CHARACTERIZATION OF THE CONTENT OF ANTIOXIDANT SUBSTANCES IN THE WINES OF SARDINIA

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Abstract

This study examined the variability of compounds with antioxidant activity in wines coming from native cultivars of Sardinia. Hydroxycinnamic acids, resveratrols, anthocyanins, flavanols and total polyphenols concentrations were determined in 27 samples of wine from different parts of the island. The data obtained enabled quantification of the concentration of antioxidant molecules present in the different cultivars. Moreover, Principal Component Analysis (PCA) elaboration enabled possible discrimination to be detected between the cultivars examined depending on certain classes of compounds.

Riassunto

Lo studio ha preso in considerazione la variabilità di composti ad attività antiossidante in vini provenienti da cultivar autoctone coltivate in Sardegna. Le concentrazioni degli acidi idrossicinnamici, del resveratrolo, degli antociani, dei flavanoli e dei polifenoli totali sono state determinate in 27 campioni di vino provenienti da diverse località dell'isola. I dati ottenuti permettono di quantificare la concentrazione di molecole antiossidanti presenti nelle diverse cultivar. Inoltre l'elaborazione attraverso l'Analisi delle Componenti Principali (ACP) ha permesso di individuare una possibile discriminazione tra le cultivar esaminate in funzione di alcune classi di composti.

Keywords: wine, polyphenols, hydroxycinnamic acid, resveratrol, HPLC.

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Introduction

Antioxidant substances, vitamins and polyphenols, normally contained in quite marked quantities in fruit, vegetables and their derivatives, have aroused and continues to arouse particular interest. Numerous studies have linked the antioxidant capacities of polyphenolic substances with the protective action they exert on the cardiocirculatory apparatus, in the prevention of tumours and inflammatory and degenerative diseases (1-7). The daily intake of polyphenols varies greatly depending on the type of food choice. In the western diet the total quantity of these substances introduced in the daily diet has been estimated to be on average 1 g/day (8).

Many of these substances are contained in wine, which is an integral part of the eating habits of the western population, in particular that of southern Europe. For this reason the consumption of wine, in recent years, has received more aware consideration.

One of the compounds contained in grapes and wines that has aroused greater interest is resveratrol.

The chemiopreventive, anti-inflammatory and anti-atherogenic properties of this compound, deriving from stilbene, have been demonstrated (5, 9-13). Resveratrol is responsible for the so-called "French paradox", explaining the apparent compatibility of a diet rich in fats with a low rate of mortality by coronary diseases (14).

The phenolic substances present in wine are also fundamental in contributing to the formation of specific characteristics, such as colour, flavour, aroma and therefore everything that concurs in its distinctiveness, ultimately making one wine different from another.

The differences in composition that can be observed are ascribable to various factors, such as varietal character, place of production, degree of ripening of grapes, culture typology and vinification technique. For these reasons there is more and more a tendency to study the composition in its minor constituents with the aim of realising better characterization, which may contribute to obtaining commercial enhancement of the product.

Our research group has for several years been conducting studies in this sector on the polyphenolic characterization of grapes and wines, with particular attention to the anthocyanin, resveratrol and hydroxycinnamic acid content (15-23).

In this work, some red and white wines which represented the principal wine production of Sardinia were taken into consideration. The wines

were characterized by determination of the polyphenolic substances (total polyphenols, flavanols, monomeric anthocyanins, hydroxycinnamic acids and resveratrols), with the aim of quantifying the mean contribution of the different substances introduced in the diet. Polyphenolic compounds have already been indicated as chemical markers for varietal differentiation of the cultivars (15-16, 18, 20, 24-25).

The data obtained from such studies were subjected to statistical elaboration (PCA) with the purpose of evaluating whether these parameters enable varietal discrimination and may therefore be effectively used for the aims of chemiotaxonomic classification.

Materials and methods

Samples

In 2003 were collected 27 samples of wines produced between 2001 and 2002 and stored in refrigerated cells at 4 °C up until the moment of analysis. These wines were representative of the principal enological production of native and traditional varieties of Sardinia. In particular, for the two principal varieties of white grape, 6 samples of Vermentino and 5 of Nuragus were collected; for the red grape varieties (Cannonau and Monica) 6 samples of each. Furthermore, one sample of other wines was collected (Carignano, Moscato, Semidano and Torbato).

The following commercial products used as standard, caffeic, p-coumaric, ferulic acid formic, trans-resveratrol, Folin-Ciocalteu reagent, (+)-catechin, malvidin-3-glucoside chloride, were supplied by Fluka. All the solvents (methanol, acetonitrile, ethyl acetate) were HPLC grade. All the solutions were obtained with distilled deionised water using Milli-Q® Millipore equipment.

Statistical analysis

Statistical analysis of the data was performed using the software package SPSS 14.00 (SPSS Inc. Chicago, IL). Data were analyzed by Principal Component Analysis. PCA was performed in order to find possible differentiation between objects according to variety and to determine at the same time which variables are principally responsible of the separation of the samples.

Hydroxycinnamate esters and hydroxycinnamic acids analysis

The HPLC analysis of hydroxycinnamates (26-27) was performed on an Agilent instrument Series 1100 equipped with Diode Array Detector (DAD) and HP ChemStation, using a reversed-phase column Hypersil ODS RP-C18 (Agilent, 250 × 2.1 mm, 5 µm particle size) and precolumn Hypersil ODS (20 × 2.1 mm, 5 µm particle size). The solvents used were: 0.5% formic acid in water (A) and 2% formic acid in methanol (B). Gradients were as follows: from 16.8% to 19% of B in 7 minutes, from 19% to 53% of B in 12 minutes (total run time 19 minutes). After each run the column was washed with 100% B for 5 minutes and equilibrated for 9 minutes prior to each analysis. Flow rate was 0.4 mL/min, injection volume 10 µL and oven temperature was 40 ºC. The detection of hydroxycinnamates was at 320 nm. The tartaric esters (caftaric, coutaric and fertaric acids) were quantified as caffeic acid equivalent while free acids were quantified as free acids equivalent (caffeic, p-coumaric and ferulic acid).

Resveratrol and its monomers analysis

The wine content of resveratrol and its monomers was determined using HPLC analysis by solid phase extraction according to Mattivi's method (28). The procedure was as follows: 100 mL of wine were centrifuged for 10 min at 10,000 rpm (ALC 3229 Centrifuge) and the supernatant filtered through 0.45 m cellulose acetate. The wine (50 mL) was loaded into a 1 g reversed-phase Sep-pak C18 cartridge (Waters), previously prepared with an aqueous solution containing 5 mL MeOH followed by 10 mL of a phosphate buffer solution (pH 7). After the sample had adsorbed, the cartridge was washed twice with 10 mL of a phosphate buffer solution (pH 7) and the content eluted with 5 mL ethyl acetate. The fraction collected in a test tube was kept in the freezer overnight in order to crystallize the residual water. One aliquot of the organic fraction separated from the ice was filtered through 0.22 m PTFE filters and injected into the HPLC following Zamboni's method (31).

All the extraction phases were carried out away from light to protect the stilbenes from photochemical isomerisation.

Analysis of resveratrols was carried out on a Waters 2695 HPLC system with a Waters 2996 DAD (Waters Corp., Milford, Massachusetts) and the Empower Software (Waters). Separation was performed using a

Zorbax column (SB-Aq, 5 μm, 2.1 mm × 150 mm, Agilent Technologies, Palo Alto, California) and a Zorbax precolumn (SB-Aq, 5 μm, 2.1 mm × 12.5 mm, Agilent Technologies). The mobile phases consisted of 0.1% acetic acid in H_2O (A) and acetonitrile (B). Separation was carried out at 40 °C in 27 min, under the following conditions: linear gradients starting at 5% B to 70% B in 25 min, to 95% B in 0.1 min, 95% B for 2 min, back to 5% B in 0.1 min. The column was equilibrated for 7 min prior to each analysis. The flow rate was 0.25 mL/min and the injection volume 4 μL. The UV-VIS spectra were recorded from 220 to 400 nm, with detection at 310 nm for trans-resveratrol that was quantified and identified using the external standard method on the basis of the retention time and UV-VIS spectra.

Since the standard for cis-resveratrol is not commercially available, it was measured at the wavelength of 282 nm (maximum absorption for this compound) with the solutions used to determine the trans isomer, after UV irradiation at 366 nm for 30 min, the time necessary to convert about 90% of trans-resveratrol into cis, calculated on the amount of residual trans at a wavelength of 310 nm.

The concentration of the standard solutions was calculated on the basis of the percentage of conversion.

Resveratrol and its isomers were quantified by multiple level calibration curve (external standard) using trans-resveratrol supplied by Sigma at a wavelength of 310 nm, the maximum absorption for this compound. Since glucoside standards are not commercially available, quantification was based on the assumption that they have the same coefficients of molar extinction as trans and cis-resveratrol, 310 and 282 nm respectively.

Total phenols analysis

Total phenols were assessed by the reduction of Folin-Ciocalteu phosphomolybdic-phosphotungstic acid reagent to blue pigments by phenols in alkaline solution (29).

The red wine was diluted (usually 10-20 times) with 0.5 M H_2SO_4 and the dilution factor adjusted to obtain a final reading between 0.3 and 0.6 AU. One millilitre of diluted wine was then slowly loaded onto the conditioned Sep-Pak, and the polar substances were removed with 2 mL of 5 mM H_2SO_4. The phenolic compounds were eluted into a 20 mL calibrated flask, with 2 ml of MeOH followed by 5 ml of distilled water. One ml of Folin-Ciocalteu reagent and, after ~ 3-4 min, 4 ml of 10% Na_2CO_3, were added and the solution was brought to 20 mL with distilled water. After 90

min at 20 °C, the adsorbance of the sample (filtered at 0.45 µm) was read at 700 nm in a 10 mm cell, against a blank test prepared by using distilled water in place of the wine. Concentrations were determined by means of a calibration curve as (+) catechin (mg/L).

Determination of flavanols by vanillin assay (vanillin index) (29)

The red wine was diluted (2-10 times, to obtain a final reading between 0.2 and 4.4 AU) with 0.5 M H_2SO_4 and 2 mL was loaded onto conditioned Sep-Pak. The column was washed with 2 mL of 5 mM H_2SO_4 and purged with air, and the flavanols were eluted with 5 mL of MeOH into a test tube.

One ml of the methanolic solution containing the flavanols was placed in a test tube (shielded from light) together with 6 mL of vanillin (4% in MeOH) and immersed in the water bath at 20 °C. When cool, 3 mL of concentrated HCl was carefully added. After exactly 15 min, the absorbance of the pink complex was read at 500 nm in a 10 mm cell against a blank prepared in the same conditions, containing MeOH instead of vanillin. Concentrations were calculated as (+)-catechin (mg/L) by means of a calibration curve.

Determination of monomeric anthocyanins by HPLC (30)

An aliquot (5 to 20 mL) of wine sample, diluted four times with water, was applied to a SPE-C18 cartridge (Waters), previously activated with methanol (5 mL) and water (10 mL). The cartridge was washed with 6 mL of 0.3% HClO4 in distilled water and then eluted with 10 mL of methanol into a 100 mL pear-shaped flask. The eluate was evaporated under reduced pressure at 35 °C and reconstituted in 1 mL of diluted methanol (27% in water and 0.3% $HClO_4$). The final samples were filtered through 0.22 µm, 13 mm PTFE syringe tip filters (Millipore, Bedfore, MA) into LC vials and immediately injected into a 1090 HPLC equipped with a diode array detector (Agilent, Waldbronn, Germany). Separation of the 15 main free anthocyanins was obtained at 40 °C on a Purosphere RP-C18 column, 250 mm × 4 mm, 5 µm, with a precolumn (Merck, Germany). The flow was 0.45 mL/min, eluent A was 0.3 % $HClO_4$ in distilled water, and B was methanol. The following binary gradient was applied, from 27 to 43% of B in 32 min, then to 68.5% of B in 13 min, to 100% B in 2 min, and isocratic 100% B for 3 min; total analysis time, 50 min. Delphinidin 3-gluco-

side, cyanidin 3-glucoside, petunidin 3-glucoside, peonidin 3-glucoside, malvidin 3-glucoside, and their relevant acetic acid and p-coumaric acid esters were identified according to Castia et al. (15) and quantified at 520 mn with a calibration curve with malvidin-3-glucoside chloride.

Results and discussion

In accordance with the literature (32), the red wines contain quantities of total polyphenols that are over ten times greater than those of the white wines (Table 1). The highest total polyphenols content was found in Carignano, followed by Monica, which presents high variability among the different producers (1328-2107 mg/L, mean 1762 mg/l), and Cannonau, which presents a similar composition among the different wineries (1466-1865 mg/L).

The total polyphenols content of the white wines is not very dissimilar between the various typologies, placed within the range 104-175 mg/L, with mean values higher in the Nuragus (145 mg/L) compared with Vermentino (126 mg/L). Content of flavanols reactive to vanillin also follows the same tendency as the total polyphenols.

With the aim of highlighting the contribution of these substances in the diet, the quantity of polyphenols introduced with the daily consumption of wine recommended by nutritionists (200 mL) is given in Table 1.

TABLE 1

CONTENT OF TOTAL POLYPHENOLS AND FLAVANOLS REACTIVE TO VANILLIN IN THE WINES OF SARDINIA. DATA EXPRESSED AS mg/L OF (+) CATECHIN

Simple	Variety	Total polyphenols as mg/L of (+) catechin	Flavanols as mg/L of (+) catechin	Quantity of polyphenols present in the maximum quantity of wine recommended (200 mL)
1	Cannonau	1708	846.2	341.6
2	Cannonau	1604	860.8	320.8
3	Cannonau	1813	881.1	362.6
4	Cannonau	1865	686.3	373.0
5	Cannonau	1466	747.4	293.2
6	Cannonau	1761	863.7	352.2
Mean	Cannonau	1703	814.0	340.6
SD	Cannonau	147	78.7	29.4
7	Monica	2033	1029.4	406.6
8	Monica	1697	744.4	339.4
9	Monica	2107	1180.6	421.4
10	Monica	1593	724.1	318.6
11	Monica	1328	729.9	265.6
12	Monica	1817	674.7	363.4
Mean	Monica	1762	847.0	352.4
SD	Monica	289	206.7	57.8
13	Vermentino	121	26.2	24.2
14	Vermentino	124	33.4	24.8
15	Vermentino	143	40.7	28.6
16	Vermentino	111	20.4	22.2
17	Vermentino	110	16.0	22
18	Vermentino	144	26.2	28.8
Mean	Vermentino	126	27.0	25.2
SD	Vermentino	15	8.9	3.0
19	Nuragus	171	36.4	34.2
20	Nuragus	175	53.8	35.0
21	Nuragus	156	24.7	31.2
22	Nuragus	113	14.5	22.6
23	Nuragus	109	26.2	21.8
Mean	Nuragus	145	31.0	29.0
SD	Nuragus	32	14.9	6.4
24	Moscato	126	22.5	25.2
25	Torbato	104	23.3	20.8
26	Semidano	116	11.6	23.2
27	Carignano	2298	1231.4	459.6

The white wines with a greater polyphenols content have flavanols tenors slightly above average, probably due to the production technology, which enables more marked extraction of the polyphenols from the skin. The data in the table show that in the red wines flavanols constitute around 50% of the total polyphenols, but 20% in the white wines.

In general, compared with the white wines, the red ones present a greater quantity of hydroxycinnamic acids (Table 2). The highest content of these compounds is found in the Cannonau typology (60-120 mg/L, mean 83 mg/L), followed by Monica (3-75 mg/L, mean 55 mg/L). The hydroxycinnamic acids most commonly represented are trans-caftaric (which in the Cannonau constitutes 44% of the total hydroxycinnamic acids, in Monica 36% and Carignano 38 %), followed by trans-caffeic and trans-coutaric. Carignano, in contrast with the other red wines, does not contain trans-caffeic acid.

In the Cannonau and Monica varieties, in particular, a high content of the derivatives of p-coutaric acid and the corresponding free p-coumaric acid is found.

Among the white wines the highest quantity of hydroxycinnamic acids is found in the Nuragus (18-52 mg/L, mean 36 mg/L) and in the Moscato sample (52 mg/L), while the Vermentino (16-28 mg/L, mean 23 mg/L), Torbato and Semidano have slightly lower values. In this case also, trans-caftaric acid is the one more strongly represented, reaching 53% of the total hydroxycinnamic acids in the Nuragus.

The red wines have significant contents of resveratrols (Table 3), in particular the Cannonau (3.3-11.6 mg/L, mean 6.15 mg/L), followed by Monica (3.1-8.4 mg/L, mean 5.3 mg/L) and Carignano (4.1 mg/L). It is interesting to note that some of the white wines also have a quite high resveratrols content, in particular the samples of Vermentino n° 13, n° 14 and n° 15 and the Moscato sample. In all the varieties studied significant presence of the bound forms of resveratrol were found (trans and cis-piceide), in general prevailing over free forms.

As far as free anthocyanins are concerned, in the wines analyzed (Table 4) they vary considerably between 10 and 182 mg/L, depending on variety, degree of ageing (with higher values in the younger wines) and apparently depending on the quality of the grapes and vinification technique. Monica wines present mean values of total anthocyanins that are higher among the red wines. In accordance with the literature (16), the acylated and p-coumarate forms of malvidin 3-glucoside prove to be the most commonly represented, constituting respectively approximately 55% and

TABLE 2

HYDROXYCINNAMIC ACIDS CONTENT IN THE WINES OF SARDINIA. DATA EXPRESSED IN mg/L.

Sample	Variety	cis-Caftaric Acid (*)	trans-Caftaric Acid (*)	cis-Cutaric Acid (*)	GRP(*)	trans-Cutaric Acid (*)	Fertaric Acid (*)	trans-Caffeic Acid	trans-p-Cumaric Acid	trans-Ferulic Acid	Total
1	Cannonau	0.73	28.61	3.56	4.30	13.09	2.92	6.74	1.60	0.34	61.89
2	Cannonau	0.72	35.38	4.38	4.03	13.00	2.91	6.26	4.13	0.35	71.16
3	Cannonau	0.00	3.05	0.34	4.03	0.88	0.97	50.85	8.98	2.33	71.43
4	Cannonau	0.51	67.40	3.49	2.64	22.53	4.18	15.01	3.93	0.74	120.43
5	Cannonau	0.39	44.43	3.30	2.48	13.01	3.94	10.08	2.61	0.46	80.70
6	Cannonau	1.40	50.36	3.97	4.26	13.78	3.91	14.28	2.40	0.46	94.82
Mean	Cannonau	0.63	38.21	3.17	3.62	12.72	3.14	17.20	3.94	0.78	83.41
SD	Cannonau	0.46	21.79	1.44	0.83	6.90	1.19	16.88	2.65	0.77	21.28
7	Monica	0.00	8.88	2.19	3.40	6.04	0.83	16.79	12.09	0.73	50.95
8	Monica	0.52	37.55	2.94	5.76	16.08	2.19	7.10	2.32	0.13	74.59
9	Monica	0.52	26.51	3.29	3.99	12.11	2.02	6.73	5.91	0.39	61.47
10	Monica	0.84	18.79	3.26	5.09	7.26	2.06	8.43	5.32	0.62	51.67
11	Monica	0.50	26.94	3.24	4.18	11.78	2.04	6.28	4.74	0.34	60.04
12	Monica	0.61	8.42	2.12	3.81	4.28	1.02	8.62	2.42	0.16	31.46
Mean	Monica	0.50	21.18	2.84	4.37	9.59	1.69	8.99	5.47	0.40	55.03
SD	Monica	0.28	11.40	0.55	0.88	4.46	0.60	3.93	3.57	0.24	14.37
13	Vermentino	0.00	9.94	1.62	0.34	3.71	2.46	3.70	1.19	0.56	23.52
14	Vermentino	0.00	6.17	1.48	0.48	1.97	2.29	1.74	0.81	0.71	15.65
15	Vermentino	0.18	8.51	2.58	0.36	0.90	2.64	4.86	2.69	1.19	23.91
16	Vermentino	0.00	8.10	1.93	3.98	4.01	2.00	1.20	0.57	0.30	22.09

15 % of total antho-cyanins.

17	Vermentino	0.34	13.17	2.11	3.02	2.42	2.26	3.23	0.65	0.51	27.71
18	Vermentino	0.28	5.92	2.58	1.44	1.96	1.38	8.77	2.77	0.29	25.39
Mean	Vermentino	0.13	8.64	2.05	1.60	2.50	2.17	3.92	1.45	0.59	23.05
SD	Vermentino	0.15	2.68	0.47	1.55	1.17	0.44	2.72	1.02	0.33	4.09
19	Nuragus	0.58	14.91	3.09	1.84	2.70	2.08	3.37	0.94	0.54	30.05
20	Nuragus	0.19	24.66	3.38	2.20	8.19	2.43	2.04	0.64	0.64	44.37
21	Nuragus	0.48	30.67	2.41	1.64	8.24	2.15	5.17	0.95	0.23	51.94
22	Nuragus	0.00	19.06	1.57	3.37	6.11	1.95	1.60	0.47	0.33	34.46
23	Nuragus	0.17	8.71	1.66	2.74	2.11	1.50	1.05	0.33	0.15	18.42
Mean	Nuragus	0.28	19.60	2.42	2.36	5.47	2.02	2.65	0.67	0.38	35.85
SD	Nuragus	0.21	7.60	0.73	0.63	2.62	0.30	1.48	0.25	0.18	11.58
24	Moscato	0.55	20.69	2.40	3.95	6.68	2.52	11.05	3.69	0.58	52.11
25	Torbato	0.00	6.35	2.29	0.81	3.24	2.55	1.82	0.91	0.45	18.42
26	Semidano	0.25	3.49	1.68	3.50	0.58	2.20	4.22	0.96	0.80	17.68
27	Carignano	0.00	15.86	2.97	4.03	11.52	1.66	0.00	5.99	0.24	42.27

(*) Expressed as mg/l of caffeic acid equivalent

TABLE 3

Sample	Variety	trans-Resveratrol glucoside (*)	cis-Resveratrol glucoside (**)	trans-Resveratrol	cis-Resveratrol	Total
1	Cannonau	1.33	1.83	1.04	0.70	4.90
2	Cannonau	3.06	2.66	3.30	2.61	11.63
3	Cannonau	1.04	1.26	0.13	0.65	3.08
4	Cannonau	1.19	0.98	0.52	1.16	3.85
5	Cannonau	0.84	0.94	0.83	1.76	4.37
6	Cannonau	1.94	2.11	1.82	3.21	9.08
Mean	Cannonau	1.57	1.63	1.27	1.68	6.15
SD	Cannonau	0.82	0.69	1.14	1.05	3.41
7	Monica	0.72	1.87	2.54	2.07	7.20
8	Monica	1.15	1.06	0.37	0.58	3.16
9	Monica	1.17	1.90	1.25	1.47	5.79
10	Monica	2.36	2.53	1.48	2.05	8.42
11	Monica	0.81	1.29	1.05	0.98	4.13
12	Monica	0.76	1.27	0.22	1.22	3.47
Mean	Monica	1.16	1.65	1.15	1.40	5.36
SD	Monica	0.62	0.55	0.84	0.59	2.14
13	Vermentino	0.41	0.21	0.40	0.88	1.90
14	Vermentino	0.39	0.45	0.37	0.68	1.89
15	Vermentino	0.65	0.93	0.64	2.68	4.90
16	Vermentino	0.18	0.11	0.07	0.22	0.58
17	Vermentino	0.09	0.24	0.06	0.92	1.31
18	Vermentino	0.01	0.03	0.00	1.74	1.78
Mean	Vermentino	0.29	0.33	0.26	1.19	2.06
SD	Vermentino	0.24	0.33	0.25	0.88	1.48
19	Nuragus	0.06	0.21	0.05	1.20	1.52
20	Nuragus	0.18	0.31	0.06	0.34	0.89
21	Nuragus	0.05	0.10	0.00	0.25	0.40
22	Nuragus	0.08	0.11	0.00	0.27	0.46
23	Nuragus	0.05	0.10	0.01	0.14	0.30
Mean	Nuragus	0.08	0.17	0.02	0.44	0.71
SD	Nuragus	0.06	0.09	0.03	0.43	0.50
24	Moscato	0.68	0.56	0.19	1.20	2.63
25	Torbato	0.19	0.17	0.14	0.43	0.93
26	Semidano	0.11	0.35	0.00	1.81	2.27
27	Carignano	0.90	1.57	0.99	0.67	4.13

(*) Expressed as mg/l of trans-resveratrol equivalent
(**) Expressed as mg/l of cis-resveratrol equivalent

RESVERATROLS CONTENT IN THE WINES OF SARDINIA.

TABLE 4

ANTHOCYANINS CONTENT IN THE WINES OF SARDINIA. DATA EXPRESSED AS mg/L OF MALVIDINE 3-GLUCOSIDE CHLORIDE EQUIVALENT

DATA EXPRESSED IN mg/L

	Cannonau								**Monica**								**Carignano**
Sample number	1	2	3	4	5	6	Mean	SD	7	8	9	10	11	12	Mean	SD	27
Delfinidin 3-glucoside	2.89	5.42	0.59	1.18	0.59	1.49	2.03	1.87	6.78	0.52	3.42	2.31	1.27	4.71	3.17	2.32	3.87
Cyanidin 3-glucoside	0.67	1.46	0.14	0.24	0.38	0.44	0.56	0.48	0.53	0.13	0.64	1.70	0.25	0.94	0.70	0.57	0.45
Petunidin 3-glucoside	4.23	8.32	0.87	1.17	1.10	2.34	3.01	2.89	13.49	0.80	5.83	3.55	2.29	5.68	5.27	4.47	7.11
Peonidin 3-glucoside	1.75	8.61	0.71	0.42	1.24	1.70	2.41	3.09	4.38	0.31	3.03	5.49	1.02	2.29	2.75	1.97	2.09
Malvidin 3-glucoside	27.28	82.59	7.73	5.02	16.80	21.26	26.78	28.57	110.65	4.54	39.72	19.68	16.39	24.92	35.98	38.34	50.96
Delfinidin 3-glucoside acylate	0.48	1.47	0.15	0.22	0.00	0.62	0.49	0.53	1.20	0.00	0.71	0.39	0.37	0.85	0.59	0.42	1.00
Cyanidin 3-glucoside acylate	0.08	0.24	0.00	0.00	0.00	0.19	0.09	0.11	0.17	0.00	0.15	0.14	0.06	0.13	0.11	0.06	0.09
Petunidin 3-glucoside acylate	0.45	1.43	0.10	0.20	0.00	0.20	0.40	0.53	1.83	0.00	0.76	0.23	0.28	0.81	0.65	0.66	0.86
Peonidin 3-glucoside acylate	0.15	1.42	0.00	0.00	0.00	0.09	0.28	0.56	1.93	0.00	0.59	0.26	0.18	0.32	0.55	0.70	0.71
Malvidin 3-glucoside acylate	4.22	19.54	2.17	3.62	1.61	4.87	6.01	6.74	26.68	3.08	9.50	3.23	4.67	5.24	8.73	9.10	13.27
Delfinidin 3 glucoside p-cumarate	0.17	0.32	0.00	0.00	0.00	0.00	0.08	0.14	1.07	0.12	0.47	0.16	0.10	0.17	0.35	0.38	0.85
Cyanidin 3-glucoside p-cumarate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Petunidin 3-glucoside p-cumarate	0.38	0.44	0.00	0.00	0.00	0.00	0.14	0.21	1.48	0.14	0.55	0.12	0.20	0.33	0.47	0.52	1.12
Peonidin 3-glucoside p-cumarate	0.29	1.32	0.19	0.13	0.18	0.30	0.40	0.45	1.54	0.10	0.70	0.58	0.25	0.21	0.56	0.53	0.72
Malvidin 3-glucoside p-cumarate	2.14	6.36	0.72	0.46	1.27	1.45	2.07	2.18	10.56	0.63	4.44	1.54	1.69	1.65	3.42	3.73	6.43
Total anthocyanins	45.18	138.94	13.37	12.66	23.17	34.95	44.71	47.85	182.29	10.37	70.51	39.38	29.02	48.25	63.30	61.62	89.53

In order to bring to light any similarities and correlations between the three types of red wines (Cannonau, Monica and Carignano), the data set of five variables (Σ-Anthocyanins, Σ-Resveratrols, Σ-Hydroxycinnamics, Σ-Flavanols and Σ-Polyphenols) and 13 objects were evaluated by Principal Component Analysis (PCA). The first component is positively correlated with the Σ-Flavanols, Σ-Anthocyanins and Σ-Polyphenols (loading values are 0.880, 0.822 and 0.767 respectively). The second component is positively correlated with the Σ-Resveratrols (loading value is 0.903) while the third component correlate with Σ-Hydroxycinnamics with loading value of 0.829.

On the individual plane of the first two components, accounting for 74 % of total variance, it was shown that there are no different characteristics between the red wine samples in relation to the cultivar (Figure 1).

The analysis highlights that the four samples are distinguished from the others for certain features: a sample of Cannonau (n° 2) is charac-

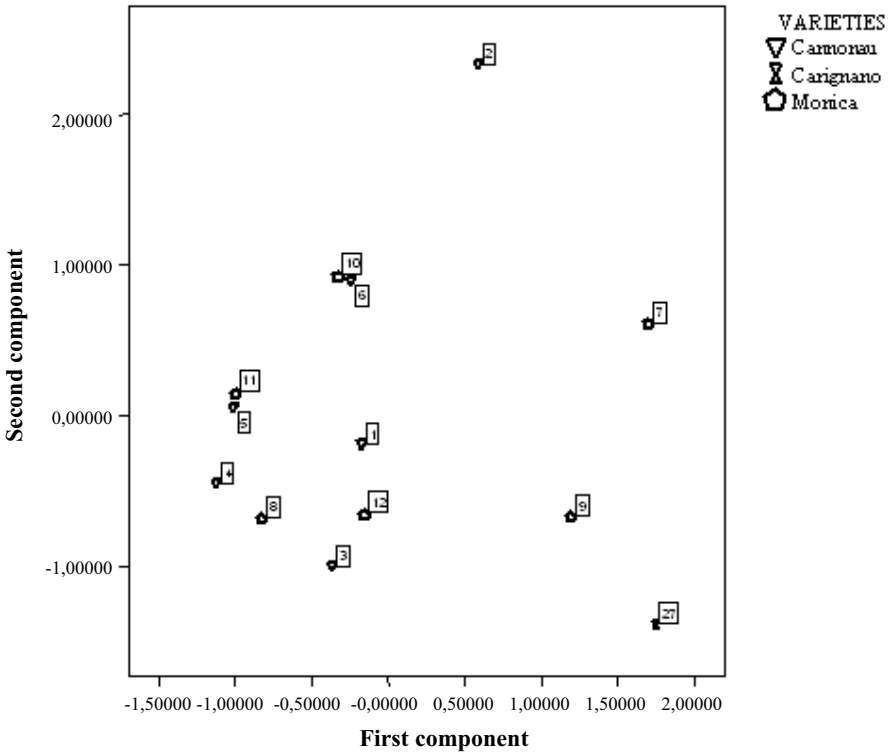
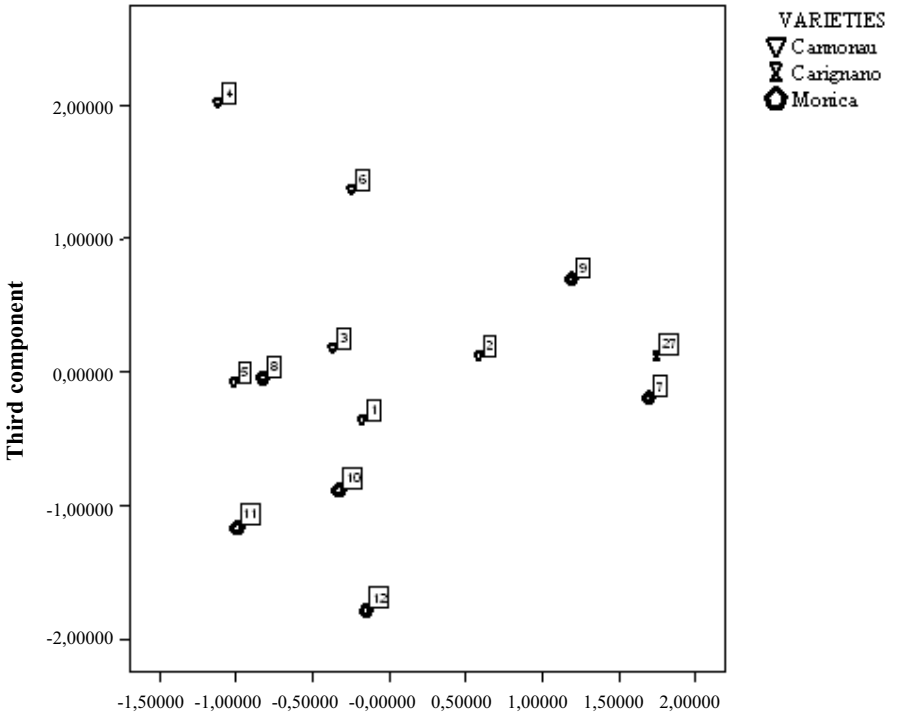


Fig. 1 - Objects identified on the plane of the first and second principal components.

terized by the highest resveratrols content (11.6 mg/L) and a very high total anthocyanins content (138.9 mg/L). The Carignano (n° 27) and the two Monica wines (n° 7 and n° 9) are distinguished by their total flavanols, total polyphenols and total anthocyanins content which are higher than average.



On the individual plane of the first and third component it was shown that the two Cannonau samples (n° 4 and n° 6) are different from the others for their higher content of total hydroxycinnamic acids, while a sample of Monica (n° 12) presents the lowest content of total hydroxycinnamic acids (Figure 2).

Fig. 2 - Objects identified on the plane of the first and third principal components.

On the other hand, on analysis of the principal components the white wines show differences that can be correlated with the cultivar. PCA carried out on a data set of 4 variables (Σ-Resveratrols, Σ-Hydroxycinna-

mics, Σ-Flavanols and Σ-Polyphenols) and 14 objects, highlighted (Table 6) that the first component is correlated with Σ-Polyphenols and Σ-Flavanols with correlation coefficient of 0.942 and 0.852 respectively, while the second is correlated with Σ-Resveratrols with correlation coefficient of 0.840, account for 80% of the total variance.

The first and second component plane identified enables it to be shown that the wines of the Vermentino typology present characteristics that are quite homogeneous amongst themselves, with the exception of one sample (n° 15), which is distinguished by a higher content of total resveratrols, whose quantity approaches the mean for red wines. Within the Nuragus typology more marked variability exists, especially as concerns the ?-Flavanols, ?-Polyphenols and ?-Resveratrols variables. The Moscato sample seems to have features more similar to the Nuragus, while the

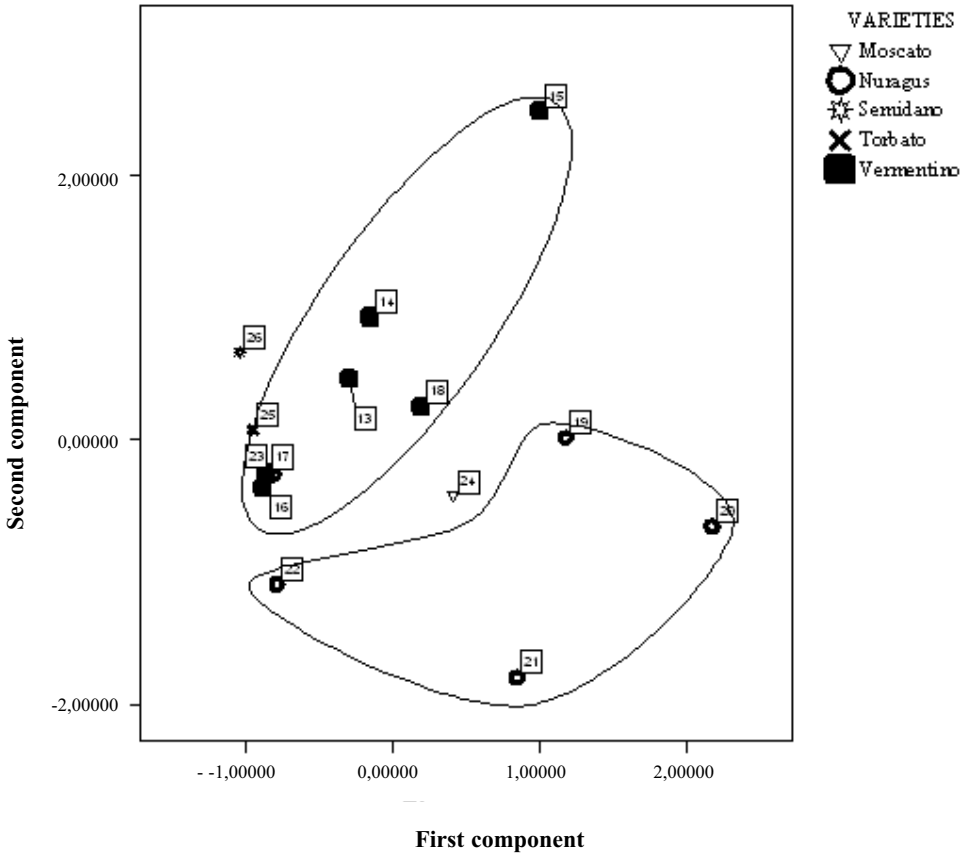


Fig. 3 - Objects identified on the plane of the first and second principal components.

Semidano and Torbato samples present greater affinity with the Vermentino. However, the plane shown by the first two principal components highlights good separation between the two most commonly represented varieties (Vermentino and Nuragus) (Figure 3).

Conclusions

The results obtained enabled characterization of the content of antioxidant substances present in the wines produced in Sardinia from predominantly native cultivars. In particular, the content was determined of hydroxycinnamic acids, resveratrol in its various forms, total polyphenols, flavanols and anthocyanins in the various monomeric forms. Moreover, the quantity of the polyphenolic substances present in 200 mL of wine (recommended daily quantity) was also shown, presenting variability in the range 459-265 mg/200 mL for the red wines and 20-35 mg/200 mL for the white wines. In the case of red wine, those quantities represents a good percentage of the total quantity of antioxidant substances recommended by nutritionists (1 g/day) that should, however, be integrated by a diet rich in fruits and vegetables, naturally abundant in polyphenolic compounds.

The multivariate statistics study enabled it to be concluded that significant differences are not highlighted between the wines produced from red grape cultivars depending on the content of antioxidant species studied. Among the white grapes, on the other hand, it was possible to differentiate between the Vermentino and Nuragus cultivars depending on the content of flavanols, total polyphenols and resveratrols.

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