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Determination of some inorganic anions and heavy metals in D.O.C. Golden and Amber Marsala wines: statistical study of the influence of ageing period, colour and sugar content

Giacomo Dugo [a], Lara La Pera [a,*], Teresa Maria Pellicanó [a],
Giuseppa Di Bella [b], Marco D'Imperio [c]

[a] Department of Organic and Biological Chemistry, University of Messina, Via Salita Sperone 31, S. Agata, 98166 Messina, Italy
[b] Department of Farmaco-Chimico, University of Messina, V.le SS. Annunziata, 98165 Messina, Italy
[c] Department of S.T.A.A.M., University of Molise, Via De Sanctis, 86100 Campobasso, Italy

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Abstract

The purpose of this paper was to determine the concentration of inorganic anions (chloride, nitrate, sulphate) and trace metal cations (cadmium, copper, lead, zinc) in different types of Marsala wines produced in the D.O.C. zone of Marsala (West Sicily), and to study the correlation between the content of these elements and the characteristics of the wines as the age, the colour, and the sugar content. The quantification of inorganic ions was executed by ion exchange chromatography with a conductivity detector and 8 mM phtalic acid, 2% acetonitile, pH 4 Tris buffer as the mobile phase, which allowed to separate sulphates from tartrates. Cadmium, copper, lead and zinc were simultaneously determined by derivative anodic stripping chronopotentiometry. The obtained results provided evidence that the concentrations of all the studied anions and cations were lower than the law limits. The analysis of variance (ANOVA) showed that ageing in wooden barrels has significantly influenced the concentration of inorganic ions (Cd^{2+}, Cl^-, NO_3^-, Pb^{2+}, SO_4^{2-}, Zn^{2+}); these relations have been further studied using multivariate statistical methods such as the principal component analysis (PCA) and the linear discriminant analysis (LDA). Finally, in order to create the model that connects the age of the wine to the concentration of some inorganic ions a multiple linear regression analysis was used. In Marsala wines, other factors, such as the sugar content and the colour did not show any influence on the concentration of the studied inorganic elements.

Keywords: Heavy metals; Inorganic anions; Ion exchange chromatography; Marsala wines; Stripping chronopotentiometry; Statistical analysis

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1. Introduction

Marsala is a popular Sicilian fortified aged wine usually served as a dessert wine, but is also an important ingredient of many Italian dishes. Marsala wine has ancient origins, but only after 1773 it was known all over the world owing to the Englishman John Woodhouse which 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, its intense perfume. Nowadays Marsala is produced in many cellars and firms located in the coastal province territory of Marsala (Trapani) that has been recognized as a D.O.C. zone (Italian Republic, DPR 2/04/1969), and is exported all over the world. Marsala wine comes in three different colours: "Oro" (Golden) and "Ambra"

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[*] Corresponding author. Tel.: +39 906765438; fax: +39 906765180.
E-mail address: laralapera20@libero.it (L. La Pera).

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(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 content 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 ranking 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, were fortified with must, alcohol and wine (13%, v/v, ethanol content), while "Vergine Soleras" Marsala was fortified only with alcohol and wine (Italian Republic, DPR 17/11/1986). Literature reports few data about the composition of Marsala wines (Di Stefano, 1985), but any information was available concerning the presence of inorganic elements such as inorganic anions and heavy metals. The aim of this paper was to bring some novelty about the inorganic composition of Marsala wines; in particular the concentrations of chloride, nitrate, sulphate, copper, cadmium, lead and zinc in different types of Golden and Amber Marsala was valued, using ion exchange chromatography and stripping chronopotentiometry. Moreover, the correlation between the levels of these inorganic elements and the characteristics of the wines such as the colour, the age and the content of reducing sugars, was assessed. The legal levels of these elements in Marsalas are 1.0 g L^{-1} for chloride (expressed as NaCl), 5.0 g L^{-1} for sulphate (expressed as K_2SO_4) (Italian Republic, DPR 12/02/1965), 0.1 mg L^{-1} for cadmium, 1.0 mg L^{-1} for copper, 0.2 mg L^{-1} for lead and 5.0 mg L^{-1} for zinc (Italian Republic, DM 29/12/1986; rule CE 466/2001 (European Community, 2001)). Actually does not exist any rule that limit the content of nitrate in wine. The presence of inorganic elements in wines depends on many factors as grapes maturity, type of soil, climatic condition, use of pesticides, wine making procedures, storage and technological factors (Salvo, La Pera, Di Bella, Nicotina, & Dugo, 2003); particularly it was demonstrated that chloride levels are particularly high for wines harvested near the coast. (Cordonnier, 1965; Gonzalez Hernández, Hardisson De La Torre, & Arias Leon, 1997). Chloride can be employed with egg albumin-type natural proteins as flocculating agent in wine clarification or used for organoleptic correction to add a certain element of taste (Cappelli & Vannucchi, 2000, chap. 27). Moreover there is an old habit practiced from some winegrowers in dry zones that raises chloride levels in wines: barrel washing with common salt or sea-water (Di Stefano, 1985). Literature reports that the chloride contents in wines is extremely variable, but in most cases does not exceed 500 mg L^{-1} (expressed as sodium chloride) (Gonzalez Hernández et al., 1997). The presence of sulphate in wines, partly depends on the use of sulphiting agents in vinification process, which is essential to prevent oxidation and to inhibit bacterial growth, conversely high concentrations of these substances can spoil a wine. During the vinification, sodium sulphite and metabisulphite dissociate forming HSO_3^- which readily oxidizes to sulphate by a complex radical mechanism, thus enhancing the sulphates levels of wine; moreover, the fraudulent addition of H_2SO_4 to wines leads to a clearly detectable increase of sulphate content (Cappelli & Vannucchi, 2000). Nitrates are naturally present in some foodstuffs, particularly in vegetables. Because of its toxicity, it is important to assess the concentration of nitrate in wines. The reduction in the human intestine of nitrate to nitrite can induce met-haemoglobinemia and under physiological conditions, nitrite reacts with secondary and tertiary amines forming carcinogenic nitrosamines (Buldini, Cavalli, & Trifiró, 1997). Of all the trace elements, lead and cadmium are important to consider in terms of food chain contamination because of their toxicity, since they cause both acute and chronic poisoning, adverse effects on the kidney, liver, heart, vascular and immune system (Heyes, 1997; Rojas, Herrera, Poirier, & Ostrosky-Wegman, 1999). Some micronutrients as copper and zinc, are essential for plant growth and human nutrition; moreover, at low concentrations, they are important in the fermentative process of wine (Ribérau-Gayon, Peynaud, & Ribérau-Gayon, 1976), while at high concentrations copper and zinc negatively influence the quality of the wine (Fernandez Pereira, 1998; Mc Kinnon & Scollary, 1997), particularly its organoleptic properties (Gennaro, Mentasti, Sarzanini, & Pesticcio, 1986). Ion exchange chromatography is wide diffused for the analysis of anions in wines since it requires a minimal handling of the sample and allows simultaneous analysis; therefore the determination of sulphates may suffer from the co-elution of some organic acids (Ding, Chen, & Luo, 1997; Gonzalez Hernández et al., 1997). In this paper the determination of chloride, sulphate and nitrate in Marsala wines was achieved with a conductivity detector without suppression, and using phtalic acid as the mobile phase. Moreover, IEC coupled with a conductivity detector is particularly useful for sweet wines, since neutral compounds such as carbohydrates, are not detected and co-eluted. The proposed method does not need extraction of the sample, the only procedure required was filtration trough C 18 cartridge. The simultaneous determination of Cd(II), Cu(II), Pb(II) and Zn(II) in Marsala wines, without any sample pre-treatment, was carried out by derivative anodic stripping chronopotentiometry, improving its analytical performances, already described in a previous paper (Salvo et al., 2003).

2. Materials and methods

2.1. Reagent

Pthalic acid, tris buffer, acetonitrile and ultra pure water for liquid chromatography were purchased from Sigma–Aldrich (Milwakee, WI, USA). Pure NaCl, $NaNO_3$ and Na_2SO_4 (Carlo Erba, Milan, Italy) were used to prepare aqueous standard solutions respectively containing 1, 10, 50 and 100 $mg\,L^{-1}$ of each anion. The SPE C18 cartridges and the Supel-ENVI carbon column, were purchased from Supelco Bellefonte, PA, USA. Ultra pure hydrochloric acid (34–37%), Hg(II) (1000 $\mu g\,mL^{-1}$, 1 M in hydrochloric acid) and Cd(II), Cu(II), Pb(II), Zn(II), Ga(III) and Pd(II) (1000 $\mu g\,mL^{-1}$, 0.5 N in nitric acid) standard solutions were purchased by Panreac Quimica (Barcelona, Spain). By dilution with ultra pure water solutions 1.0 $\mu g\,mL^{-1}$ Cd(II), 1.0 $\mu g\,mL^{-1}$ Cu(II), 1.0 $\mu g\,mL^{-1}$ Pb(II), 1.0 $\mu g\,mL^{-1}$ Zn(II), and 10 $\mu g\,mL^{-1}$ Ga(III) were prepared.

2.2. Samples

Eighteen samples of six different types of Marsala wines were studied. Particularly three samples for each type of wine, produced in three different firms in the D.O.C. zone of Marsala (Trapani, Sicily) were analysed. The vines grew on a dry calcareous soil near the coast. In all the considered crop years (1993, 1997, 2000, 2001), the grapes were harvested in the period 20 September–15 October; the newly cropped *Vitis vinifera* fruits were crushed, destemmed and subjected to soft pressing in contact with the vinasses to obtain 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 let to mature in oaken barrels. Three months after the end of the aging period, the wines were bottled and let to refine in the bottles 2 months before uncorking and consuming. All the information concerning the studied samples are given in Table 1.

2.3. Statistical analysis

The statistical elaboration of the data was performed by two software: SPSS for Windows (version 6.0; 1993) and STATISTICA package for Windows (version 5.1, 1997). The statistical procedure is reported in previous papers (Mannina et al., 2003). The analysis of variance (ANOVA) was performed both grouping Marsala wines according to their ageing period – (1) 12 months, (2) 24 months, (3) 60 months and (4) 132 months – and according to their colour Golden or Amber to value if the age of the wines and variety of the grapes significantly influenced the contents of Cd(II), Cu(II), Pb(II), Zn(II), Cl^-, NO_3^- and SO_4^{2-}. The multiple regression analysis was employed to correlate the content of the inorganic elements both to the age of the wines and the sugar content. Moreover, the principal component analysis (PCA) and linear discriminant analysis (LDA) and were further used to find out the simplest statistical model able to describe the data set satisfactory.

2.4. Sample pre-treatment

Prior to the ion exchange chromatographic analysis of chloride, nitrate and sulphate in Marsala wines, the samples were filtered trough an SPE C 18 cartridge to remove interfering and potentially column contaminant organic compound, and then diluted with ultra-pure water (1:10). For the simultaneous chronopotentiometric determination of cadmium, copper, lead and zinc were acidified to pH 2 with 5 M HCl, filtered trough a Carbon column and analysed (Salvo et al., 2003).

2.5. Ion exchange chromatographic analysis

The ion cromatograph used was an IC 761 (Metrohm, Switzerland) equipped with a double piston pump

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

Group	Type	Grape variety	Production year	Ageing (months)	Alcohol %	Sugars ($g\,L^{-1}$)
ORO	Fine Dolce	Grillo	2001	12	17	120
	Vergine Secco	Grillo	1997	60	18	10
	Vergine Stravecchio Secco	Grillo	1993	132	19	5
AMBRA	Fine Dolce	Grillo-Cataratto	2001	12	17	120
	Superiore Dolce	Grillo-Inzolia-Cataratto	2000	24	17	120
	Superiore Riserva Secco	Grillo-Cataratto	1997	60	18	39

Three samples for each type of Marsala were analysed.

and a thermo-stabilized (20 °C) conductimetry detector. A Metrosep Anion Dual 1 column (3 × 150 mm) and pre-column, packed with quaternary ammonium polymethacrylate, were used. A mobile phase 8 mM phtalic acid, 2% acetonitile, pH 4. Tris buffer was used flowing at 0.5 mL min^{-1}; the injection volume was 20 μL and the pressure 5.7 MPa.

2.5.1. Analytical performances

For quantification of chloride, nitrate and sulphate, four points calibration curves ($r = 0.995$) were obtained in the range 0–100 mg L^{-1}. The detection limits, defined as the signal height at a signal/noise ratio S/N = 3 and the repeatability of the method, valued by 10 replicates of the same Marsala, are given in Table 2. To value the accuracy of the ion exchange chromatographic determination of inorganic anions in Marsala wines, recoveries were executed from a hydro-alcoholic solution, 18% ethanol, 100 g L^{-1} saccharose, 4.0 g L^{-1} tartaric acid, spiked at different levels with chloride, nitrate and sulphate standard solutions. The obtained recoveries are shown in Table 3. As Fig. 1 illustrates, the retention times were 5.0 min for chloride, 7.8 min for nitrate and 11.5 min for sulphate.

2.6. Derivative anodic chronopotentiometric stripping analysis (dASCP)

Metals analysis were carried out by a PSA ION 3 potentiometric stripping analyser (Steroglass, S. Martino in Campo, Perugia, Italy). The analyser was equipped with a conventional three-electrodes cell. The plating process with mercury of the glassy carbon working electrode, the reference and the auxiliary electrodes, were described in previous papers (Dugo, La Pera, Lo Turco, Mavrogeni, & Alfa, 2003; La Pera, Lo Curto, Visco, La Torre, & Dugo, 2002). The electrochemical parameters for the simultaneous determination of Cd(II), Cu(II), Pb(II) and Zn(II) were: −1200 mV electro-deposition potential for 240 s; 0 mV the final acquisition potential, and 120 rpm the agitation speed. For the analysis of Marsalas wine, 0.3 mL of the sample were placed into the electrochemical cell, together with

Table 2
Calibration curves ($y = Kx$) and analytical parameters for inorganic ions analysis by ion exchange chromatography

	Cl^-	NO_3^-	SO_4^{2-}
$y =$	$10.6x$	$12.9x$	$18.8x$
R^2	99.5	99.5	99.6
Retention times (min)	5.0	7.8	11.5
Noise (nS cm^{-1})	5.00	5.00	6.00
Sensitivity* (μS cm^{-1})	45.0	80.0	50.0
Detection limits (mg L^{-1})	0.17	0.09	0.18
Repeatability %** ($n = 10$)	1.1	2.6	2.0

* Peak height corresponding to a concentration of 0.5 mg L^{-1}.

** Expressed as rsd% of 10 measurements on the same Marsala wine sample.

Table 3
Recoveries obtained for IEC determinations of chloride, nitrate, sulphate

	Added (mg L^{-1})	Found (mg L^{-1})	Recovery %
Chloride	10.0	9.71 ± 0.1	97.10 ± 1.11
Nitrate	10.0	9.53 ± 0.2	95.30 ± 2.50
Sulphate	10.0	9.73 ± 0.2	97.30 ± 1.91
Chloride	50.0	48.01 ± 0.51	96.00 ± 1.43
Nitrate	50.0	47.72 ± 1.21	95.40 ± 2.51
Sulphate	50.0	48.33 ± 0.91	96.61 ± 1.90
Chloride	100.0	98.05 ± 1.10	98.05 ± 1.10
Nitrate	100.0	96.12 ± 2.51	96.12 ± 2.51
Sulphate	100.0	98.95 ± 1.81	98.95 ± 1.81

Each result is the mean of three determinations.

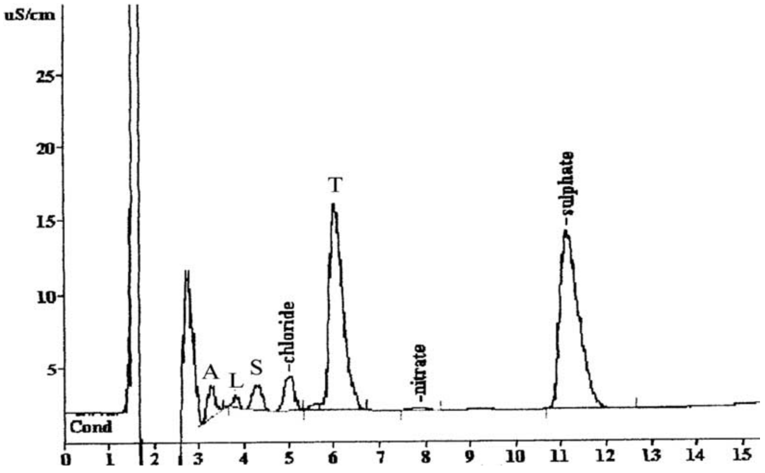

Fig. 1. Chromatogram of a Marsala wine sample; elution with 8 mM phtalic acid, 2% acetonitile, pH 4, Tris buffer at 0.5 mL min^{-1}; the injection volume was 20 μL and the pressure 5.7 MPa. A, acetate; L, lactate; S, succinate; T, tartrate.

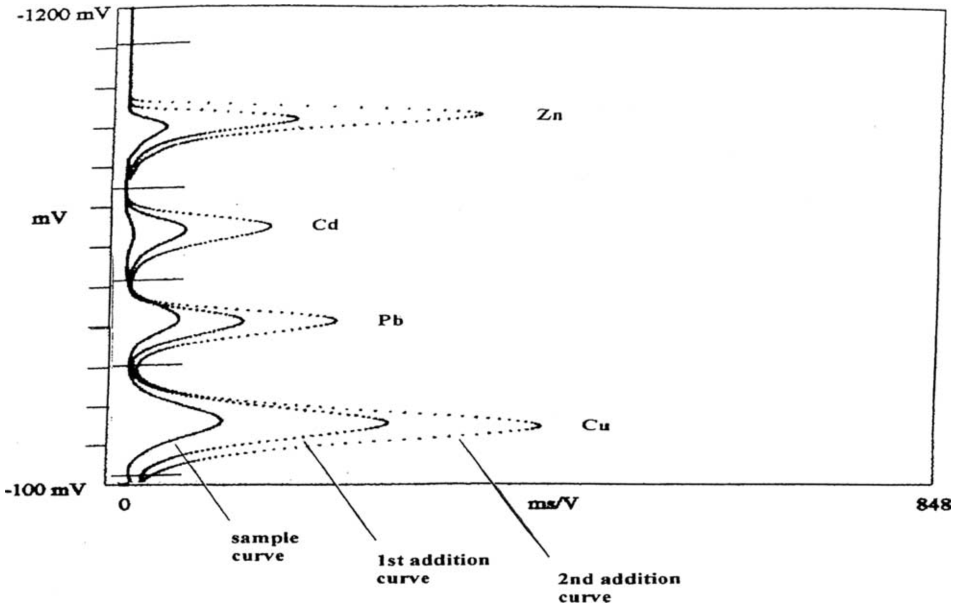

Fig. 2. Potentiometric curves of Zn, Cd, Pb and Cu. The deposition potential was −1200 mV for 240 s.

10 mL of ultrapure 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) (La Pera et al., 2002); Fig. 2 shows, the E vs. dt/dE potentiometric curves (Dugo et al., 2003). The quantitative analysis was done by the multiple point standard addition method, calibration curves with $r > .995$ were obtained (Renman & Jagner, 1997). Each analysis lasted about 45 min.

2.6.1. Analytical performances

The analytical performances of the chronopotentiometric method for the simultaneous analysis of Cd(II), Cu(II), Pb(II) and Zn(II) in Marsala wines, were tested on a hydro-alcoholic solution, 18% ethanol, 100 g L^{-1} saccharose, 4.0 g L^{-1} tartaric acid, containing 10 μg L^{-1} of Cd(II), 10 μg L^{-1} of Cu(II), 10 μg L^{-1} of Pb(II) and 10 μg L^{-1} of Zn(II). Detection limits were calculated using the expression $3\sigma/S$, where σ was the standard deviation of the analytical response, S was the sensibility represented by the slope of the calibration curves (Winefordner & Long, 1983). Recoveries were performed spiking at different levels with metal cations, the hydro-alcoholic solution mentioned above, which was passed trough the carbon column and analysed by dASCP. Repeatability was assessed by performing 10 analysis of a sample of Marsala wine. The obtained results are reported in Table 4.

3. Results and discussions

3.1. Analytical methods

The determinations of inorganic elements such as chloride, nitrates, sulphates, cadmium, copper, lead and zinc in Marsala wines is of great concern both from the enological and the toxicological point of view, moreover the correct knowledge of those parameters is required by the law. Ion exchange chromatography for sulphates analysis in wine is complicated by the co-elution of organic acids, but can be achieved using a quaternary ammonium poly-methacrylate

Table 4
Calibration curves equations ($y = Sx + q$) and analitical parameters for dASCP determinations of cadmium, copper, lead and zinc

	Cd	Cu	Pb	Zn
$y =$	$48861004x + 8231$	$148107762x + 38303$	$38340503x + 7116$	$56081754x + 13181$
R^2	0.998	0.995	0.996	0.999
σ (ms)	827	3900	691	1330
LOD (μg L^{-1})	0.05	0.07	0.05	0.07
LR[*] (μg L^{-1})	0–6000	0–4000	0–6000	0–4000
Repeatability[**]%	1.1	2.0	1.5	1.0
Recovery %	98.4 ± 1.1	96.8 ± 1.2	99.8 ± 0.8	97.0 ± 1.2

[*] LR, linearity range.

[**] Expressed as rsd% of 10 measurements on the same Marsala wine sample.

column, phtalic acid, 2% acetonitrile as the mobile phase and a conductivity detector without chemical suppression. In this conditions, repeatability ranging from 1.1% to 2.6% and detection limits of 0.17, 0.09 and 0.18 mg L^{-1}, respectively, for chloride, nitrate and sulphate were achieved (Table 3), which are much lower than those reported by literature: 0.31, 0.70 and 1.90 mg L^{-1} (Mongay, Pastor, & Olmos, 1996). A high analytical sensitivity is particularly important for nitrates analysis since are present in wines in very low amounts. As Fig. 1 shows, the proposed chromatographic method is also suitable for the separation of acetate ($t_R = 3.2$), lactate ($t_R = 3.8$), succinate ($t_R = 4.4$) and tartrate ($t_R = 6.0$).

A recent study demonstrated that derivative anodic stripping chronopotentiometry at a glassy carbon mercury film electrode, was well suited for the simultaneous analysis of cadmium, copper, lead in wines, whereas zinc was determined separately. Without any preliminary extraction procedure, detection limits lower than 2.0 μg L^{-1} were obtained. Moreover the reliability of this techniques was confirmed by graphite furnace atomic absorption spectroscopic analysis of red and white wine samples and the obtained results agreed within 5% (Salvo et al., 2003). In this paper, the simultaneous analysis of the four metals was achieved, improving the analytical sensitivity by increasing the electrolysis time up to 240 s. The analytical response was also enhanced by passing the wine sample trough the carbon column prior to the chronopotentiometric analysis, in order to remove electro-active organic compounds. Therefore the detection limits obtained in this study were lower then 0.07 μg L^{-1} and the accuracy, expressed as recovery percent, ranged from 96.8% to 99.8% (Table 4). The dependence

Table 5
Concentrations of inorganic anions and heavy metals in different types of Golden and Amber Marsala wines

	Cl^{-*} (mg L^{-1})	NO_3^- (mg L^{-1})	SO_4^{2-**} (mg L^{-1})	Cu^{2+} (mg L^{-1})	Zn^{2+} (mg L^{-1})	Pb^{2+} (μg L^{-1})	Cd^{2+} (μg L^{-1})
FAD							
1	90.1	0.0	535.5	0.6	0.3	22.5	1.5
2	110.4	1.0	740.3	0.7	0.4	27.0	2.5
3	101.3	0.8	632.1	0.8	0.5	24.8	2.0
M ± SD	100.6 ± 10.2	0.6 ± 0.5	636.0 ± 102.5	0.7 ± 0.1	0.4 ± 0.1	24.8 ± 2.3	2.0 ± 0.5
SAD							
1	45.1	1.5	1009.0	0.7	0.3	35.0	3.8
2	66.3	3.5	1077.0	0.5	0.6	58.5	5.9
3	55.2	2.1	1049.0	0.8	0.7	42.6	4.6
M ± SD	55.5 ± 10.6	2.4 ± 1.0	1045.0 ± 34.2	0.7 ± 0.1	0.5 ± 0.2	45.4 ± 12.0	4.8 ± 1.1
SRAS							
1	80.5	3.5	1301.5	0.6	1.8	34.8	6.5
2	125.0	4.6	1670.5	0.9	2.1	52.6	8.9
3	102.9	5.7	1501.0	0.9	2.0	37.9	7.8
M ± SD	102.8 ± 22.3	4.6 ± 1.1	1491.0 ± 184.7	0.8 ± 0.2	2.0 ± 0.2	41.8 ± 9.5	7.7 ± 1.2
FOD							
1	51.7	1.5	833.8	0.7	0.2	16.9	1.3
2	70.8	2.6	1040.0	0.8	0.5	19.5	3.3
3	64.8	3.6	933.8	0.8	0.4	19.8	2.0
M ± SD	62.4 ± 9.8	2.6 ± 1.1	935.9 ± 103.1	0.8 ± 0.1	0.4 ± 0.2	18.7 ± 1.6	2.2 ± 1.0
VOS							
1	60.9	3.6	1450.0	0.5	1.5	42.2	8.6
2	108.2	4.0	1345.0	0.7	2.0	68.9	11.0
3	80.9	6.6	1560.0	0.5	2.3	60.0	9.5
M ± SD	83.3 ± 23.7	4.7 ± 1.6	1451.7 ± 107.5	0.6 ± 0.1	1.9 ± 0.4	57.0 ± 13.6	9.7 ± 1.2
VOSS							
1	100.1	6.6	1672.4	1.0	2.5	180.0	10.0
2	142.0	8.3	1472.0	0.9	3.0	198.0	15.3
3	116.0	5.5	1880.3	1.0	3.7	130.0	12.0
M ± SD	119.4 ± 21.2	6.8 ± 1.4	1674.9 ± 204.2	0.9 ± 0.1	3.1 ± 0.6	169.3 ± 35.2	12.4 ± 2.7

FOD, Fine Ambra Dolce; SAD, Superiore Ambra Dolce; SRAS, Superiore Riserva Ambra Secco; FOD, Fine Oro Dolce; VOS, Vergine Oro Secco; VOSS, Vergine Oro Secco Stravecchio. Three samples for each type of Marsala were analysed.

* Expressed as NaCl.

** Expressed as K_2SO_4.

of the analytical sensitivity of the simultaneous analysis of Cd(II), Cu(II), Pb(II) and Zn(II) in food samples, on the electrochemical parameters was accurately described in a previous paper (Dugo, La Pera, La Torre, & Giuffrida, 2004).

3.2. Analysis of Marsala wines: influence of the colour

As Table 5 shows, the concentrations of chloride, nitrate, sulphate, copper, zinc, lead and cadmium, found in eighteen Marsala wines, are well below the legal limits established by the European Community for these elements. Among the studied anions, sulphate was the one present in the highest amounts (535.5–1880.3 mg L^{-1}, expressed as K_2SO_4), followed by chloride (45.1–116.0 mg L^{-1}, expressed as NaCl) and nitrate (0–8.3 mg L^{-1}). Among the studied heavy metals, copper and zinc were those present in the highest concentrations (respectively 0.6–0.9 and 0.4–3.1 mg L^{-1}), followed by lead (22.5–198.0 μg L^{-1}), whereas only traces of cadmium were detected (<20.0 μg L^{-1}). Figs. 3 and 4 provide evidence that the average concentrations of nitrates, sulphates, zinc, lead and cadmium were significantly higher in Golden Marsala wines then in Amber ones; chloride and copper levels did not show significant variations. The observed differences may depend on the grape varieties used in winemaking: Golden Marsalas of this study were produced from Grillo grapes, whereas Amber Marsalas were produced by mixing Grillo, Inzolia and Cataratto varieties.

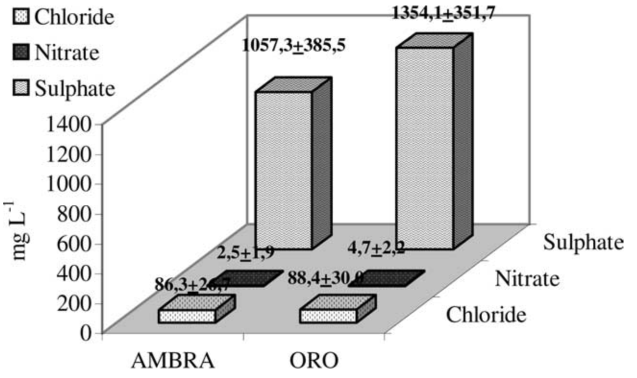

Fig. 3. Mean content of anions found in Amber (Ambra) and Golden (Oro) Marsala wines.

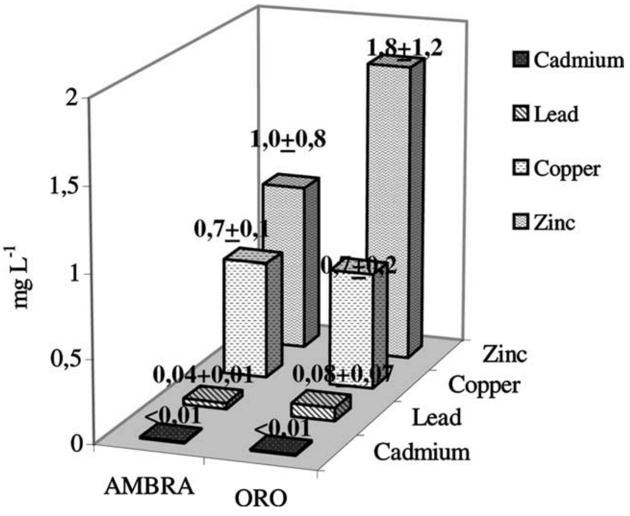

Fig. 4. Mean content of heavy metals found in Amber (Ambra) and Golden (Oro) Marsala wines.

Table 6
Analysis of variance considering the age of the wine as the discriminant factor

Variable	*F* Fischer	*p* level	Differences between the groups*
Cd^{2+}	39.4048	0.0000	$1 \neq 2 \neq 3 \neq 4$
Cl^{-}	4.7572	0.0173	$2 \neq 4$
Cu^{2+}	3.0199	0.0653	–
NO_3^{-}	13.8510	0.0002	$3 \neq 1–2$; $4 \neq 1–2–3$
Pb^{2+}	57.3364	0.0000	$3 \neq 1$; $4 \neq 1–2–3$
SO_4^{2-}	29.0389	0.0000	$1 \neq 2–3–4$; $2 \neq 3–4$
Zn^{2+}	69.6443	0.0000	$3 \neq 1–2$; $4 \neq 1–2–3$

* (1) 12 months aged; (2) 24 months aged; (3) 60 months aged; (4) 132 months aged.

3.3. Influence of aging period

Table 6 shows that the concentration of all the studied inorganic elements, except copper, vary significantly with the age of the wine but it is influenced by the colour of the wine (Golden or Amber). Data regarding all studied samples were processed using a PCA and an LDA procedure. Results of the PCA (Fig. 5) and to a major extent those relative to LDA (Fig. 6) show a good separation of the four groups representing various ages of the wine. In particular, the LDA map can be used as a graphic model in order to establish the age of a wine. In this case it seems that Root 1 expresses remarkably well the effect of the ageing process. The linear regression analysis provides a strong positive correlation between the age of the wines, assigned as a dependent variable, and the concentration of the inorganic ions, the independent variables, and described by the following equation:

$$\begin{aligned}&\text{Ageing period (months)}\\&\quad = 0.373\mathrm{Pb}^{2+} + 23.010\mathrm{Zn}^{2+} - 3.845\\&\quad (R^2 = 0.9723;\ F \text{ ratio} = 263,7145;\ p = 0.0000;\\&\qquad \mathrm{DW} = 2.8784),\end{aligned}$$

where Pb^{2+} and Zn^{2+} were, respectively, expressed in μg L^{-1} and mg L^{-1}. The Durbin–Watson test (DW) was used to detect errors of the linear regression, and to provide a very good reliability of the statistical model. The regression equation shows that, among all variables, Pb^{2+} and Zn^{2+} show the best correlation with the aging

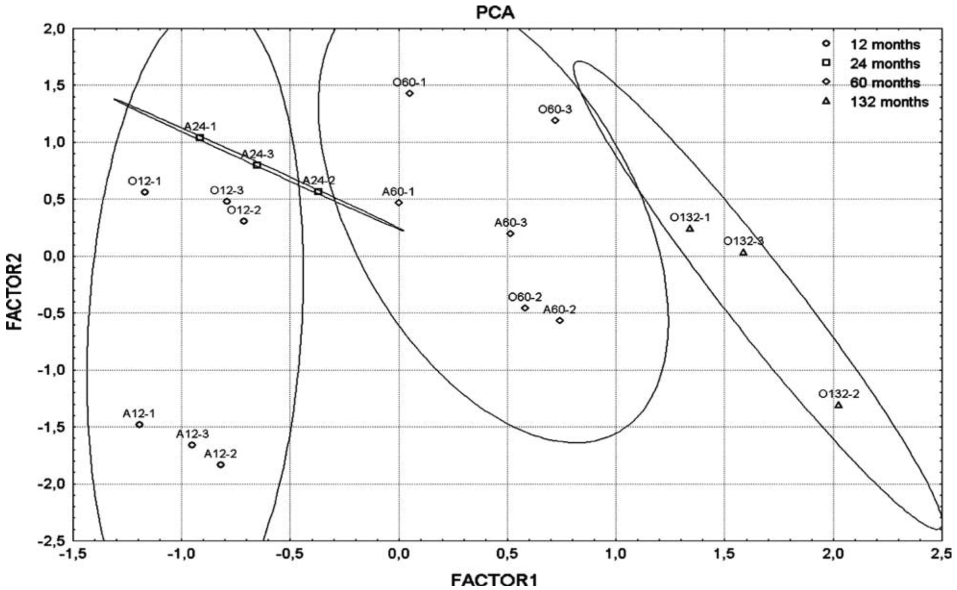

Fig. 5. Principal component analysis plot from inorganic composition of 18 Marsala samples grouped according to their age and colour. The used variables were Cd^{2+}, Cl^{-}, NO^{3-}, Pb^{2+}, SO_4^{2-} and Zn^{2+} levels.

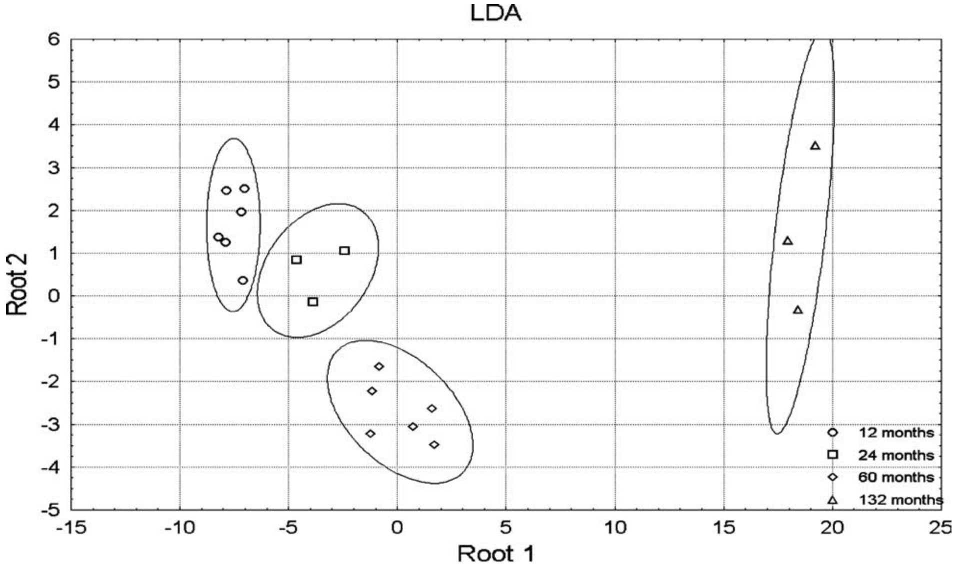

Fig. 6. Linear discriminant analysis (LDA) plot from inorganic composition of 18 Marsala samples grouped according to their age. The used variables were Cd^{2+}, Cl^{-}, Cu^{2+}, NO^{3-}, Pb^{2+}, SO_4^{2-} and Zn^{2+} levels.

period. According to linear regression analysis the correlation between the sugar content of wines and the concentrations of inorganic elements is not significant.

4. Conclusion

The present study bring some novelty about the presence of some inorganic element, of toxicological and legal concern in Marsala wines. Particularly, it describes the use of two practice, sensitive and accurate analytical methods as ion exchange chromatography to determine the concentrations of chloride nitrate, sulphate, and derivative anodic stripping chronopotentiometry for copper, zinc, lead and cadmium analysis in Marsala wines. The obtained data provide evidence that the content of chlorides, nitrates, sulphates, copper, zinc, lead and cadmium in Marsala wines, are well below legal limits. The statistical elaboration of the results provide evidence that the age of the wine significantly influences the concentrations of inorganic cations and anions in Marsala samples, which increased with prolonging the maturation period. In particular, the increase of sulphate and nitrate levels, can be the result of the lightly oxidizing environment represented by the wooden barrels used to mature the wines. The increase of metals concentration with prolonging maturation time in oaken barrels, can be the result of the extraction of inorganic elements naturally present in the wood. Other factors, as the sugar content and the colour of the wines, did not influenced the concentration of the studied inorganic elements in Marsala wines.

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