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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,