilasi, Pellicanò, & Dugo, 2006). Phenolic compounds in Marsala samples were identified using a direct-injection chromatographic method already used for the determination of this class of compound in Sicilian red wines (La Torre et al., 2006). Compounds were separated on a 150 mm × 2.1 mm, 5 μm particle size, Supelco Discovery C18 column; a Supelco guard column packed with the same stationary phase was also used. The mobile phase for gradient elution was prepared in water, from pH 3, with formic acid (solvent A) and acetonitrile, pH 3, with formic acid (solvent B): 0.01–20.00 min, 5% B isocratic; 20.01–50.00 min, 5–40% B; 50.01–55.00 min, 40–95% B; 55.01–60.00 min, 95% B isocratic. The gradient was reduced to initial condition in another 5 min; 10 min of equilibration was required before the next injection. The flow-rate was 0.2 ml/min and the analyses were performed at 20 °C. The conditions of the MS detector, the quantitative analysis and peaks identification, are described in a previous paper (La Torre et al., 2006).

Table 1
Description of the studied Marsala wine samples produced in the C.D.O. zone of Marsala

Type	Production year	Alcohol (%)	Total sugars (g/l)	Acidity	pH
Fine Ambra Secco (FAS)	2004	18	40	3.7	3.5
Fine Oro Dolce (FOD)	2004	18	125	4.0	3.6
Superiore Ambra Secco (SAS)	2003	18	39	5.0	3.6
Superiore Riserva (SR)	2001	18	110	5.2	3.6
Vergine Soleras (V)	2000	19	10	3.9	3.6

2.3. HPLC-ELSD analysis of carbohydrates

Carbohydrate analysis was performed using a liquid chromatography system equipped with two pumps, 10 Avp, a vacuum degasser, a 20 μl manual injector and an ELSD LT detector (Shimadzu, Milan, Italy). N_2 generated from a Chrom-gas generator (Parker-Balston Corp., Haverhill, MA) was used as the carrier gas to transport the analyte substance from the drift tube into the detection chamber of the ELSD. The overall system operated under the control of the CLASS VP software package (Shimadzu, Milan, Italy) (La Pera, Di Bella, Magnisi, Lo Turco, & Dugo, 2007).

Sugars were separated on a Prevail Carbohydrate ES column (250 mm × 4.6 mm, 5 μm particle size) (Alltech Italia, Milan, Italy) packed with a rugged hydrophilic polymeric gel; a guard column of the same material was also used. The separation was achieved at ambient temperature. The mobile phase for isocratic elution was a mixture of water–acetonitrile (20:80, v/v) at a flow-rate of 1.0 ml/min; the total run time was 20 min. The temperature of the ELSD drift tube was 40 °C, the carrier gas pressure was 250 kPa at a flow-rate of 2.0 ml/min. Calibration was carried out using the external standard method by preparing four aqueous solutions of carbohydrates of different concentrations in the range of 30–2500 mg/l. The method was precise (3–6% expressed as relative standard deviation of nine measures), highly reproducible (2.4–5.7%, expressed as stability of the results on five consecutive days) and sensitive (detection limits lower than 36 μg/l were obtained) (La Pera et al., 2007).

2.4. Chronopotentiometric stripping analysis of heavy metals

The analysis of heavy metals was carried out using an Ion 3 stripping chronopotentiometric analyzer (Steroglass, Perugia, Italy) equipped with a three electrode cell: the working electrode was a glassy carbon one, coated with a thin mercury film; the reference electrode was an Ag/AgCl electrode (3 M KCl) and a platinum wire was used as the auxiliary electrode. Before each analysis, the carbon surface of the working electrode was covered with a Hg film, through electrolysis of a Hg(II) 1000 mg/l solution (20 ml), 1 M in HCl, using a potential of −950 mV for 1 min (plating procedure). For the analysis of Marsala wines, 0.3 ml of the sample was placed into the electrochemical cell, together with 10 ml of ultra pure water, 1.0 ml of 1.0 mg l^{-1} Hg(II) as chemical oxidant and 0.1 ml of 1.0 mg l^{-1} Ga(III). The analytical procedure for the chronopotentiometric metals analysis in the wine samples is described in previous papers (Dugo et al., 2005; La Pera & Dugo, 2005, chap. 5; La Pera, Dugo, La Torre, Vilasi, & Pellicanò, 2004).

2.5. Reagents

Acetonitrile and H_2O for HPLC were purchased from Carlo Erba (Milano, Italy). Formic acid, (−)-epicatechin, (+)-catechin, gallic acid, 3,4-dihydroxybenzoic acid (protocatechuic acid), 4-hydroxy-3-methoxybenzoic acid (vanillic acid), 4-hydroxy-3,5-dimethoxybenzoic acid (syringic acid), 3,4-dihydroxycinnamic acid (caffeic acid), 4-hydroxy-3-methoxycinnamic acid (ferulic acid), 4-hydroxycinnamic acid (*p*-coumaric acid), tyrosol (2-(4-hydroxyphenyl) ethylalcohol), and *trans*-resveratrol were purchased from Sigma-Aldrich (Milano, Italy). The other phenolic compounds,

procyanidin B1, procyanidin B2, ethylgallate, quercetin, isoquercitrin (quercetin-3-*O*-glucoside), kaempferol, kaempferol 3-*O*-glucoside, rhamnetin, isorhamnetin, isorhamnetin 3-*O*-glucoside, rutin (quercetin-3-*O*-rutinoside), myricetin and malvidin-3-glucoside, were obtained from Extrasynthese (Genay, France). Stock solutions of the individual standards were prepared by dissolving 100 mg of standard in aqueous formic acid (pH 3)/methanol (90:10). All the solutions were stored in the dark at −4 °C. The stock solution of *cis*-resveratrol was produced by UV irradiation of *trans*-resveratrol in methanol for 120 min at 366 nm (Trela & Waterhouse, 1996).

The standards of L-rhamnose, D-xylose, D-fructose, D-glucose, saccharose, D-lactose and D-mannose were furnished by Sigma-Aldrich (Milano, Italy). Stock solutions of each carbohydrate were prepared as an aqueous solution at a concentration of 2500 mg/l. The standard mixture of sugars was prepared using these stock solutions. All the solutions were stored in the dark at −4 °C. All the solutions were filtered through a 0.45 μm glass-microfiber GMF Whatman chromatographic filter (Aldrich, Milano, Italy) before HPLC analysis and mobile phase solvents were degassed before use. Ultra pure hydrochloric acid (34–37%), Hg(II) (1000 $\mu g\ ml^{-1}$, 1 M in hydrochloric acid) and of Cd(II), Cu(II), Pb(II) and Zn(II) (1000 $\mu g\ ml^{-1}$, 0.5 N in nitric acid) standard solutions were purchased from Panreac Quimica (Barcellona, Spain). By dilution with pure ultra water, solutions of 1.0 $\mu g\ ml^{-1}$ Cd(II), 2.5 $\mu g\ ml^{-1}$ Cu(II), 1.0 $\mu g\ ml^{-1}$ Pb(II), 2.5 $\mu g\ ml^{-1}$ Zn(II) were prepared.

For the simultaneous chronopotentiometric determination of cadmium, copper, lead and zinc, Marsala wines were acidified to pH 2 with 5 M hydrochloric acid, and filtered trough a carbon column to remove the electron-active organic compounds. The Supel-ENVI carbon column was purchased from Supelco (Bellefonte, PA, USA).

2.6. Statistical analysis

To ascertain the significance of difference in polyphenol, metal and sugar levels between different types of Marsala wines, the data were statistically evaluated by analysis of variance (ANOVA), grouping wine samples according to their typology: Marsala Vergine (V), Superiore Ambra Secco (SAS), Superiore Riserva (SR), Fine Oro Secco (FOS) and Fine Oro Dolce (FOD).

Stepwise forward canonical discriminant analysis (CDA) was separately performed on data expressing polyphenols, metals and minor sugar levels in wines (independent variables), in order to classify different types of Marsala (grouping variable): V, SAS, SR, FOS and FOD.

The statistical elaboration of the data was performed by two softwares: SPSS for Windows (version 12.0, 2003) and STATISTICA package for Windows (version 6.0, 2000).

3. Results and discussion

3.1. Polyphenol content

Polyphenols have become an intense focus of research interest because of their perceived health-beneficial effects, such as by anti-inflammatory, anti-atherogenic, anti-microbial and anti-carcinogenic (Bravo, 1998) effects. Polyphenols in grapes and wine have aroused much attention but Marsala wine has not been investigated intensively up to now.

Phenolic compounds belonging to different classes – phenolic acids (gallic acid, protocatechuic acid, vanillic acid, syringic acid, caffeic acid, ferulic acid, *p*-coumaric acid, and tyrosol) flavan-3-ols ((−)-epicatechin, (+)-catechin, procyanidins B1 and B2), flavonols (quercetin, isoquercitrin, kaempferol, kaempferol 3-*O*-glucoside, rhamnetin, isorhamnetin, isorhamnetin 3-*O*-glucoside, rutin, myricetin) and stilbenes (*cis*- and *trans*-resveratrol, *cis*- and *trans*-piceid) – were analyzed in five different types of Marsala wines. The obtained data provided evidence that, of all the studied polyphenolic substances, phenolic acid derivatives were the most abundant: they represented 76% of the total phenolic fraction in Marsala Vergine, 68% in Superiore Ambra Secco and Superiore Riserva, 64% in Fine Oro Secco and 50% in Fine Oro Dolce. Gallic acid and tyrosol were the most abundant phenolic acids in all the Marsala wines (Table 2), followed by protocatechuic acid, vanillic acid and caffeic acid. Flavan-3-ols represented approximately 25–30% of the polyphenolic fraction in Fine Ambra Secco, Fine Oro Dolce and Superiore Ambra Secco Marsalas, 21% in Superiore Riserva and 16% in Vergine Marsalas; up to 50% of the flavan-3-ols fraction consisted of dimeric procyanidins in Fine Ambra Secco, Superiore Ambra Secco and Vergine Marsalas. The monomers, (+)-catechin and (−)-epicatechin, were the major flavan-3-ols present in Fine Oro Dolce, Superiore Ambra Secco and Superiore Riserva wines, whereas procyanidin B1 was the most abundant dimeric flavanol in Fine Ambra Secco and Vergine Soleras Marsalas.

The concentrations of the studied flavonols were very low in all the wines: isoquercetrin was present in all of the studied wines in concentrations ranging from 1 to 3 mg/l except Marsala Vergine.

Among stilbenes, *trans*-resveratrol has recently received great attention due to its presence in red wine and its proposed protective effect against atherosclerosis, certain cancers and all the pathologies characterized by cellular oxidative stress (Bravo, 1998; Jang et al., 1997). The studied Marsala wines are produced from white grapes; therefore very low levels of stilbenes are expected to be found. *trans*-Resveratrol occurs only in Superiore Ambra Secco and Vergine Marsalas at concentrations lower than 0.3 mg/l. Trace levels of *cis*-resveratrol were found only in Marsala Vergine, whereas small amounts of *trans*- and *cis*-piceid were detected in Superiore Ambra Secco, Fine Ambra Secco, Fine Oro Dolce and Vergine Marsalas.

The ANOVA performed on data expressing polyphenol concentration, showed that, at the 0.05 level, six out of 26 studied phenols did not vary significantly in different types of Marsala: vanillic acid, isorhamnetin 3-*O*-glucoside, kaempferol-3-O glucoside, myricetin, *cis*-resveratrol and *cis*-piceid. The Partial Wilks' Lambda test indicated the variables that contributed most to the discriminant model: *p* coumaric acid, tyrosol, *trans*-resveratrol, ethyl gallate, catechin, syringic acid, procyanidin B1, kaempferol, ferulic acid, protocatechuic acid, quercetin; all the other variables were not considered in the discriminant model. To establish to which typology of Marsala a sample belongs, five classification functions in the form $L = b_1x_1 + b_2x_2 + \cdots + b_nx_n + c$ were created, where L is the latent variable formed by the discriminant function; the b's are discriminant coefficients (partial coefficients) that reflect the unique contribution of each polyphenol to the classification of Marsalas, the x's are discriminating variables, and c is a constant. A Scatterplot (Fig. 1) relative to two discriminant functions shows a good separation among different types of Marsala wines. Moreover, the classification matrix (Table 3) shows that 100% of total samples are correctly classified relating to the five functions.

3.2. Analysis of carbohydrates

The determination of the carbohydrates was done by high performance liquid chromatography (HPLC), using an evaporative light scattering detector (ELSD). The separation and quantification of L-rhamnose, D-xylose, D-fructose, D-glucose, saccharose, D-lactose and D-maltose was achieved within 20 mins without pre-treating the sample. The advantages of using HPLC coupled with a ELSD detector and the performances of this technique are widely discussed in a previous paper: detection limits lower than 0.04 g/l

Table 2
Concentrations of polyphenols (mg/l ± SD) in different types of Marsala wines

Standard	Superiore Ambra Secco ($n = 3$)	Fine Ambra Secco ($n = 3$)	Superiore Riserva ($n = 3$)	Vergine Soleras ($n = 3$)	Fine Oro Dolce ($n = 4$)
Gallic acid	29.1 ± 1.34	14.3 ± 0.64	26.7 ± 0.30	22.2 ± 0.28	10.9 ± 0.75
Protocatechuic acid	2.81 ± 0.10	3.13 ± 0.37	3.39 ± 0.05	5.02 ± 0.08	1.86 ± 0.14
Tyrosol	26.0 ± 2.28	30.7 ± 0.84	20.0 ± 0.70	35.6 ± 1.20	2.42 ± 1.69
Vanillic acid	6.25 ± 2.60	3.57 ± 0.40	5.27 ± 0.20	4.38 ± 0.15	5.99 ± 0.87
Syringic acid	1.60 ± 0.26	0.86 ± 0.33	0.26 ± 0.01	0.85 ± 0.02	0.71 ± 0.12
Caffeic acid	3.62 ± 0.03	2.56 ± 0.16	3.73 ± 0.12	1.99 ± 0.06	0.46 ± 0.20
Ferulic acid	0.19 ± 0.01	0.28 ± 0.06	0.17 ± 0.00	0.66 ± 0.01	0.08 ± 0.03
p-Coumaric acid	0.89 ± 0.01	0.16 ± 0.02	1.05 ± 0.02	0.19 ± 0.00	⩽0.03
Procyanidin B1	7.86 ± 0.84	12.34 ± 7.10	2.66 ± 0.05	7.06 ± 0.12	1.06 ± 0.21
Procyanidin B2	5.39 ± 1.40	3.59 ± 0.95	3.71 ± 0.05	3.61 ± 0.04	1.22 ± 0.14
(+)-Catechin	2.99 ± 1.00	5.87 ± 1.00	3.90 ± 0.03	2.20 ± 0.02	7.50 ± 0.56
(−)-Epicatechin	8.60 ± 0.39	4.26 ± 0.75	8.73 ± 0.06	1.99 ± 0.01	2.49 ± 0.66
Ethylgallate	4.71 ± 0.42	3.01 ± 0.60	6.10 ± 0.06	6.08 ± 0.07	3.58 ± 0.52
Rutin	⩽0.003	⩽0.003	0.69 ± 0.01	⩽0.003	0.99 ± 0.17
Isoquercitrin	1.08 ± 0.08	2.08 ± 0.06	2.45 ± 0.05	⩽0.003	3.09 ± 0.36
Isorhamnetin-3-*O*- glucoside	⩽0.002	⩽0.002	⩽0.002	⩽0.002	⩽0.002
Kaempferol-3-*O*- glucoside	⩽0.005	⩽0.005	⩽0.005	⩽0.005	⩽0.005
Myricetin	⩽0.09	⩽0.09	⩽0.09	⩽0.09	⩽0.09
Quercetin	0.95 ± 1.14	⩽0.003	⩽0.003	⩽0.003	2.19 ± 0.31
Kaempferol	0.13 ± 0.00	⩽0.002	⩽0.002	⩽0.002	0.13 ± 0.02
Isorhamnetin	0.10 ± 0.01	⩽0.002	⩽0.002	⩽0.002	0.08 ± 0.02
Rhamnetin	⩽0.002	⩽0.002	⩽0.002	⩽0.002	⩽0.002
trans-Resveratrol	0.28 ± 0.04	⩽0.001	⩽0.001	0.16 ± 0.01	0.05 ± 0.02
cis-Resveratrol	⩽0.004	⩽0.004	⩽0.004	0.04 ± 0.00	⩽0.004
trans-Piceid	0.28 ± 0.16	0.40 ± 0.02	–	0.66 ± 0.01	0.06 ± 0.01
cis-Piceid	0.03 ± 0.02	0.04 ± 0.01	–	0.05 ± 0.00	
Sum	86.72	44.85	102.29	88.87	92.76

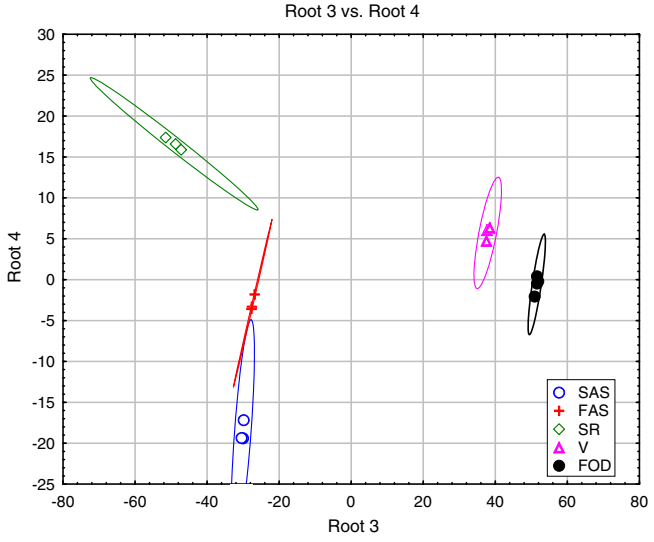

Fig. 1. 2D scatterplot of canonical scores resulting from applying the discriminant functions to the data expressing polyphenol levels in Marsala wines.

Table 3
Classification matrix for polyphenols

	%	SAS	FAS	SR	V	FOD
SAS	100	3	0	0	0	0
FAS	100	0	3	0	0	0
SR	100	0	0	3	0	0
V	100	0	0	0	3	0
FOD	100	0	0	0	0	4
Total	100	3	3	3	3	4

were obtained for all the studied sugars; intra-and inter-day precision were within 4%, expressed as RSD% (La Pera et al., 2007). The obtained results (Table 4) gave evidence that fructose and glucose were the most important sugars in all the studied wines. Residual sugars, including rhamnose, xylose, maltose and lactose, were detected in the studied wines at concentrations lower than 0.5 g/l, whereas saccharose levels were lower than the LOD in all the samples.

The ANOVA performed on data expressing carbohydrate concentrations, showed that, at the 0.05 level, all the studied compounds, except xylose and maltose varied significantly in different types of Marsala. The Partial Wilks' Lambda test indicated that the variables that contributed most to the discriminant model were lactose, fructose and glucose ($2 \leqslant F \leqslant 4$), followed by maltose and lactose ($1 \leqslant F \leqslant 2$), whereas xylose was the variable that contributed the least to the statistical model. To establish to which typology of Marsala a sample belongs according to its sugars content, five classification functions were created. Scatterplots (Fig. 2) relative to two discriminant functions show a good separation among different types of Marsala wines. Furthermore a classification matrix identical to that obtained for polyphenols (Table 3) confirmed that the classification functions allowed the correct classification of 100% of total samples. In particular, the contribution of residual sugars to the statistical model allowed a good discrimination among Marsalas having a similar total sugar content (SAS and FAS, SR and FOD).

3.3. Heavy metals content

The determination of cadmium, lead, copper and zinc in Marsala wines is of great interest from both enological and toxicological points of view; moreover, correct knowledge of these parameters is required by the law (European Community, 2001; Italian Republic, 1986). Table 5 shows the mean concentrations of heavy metals. The obtained results provide evidence that zinc was the most abundant metal, followed by copper and lead, whereas cadmium levels were lower than 3 ppb in all the studied wines. The analysis of variance and the Partial Wilks' Lambda test showed that zinc, copper and lead concentrations varied signifi-

Table 4
Sugar concentrations (g/l) in Marsala wines determined by HPLC-ELSD

	Rhamnose	Xylose	Fructose	Glucose	Saccharose	Lactose	Maltose
SAS	0.32 ± 0.02	0.24 ± 0.22	18.4 ± 1.40	19.4 ± 2.42	<0.007	0.036	0.28 ± 0.04
FAS	0.33 ± 0.05	0.40 ± 0.04	4.33 ± 0.15	4.07 ± 0.21	<0.007	0.26 ± 0.01	0.21 ± 0.06
SR	0.39 ± 0.04	<0.023	54.9 ± 2.20	55.1 ± 1.90	<0.007	0.036	0.26 ± 0.04
V	0.26 ± 0.03	0.42 ± 0.04	1.36 ± 0.02	1.27 ± 0.05	<0.007	0.036	0.35 ± 0.05
FOD	0.42 ± 0.05	<0.023	57.5 ± 6.38	67.5 ± 8.29	<0.007	0.12 ± 0.21	0.20 ± 0.07

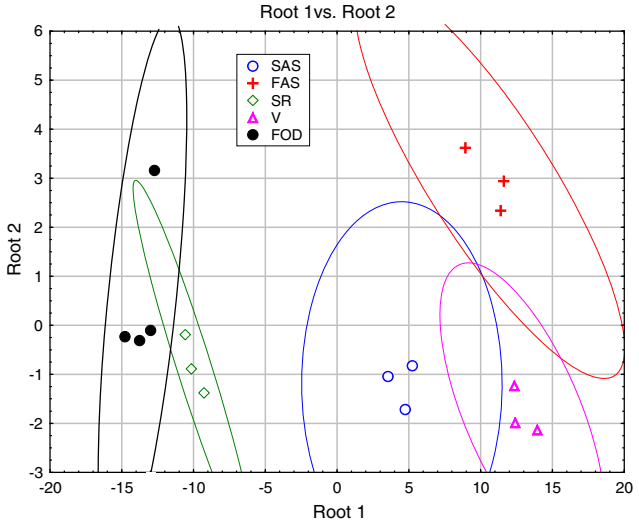

Fig. 2. 2D scatterplot of canonical scores resulting from applying the discriminant functions to the data expressing carbohydrate levels in Marsala wines.

Table 5
Mean concentrations (±SD) of heavy metals in Marsala wines

	Cd (ppb)	Pb (ppb)	Cu (ppb)	Zn (ppb)
SAS	1.5 ± 0.3	30.1 ± 5.4	77.0 ± 15.1	1170 ± 301
FAS	1.6 ± 1.3	73.1 ± 5.1	152.0 ± 30.1	777 ± 25.4
SR	1.4 ± 0.4	47.7 ± 4.0	230 ± 20.3	1508 ± 68.4
V	1.5 ± 0.2	21.4 ± 5.3	74.7 ± 9.5	933 ± 57.0
FOD	1.2 ± 0.2	10.2 ± 4.0	500 ± 20.4	1340 ± 494

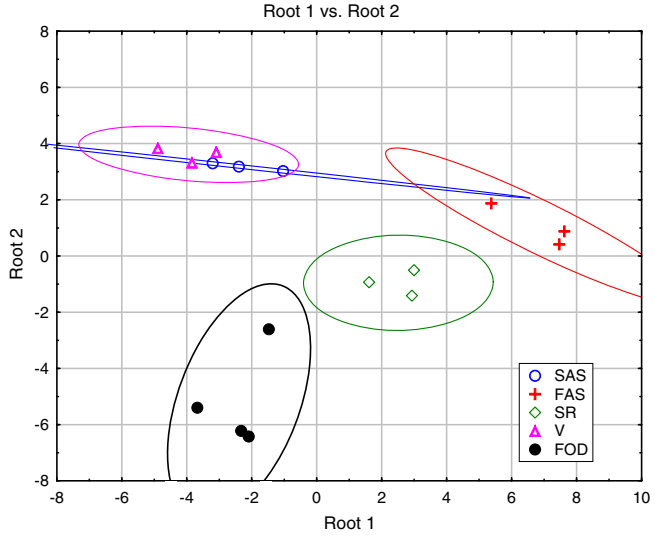

Fig. 3. 2D scatterplot of canonical scores resulting from applying the discriminant functions to the data expressing heavy metal levels in Marsala wines.

Table 6
Classification matrix for heavy metals

	%	SAS	FAS	SR	V	FOD
SAS	66. 7	2	0	0	1	0
FAS	100.0	0	3	0	0	0
SR	100.0	0	0	3	0	0
V	100.0	0	0	0	3	0
FOD	100.0	0	0	0	0	4
Total	93.7	2	3	3	4	4

cantly in different types of Marsalas, whereas cadmium level variations were not statistically important. Scatterplots (Fig. 3) relative to two discriminant functions show a good separation among FOD, SR and FAS wines. Furthermore the classification matrix gave evidence that almost 93.7% of all the studied samples were correctly classified. In particular, SAS and V Marsalas were not discriminated using heavy metal concentrations (see Table 6).

3.4. Overall statistical analysis

Finally, an overall analysis, including all the variables – polyphenols, carbohydrates and heavy metals – was performed. The analysis of variance and the Partial Wilks' Lambda test showed that the variables with the highest discriminant power were: tyrosol, caffeic acid, procyanidin B1, catechin, quercetin, kaempferol, lactose, rhamnose, zinc, copper and lead. Both the classification matrix and the scatterplot (Fig. 4) showed that 100% of the studied Marsalas can be correctly differentiated according to their typology.

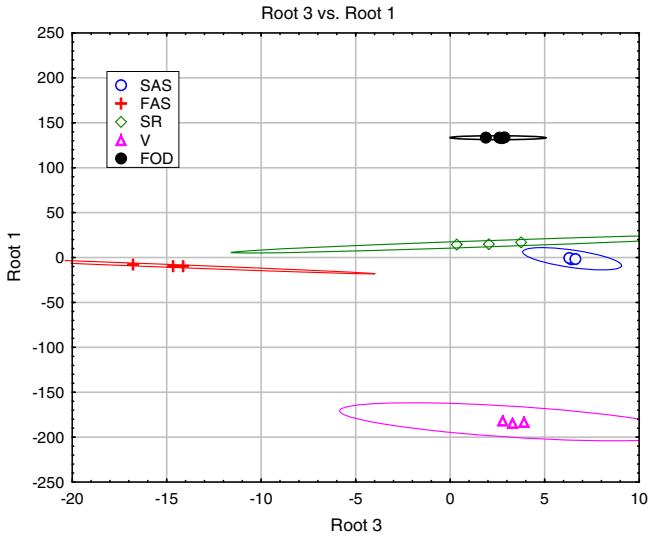

Fig. 4. 2D scatterplot of canonical scores resulting from applying the discriminant functions to the variable explaining the highest discriminant power: tyrosol, caffeic acid, procyanidin B1, catechin, quercetin, kaempferol, lactose, rhamnose, zinc, copper and lead.

4. Conclusion

In the past 10 years, thousands of papers have been published about the composition of wine and in particular about its polyphenolic pattern; even so very few data were found about Marsala wines. The purpose of this research was to bring some attention to the presence of carbohydrates, polyphenols and heavy metals in different types of Marsala wines and to perform statistical classifications by stepwise forward canonical discriminant analysis (CDA). The obtained results provide evidence that Fine Ambra Secco, Superiore Ambra Secco, Vergine, Fine Oro Dolce and Superiore Riserva Marsalas can be correctly classified according to their typology by separately applying canonical discriminant analysis on data expressing carbohydrate, polyphenol and heavy metal concentrations. Moreover, an overall statistical model showed that the variables with the highest discriminant power were: tyrosol, caffeic acid, procyanidin B1, catechin, quercetin, kaempferol, lactose, rhamnose, zinc, copper and lead.

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