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CHARACTERIZATION OF GRAPES CULTIVATED IN SARDINIA: CHEMOMETRIC METHODS APPLIED TO THE ANTHOCYANIC FRACTION

CARACTÉRISATION DE CULTIVAR EN SARDAIGNE : MÉTHODES CHÉMOMÉTRIQUES APPLIQUÉES A L'ANALYSE FRACTIONNÉE DES ANTHOCYANES

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SUMMARY

A characterization of red varieties of *Vitis vinifera* cultivated on the island of Sardinia was done on the basis of their anthocyanin profile obtained by means of HPLC analysis.

A comparison between the data on Sardinian varieties and those on continental ones, contained in a reference data bank, was performed. The chemometric method employed was Linear Discriminant Analysis (LDA). The variability of anthocyanin patterns of some varieties, related to different growing areas, ripening stages and training system was tested in relation to this classification method.

This approach showed similarities and differences among varieties, which can usefully support ampelographic and ampelometric description for their characterization.

Key-words: *grapes, anthocyanins, discriminant analysis, chemotaxonomy.*

RÉSUMÉ

La caractérisation de variétés rouges de Vitis vinifera cultivées en Sardaigne est faite sur la base de profils anthocyaniques obtenus à partir d'analyses en CLHP.

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Ces caractéristiques nous ont permis d'effectuer une comparaison entre les variétés de Sardaigne et les données sur d'autres variétées continentales en utilisant notre banque de données. La méthode chémométrique employée est l'Analyse Discriminante Linéaire (LDA). Avec cette méthode de classification nous avons testé la variabilité des profils anthocyaniques de quelques variétés due aux différentes aires de culture, au stade de maturité et aux modes de conduite.

Cette première approche a montré des similitudes et des différences entre variétés qui peuvent appuyer utilement la description ampélographique pour une caractérisation complète de ces cultivars.

Mots clés : *raisins, anthocyanines, analyse discriminante, chimotaxonomie.*

1 - INTRODUCTION

The characterization of the monomeric anthocyanins in grapes was greatly improved since the development of HPLC analysis (WULF and NAGEL, 1978).

Percentage values of singular anthocyanins resulted of great interest because of their keeping considerably constant within a given variety.

Differences among genotypes (SINGLETON and ESAU, 1969) were estimated on the basis of the different quantitative ratios between di-substituted (cyanidin-3-monoglucoside and peonidin-3-monoglucoside) and tri-substituted (delphinidin-3-monoglucoside, petunidin-3-monoglucoside and malvidin-3-monoglucoside) anthocyanins, different methylation of the phenolic functions and the presence or absence of esterificated anthocyanins. In that way, groups were identified using either the similarity of the anthocyanin profile or statistical procedures (WENZEL *et al.*, 1987; ROGGERO *et al.*, 1988; SCIENZA *et al.*, 1985; BAKKER and TIMBERLAKE, 1985). At the same time, synonymy and/or phylogenesis problems for some varieties could be solved. ROGGERO *et al.* (1988) and MATTIVI *et al.* (1989b) found very similar anthocyanin profiles for varieties with partial synonymy (families of Gamay, Pinot, Trollinger, etc.).

In the present work, anthocyanins of some varieties cultivated on the island of Sardinia (Italy) were analysed. The data included in the present paper refer to the first experimentation year.

The historical situation of viticulture in this region plays a relevant role: most of its old varieties are thought to have been imported either during the different foreign dominations or from the countries having the main exchanges with Sardinia.

The aim was to:
a) study and characterize the anthocyanin profile of grapes;
b) compare and group varieties, especially those of presumably autochtonous origin, by means of a mathematical-statistical approach, which uses the profile

data of a wide number of Italian and foreign varieties, recorded and grouped in a reference bank.

Some phylogenetic considerations about Sardinian varieties, assuming a French, Spanish or Tuscan origin, as referred in Italian ampelography, were finally discussed.

2 - MATERIALS AND METHODS

Some varieties of red grapes grown in different areas of Sardinia, were sampled from monovarietal non-clonal vineyards and/or from ampelographic collections, at technological ripeness in 1988.

Furthermore, Cannonau and Cabernet-sauvignon grapes were picked at different ripening stages (August-October) in the same clonal vineyard (Alghero), from two different training systems: tunnel and espalier (it. "tendone" and "spalliera"). All treatments had a balanced clayey-sandy soil, the same root-stock (Kober 5BB, Cosmo sel.), and the same cultivation technique (e.g. soil fertilization, irrigation, etc.). The Cannonau vineyard was 15 years old and the Cabernet vineyard 18 years old.

The sampling of about 1.5-2 kg of grapes was performed according to FREGONI (1985). Berries, cut from stalk with their pedicle, were stored frozen at –20°C until the analysis, which was carried out in March 1989.

Anthocyanins were extracted from the skins of 20 frozen berries, soaked for 12 hours at room temperature in 100 ml methanol acidified with 0.1% HCl 12N and then for two hours in 50 ml of the same solution. By using the above mentioned proportions between skins and solvent, the double extraction succeeded in removing practically all the colour from the skins. Both solutions were mixed and evaporated to dryness in a rotary evaporator at 35°C and the residue redissolved in a solution suitable for HPLC analysis (methanol: 0.3% perchloric acid in water, 27:73, i.e. the starting solution of the HPLC gradient elution). In one case, the pulp was also extracted in the same way. In order to verify the performance of different sample preparation methods as reported in the literature, analyses were carried out by comparing the above method with some of the conditions proposed by other authors. That will be discussed in detail later on.

Some extracts were analyzed in two laboratories, in order to carry out an intercalibration of the analytical procedure.

The separation of the anthocyanins was made in a chromatograph Hewlett Packard 1090M with diode-array detector and 79994A analytical workstation, and by a HPLC Varian 5020, both equipped with a C18 Hypersil ODS 5 µm column. Eluants were: solvent A = 0.3% perchloric acid in water; solvent B = methanol. Flow rate was 0.4 ml/min. The initial solution was 27% solvent B. After injection a step-wise gradient was introduced as follows: (1) increase

solvent B to 36% in 11 minutes, (2) to 45.2% in 7 minutes, (3) then to 51.2% in 3 minutes, (4) lastly to 64% in 5 minutes. Detection was performed at 520 nm Temperature was maintained at 40°C.

The identification of chromatographic peaks was made on extracts purified as reported by PIERGIOVANNI and VOLONTERIO (1980) starting from varieties whose anthocyanic composition was already known (PIERGIOVANNI and VOLONTERIO, 1981). The attribution of peaks (table 1) was based on the information deriving from UV-VIS spectra of each pigment, as well as from retention times in reverse phase column, which are inversely correlated to the polarity, as known from the literature (WULF and NAGEL, 1978; PIERGIOVANNI and VOLONTERIO, 1980; ROGGERO *et al.*, 1988). The joint use of HPLC and diode-array detector, as shown by HEBRERO *et al.* (1988), affords a simple and rapid identification of tested compounds, except for a non relevant peak (generally lower than 0.5%), which elutes between the two p-coumaric esters of delphinidin-3-monoglucoside and cyanidin-3-monoglucoside. That peak could not be identified definitively, but — because of its spectral characteristics and its eluting position — can correspond to the caffeic acid ester of malvidin-3-monoglucoside, a compound already reported by the above mentioned authors.

The analytical data thus obtained were processed using the statistic package SPSS (SPSS Inc., Chicago, Illinois) on computer DEC VAX 8250 VMS 5-02.

Table 1

Absolute retention times, spectral characteristics and identification of the chromatographic peaks

Absolute retention time (min)	Experimental maxima adsorption wavelenghts (nm), with relative intensities	Anthocyanins
5.02	522 (100), 344 (10), 276 (61)	Delphinidin-3-monoglucoside
6.32	516 (100), 327 (10), 278 (69)	Cyanidin-3-monoglucoside
7.53	526 (100), 346 (11), 276 (61)	Petunidin-3-monoglucoside
9.09	516 (100), 327 (10), 278 (70)	Peonidin-3-monoglucoside
10.11	526 (100), 346 (11), 276 (62)	Malvidin-3-monoglucoside
13.55	528 (100), 346 (11), 276 (60)	Delphinidin-3-monoglucoside acetate
15.80	520 (100), 326sh (13), 280 (72)	Cyanidin-3-monoglucoside acetate
17.17	529 (100), 346 (11), 277 (61)	Petunidin-3-monoglucoside acetate
18.96	521 (100), 326sh (13), 280 (72)	Peonidin-3-monoglucoside acetate
19.62	530 (100), 348 (10), 278 (62)	Malvidin-3-monoglucoside acetate
20.39	532 (100), 312sh (69), 280 (93)	Delphinidin-3-monoglucoside p-coumarate
21.57	534 (100), 328 (62), 281 (88)	Malvidin-3-monoglucoside caffeoate
21.87	525 (100), 312 (77), 282 (109)	Cyanidin-3-monoglucoside p-coumarate
22.64	534 (100), 312sh (73), 282 (96)	Petunidin-3-monoglucoside p-coumarate
23.83co	524 (100), 314 (79), 282 (109)	Peonidin-3-monoglucoside p-coumarate
24.00co	534 (100), 312sh (80), 282 (102)	Malvidin-3-monoglucoside p-coumarate

sh: shoulder. co: coeluting peaks.

3 - RESULTS AND DISCUSSION

3.1 Considerations about the sample preparation phase

One of the problems to be solved as for the employment of anthocyanins in the classification of grapes lies in the analysis methods, which should give homogeneous and comparable results; a critical phase in this regard can be represented by the sample preparation before the HPLC analysis.

In this connection the extractant can be methanol (BAKKER and TIMBERLAKE, 1985) or more often methanol acidified with formic acid (WULF and NAGEL, 1978; PIERGIOVANNI and VOLONTERIO, 1980; SCIENZA *et al.*, 1985; WENZEL *et al.*, 1987) or with hydrochloric acid (ROGGERO *et al.*, 1984-1986-1988; HEBRERO *et al.*, 1988; MATTIVI *et al.*, 1989).

The extraction time can be short and combined with the homogenization of skins (WULF and NAGEL, 1978; PIERGIOVANNI and VOLONTERIO, 1980; SCIENZA *et al.*, 1985; BAKKER and TIMBERLAKE, 1985; ROGGERO *et al.*, 1984-1986-1988), or otherwise it can last longer with (HEBRERO *et al.*, 1988) or without homogenization (WENZEL *et al.*, 1987; MATTIVI *et al.*, 1989). A concentration phase in the rotavapor can be included (WULF and NAGEL, 1978; PIERGIOVANNI and VOLONTERIO, 1980; SCIENZA *et al.*, 1985; ROGGERO *et al.*, 1986; HEBRERO *et al.*, 1988; MATTIVI *et al.*, 1989) or not (BAKKER and TIMBERLAKE, 1985; ROGGERO *et al.*, 1984-1988).

The samples are injected in the HPLC without further purification, except in the first works carried out in Italy, that included a clean-up step on a reverse phase cartridge (PIERGIOVANNI and VOLONTERIO, 1980; SCIENZA *et al.*, 1985).

Among the reported problems there is the formation of artefacts when formic acid is added (BAKKER and TIMBERLAKE, 1985) and the hydrolysis of anthocyanin acetates, which is brought about by mineral acids (ANDERSON *et al.*, 1970; BAKKER and TIMBERLAKE, 1985). Formic acid is no more employed during the extraction phase in the most recent works.

A comparative trial between extractants was made using an homogeneous sample of berries from the variety Marzemino. This variety was chosen because of its high content of anthocyanin acetates, both in absolute and in relative quantities.

Five extractions were carried out as reported in the above materials and methods section, using methanol acidified with HCl 12N 0.1%. Five more extractions were carried out under the same conditions, but with non-acidified methanol. Each extract underwent HPLC analysis, before and after dry concentration at 35°C in rotavapor.

The results are summarized in table 2. They show clearly that both non-acidified and acidified methanol lead to equivalent extraction yields. Acidified extractants causes a statistically significant partial hydrolysis of acetates, and at the same time an increase of the relevant free monoglucosides. On the other hand, p–coumarate forms are not affected. Hydrolysis happens at the extraction stage and even more at the concentration in rotavapor. As for our sample, acetate forms were underestimated by 5% (from 22% to 17% of total anthocyanins).

Table 2

Influence of the sample preparation phase on the analytical determination of anthocyanin profile of Marzemino grapes (five repetitions each)

Differences with observed significance level (t-test) lower than 0.05 are quoted (a: comparison solvent 1/solvent 2, without concentration; b: comparison solvent 1/solvent 2, with concentration; c: comparison without and with concentration, solvent 1; d: comparison without and with concentration, solvent 2).

	Percentage areas at 520 nm with the corresponding standard deviation				
	Without concentration		With concentration		
	Solvent 1	Solvent 2	Solvent 1	Solvent 2	t-test ($P < 0.05$)
Delphinidin-3-monoglucoside	18.18/0.38	19/08/0.51	18.27/0.16	19.52/0.44	a b
Cyanidin-3-monoglucoside	1.17/0.05	1.28/0.09	1.16/0.03	1.37/0.07	b
Petunidin-3-monoglucoside	11.08/0.09	11.51/0.20	11.11/0.04	11.93/0.18	a b d
Peonidin-3-monoglucoside	2.38/0.09	2.48/0.10	2.46/0.04	2.57/0.08	b
Malvidin-3-monoglucoside	34.03/0.41	34.31/0.53	34.37/0.39	36.65/0.54	b d
Sum of acetate forms	21.97/0.12	20.25/0.37	21.94/0.14	17.05/0.28	a b d
Sum of p-coumarate forms	10.83/0.16	10.75/0.11	10.37/0.20	10.60/0.15	c
Total cromatographic area	5056/449	4805/224	4957/296	5133/206	d

Solvent 1: methanol. Solvent 2: methanol acidified with 0.1% HCl 12N. Std. dev.: standard deviation.

This can be regarded as the maximum expected error; in most cases the differences between the results given by the different tested methods are less important, because the 120 varieties we had previously investigated (table 4) have a mean acetates content which is 5% of total anthocyanins, whereas the median is situated at 3% (MATTIVI, unpublished results).

Moreover, it is worth considering that the year-related variability can produce definitely higher differences than those connected with the method used. For example, in two years with different weather conditions, grapes from the variety Teroldego showed a mean acetate anthocyanins content of 19.16% of total anthocyanins (with standard deviation = 1.23) in 1987 and of 9.38% (with standard deviation = 2.68) in 1988 (SCIENZA *et al.*, 1989).

Ascertained that the obtained values of anthocyanic profile differ according to the employed preparative stage, it is nevertheless possible to conclude that all four preparation methods provide a highly reproducible result, as can be derived from standard deviations in table 2.

The method described in the above materials and methods section was chosen for the present work, in order to keep a continuity in the historical series of acquired observations, and in order to have the possibility as well to compare in a more direct way the data with those obtained in previous works (MATTIVI *et al.*, 1989) relating to other Italian varieties.

3.2 Intercalibration

Intercalibration was made by analysing in two laboratories the same extracts of 17 samples of Sardinian grapes, chosen in order to cover the widest range of

Table 3
Intercalibration results: correlation coefficients (r), slope and intercept (with the relative percent errors) obtained comparing 17 samples

	r	Slope	Err. %	Intercept	Err. %
Delphinidin-3-monoglucoside	0.929	0.883	(0.090)	–0.010	(0.534)
Cyanidin-3-monoglucoside	0.995	0.874	(0.022)	–0.204	(0.169)
Petunidin-3-monoglucoside	0.957	0.920	(0.072)	0.259	(0.463)
Peonidin-3-monoglucoside	0.994	1.021	(0.030)	–1.620	(0.796)
Malvidin-3-monoglucoside	0.985	1.039	(0.047)	0.098	(2.195)
Sum of acetate forms	0.890	1.660	(0.219)	–0.582	(0.690)
Sum of p-coumarate forms	0.937	0.871	(0.084)	2.440	(1.639)

anthocyanin profile variability. Because of the distance between the two laboratories (about 800 km), a time longer than usual elapsed between extraction and analysis.

Generally, the pigment pattern consists of 15 main anthocyanins and can be described by seven parameters, so retaining the useful information and eliminating possible redundant data (MATTIVI *et al.*, 1989b).

The intercalibration *(table 3)* showed good correlations for all the peaks considered, with the exception of the percentage value of the acetic esters, usually in the lowest quantity and that — in this test with prolonged extract conservation time — underwent a partial degradation by acid hydrolysis.

Therefore they are the most influenced by analytical absolute error.

The differences between the two laboratories, systematic more than casual, are in any case limited, i.e. between 2% and 13%, except for the acetic esters.

3.3 Statistical evaluation

The study of the varieties cultivated in Sardinia started from their comparison with the data of the most important Italian and foreign varieties, formerly classified and included in a data bank (MATTIVI *et al.*, 1989a; MATTIVI *et al.*, 1989b).

Briefly, the reference classification was based on the analytical data of 450 samples from 120 varieties cultivated in Italy, and employed the five free forms of anthocyanins and the sum of the two types of esters (acetates and hydroxycinnamates) *(table 4)*. Among these varieties, nine groups were singled out: the first (group of Pinot) is distinguishable on the basis of the complete absence of anthocyanic esters; the other eight groups were identified through the statistical technique of cluster analysis. After that, some canonical functions allowing to assign each sample to one of the eight groups were produced. This result was obtained through two subsequent Discriminant Analysis procedures. The first one assigned the samples to groups 1–7, the second one further subdivided group 1 in the subgroups 1a and 1b.

The same parameters were considered for the Sardinian grapes *(tables 5, 6 and 7)* and were computed in two steps by the first and second above mentioned discriminant functions in order to assign samples to groups *(tables 8a, 8b and 8c)*.

Table 4
Data bank: list of classified varieties

Group 0

Pinot noir, Pinot gris, Pinot x Dekrot, Pinot tête de nègre.

Group 1a

Ancellotta, Barbera, Bombino, Braubana, Cabernet franc, Cabernet sauvignon, Cabrusina, Codelonghe, Colorino pisano, Croatina, Fortana, Fumat, Fumin, Givan, Lagrein, Lambrusco di Sorbara, Lambrusco grasparossa, Lambrusco maestri, Lambrusco salamino, Malbo gentile, Malvasia di Casorzo, Malvasia nera di Lecce, Malvasia nera di Pisa, Mariabino, Marzemino, Merlot, Negrat, Nera grossa, Petit Verdot, Refoscone, Ribolla nera, Teroldego, Vien de nus, 107-2 (Merlot x Marzemino), 107–3 (Merlot x Marzemino), 95–5 (Cab. Franc x Merlot).

Group 1b

Aleatico, Bonamico, Burghisana, Canaiolo, Cesanese comune, Ciliegiolo, Colorino, Corvina, Fortana nera (Brugnola), Gamay, Grillone, Kolor, Lambrusco di Alessandria, Lambrusco marani, Moscato violetto, Mourvedre, Negrara, Neyret, Pomela schiava, Rafosal, Rondinella, Rossara, Uva rosa, 200–496.

Group 2

Aglianico, Albanina, Aramon, Balsamina, Canena, Cornacchia, Groppello ruberti, Malbech, Negretto, Pavana, Schiava lombarda, Syrah, Tosca, Turca, Incrocio Bruni 147.

Group 3

Bonarda, Brugnola, Casetta, Corvino, Cuneute, Denela, Dindarella, Forgiarin, Jagodinka, Lambrusco oliva, Molinara, Oseleta, Pelara, Picolit nero, Pignul, Quaiara, Rossetta di montagna, Rossiola, Simesara, Sangiovese (Brunello area), Sangiovese (Prugnolo area), Sangiovese (Chianti gallo nero area), Sangiovese (Chianti putto area), Uva d'oro, Vercluna.

Group 4

Cianorie, Colorino di Lucca, Lambrusco a foglia frastagliata, Forselina, Groppello, Malvasia nera di Brindisi, Rossignola.

Group 5

Dekrot, Tocai rosa.

Group 6

Mammolo pisano, Moscato d'Adda, Moscato rosa, Muscat rouge, Nebbiolo, Schiava gentile, Schiava grossa, Trollingher.

Group 7

Tenerone.

Table 5
Anthocyanin profile (percentage areas at 520 nm) of Cabernet-sauvignon grapes sampled during the ripening

Date of harvesting	Delphinidin-3-mono glucoside	Cyanidin-3-mono glucoside	Petunidin-3-mono glucoside	Peonidin-3-mono glucoside	Malvidin-3-mono glucoside	Sum of acetate forms	Sum of p-coumarate forms
Tunnel training system							
22/08	5.22	0.64	4.43	5.09	44.03	26.10	13.62
29/08	6.10	0.66	4.70	5.07	40.71	27.06	14.43
05/09	15.71	0.65	4.24	5.16	38.78	21.16	13.31
12/09	14.11	0.77	5.36	5.80	43.14	16.20	13.83
19/09	6.09	0.65	4.97	5.45	49.09	17.10	15.78
26/09	14.35	0.78	5.74	5.89	42.97	16.91	12.84
05/10	8.43	0.64	5.74	5.13	51.62	14.47	13.46
12/10	5.16	0.41	4.18	4.54	57.24	12.09	14.97
19/10	6.33	0.48	6.32	5.56	53.70	14.84	12.09
Espalier training system							
22/08	15.33	1.33	5.48	6.98	36.14	22.87	10.64
29/08	13.32	1.37	6.38	6.60	37.32	23.90	10.07
05/09	16.43	1.02	6.49	6.55	40.76	17.17	10.84
12/09	13.69	1.34	6.60	8.03	42.59	15.95	11.55
19/09	14.88	0.96	5.86	6.83	42.63	17.31	11.17
26/09	9.19	0.88	5.73	6.95	49.00	14.55	12.38
05/10	18.79	0.89	5.37	5.74	43.44	14.73	10.46
12/10	9.27	0.91	6.31	6.75	50.65	14.37	11.01

Table 6
Anthocyanin profile (percentage areas at 520 nm) of Cannonau grapes sampled during the ripening

Date of harvesting	Delphinidin-3-mono glucoside	Cyanidin-3-mono glucoside	Petunidin-3-mono glucoside	Peonidin-3-mono glucoside	Malvidin-3-mono glucoside	Sum of acetate forms	Sum of p-coumarate forms
Tunnel training system							
22/08	1.21	0.38	2.46	6.32	52.39	2.67	30.19
29/08	2.05	1.60	3.03	7.16	53.36	4.06	26.19
05/09	1.65	0.59	3.60	7.10	57.22	1.69	25.12
12/09	1.29	2.03	2.28	7.91	52.20	3.36	27.43
19/09	1.15	0.65	1.99	6.90	53.50	2.03	30.07
26/09	2.92	0.97	3.74	7.99	59.66	1.87	20.48
05/10	2.76	2.16	3.37	18.05	50.56	3.74	17.62
12/10	2.99	1.79	3.84	15.97	53.17	3.57	16.76
19/10	0.81	0.82	1.87	8.43	54.32	2.47	27.77
Espalier training system							
22/08	2.07	1.52	3.41	6.95	59.09	3.71	20.99
29/08	2.80	1.01	4.10	11.62	58.03	3.10	17.18
05/09	2.18	2.75	3.43	17.24	52.91	2.99	16.60
12/09	2.80	1.19	3.18	14.38	57.43	2.11	17.41
19/09	1.97	2.04	2.91	21.69	50.94	2.71	16.16
26/09	1.35	2.08	2.22	24.29	48.07	3.98	15.49

Table 7

Anthocyanin profile (percentage areas at 520 nm) of grapes from different varieties picked at ripeness

Variety	Growing area	Delphinidin-3-monoglucoside	Cyanidin-3-monoglucoside	Petunidin-3-monoglucoside	Peonidin-3-monoglucoside	Malvidin-3-monoglucoside	Sum of acetate forms	Sum of p-coumarate forms
Alicante	Mandas	7.44	1.98	7.98	10.65	48.74	6.36	14.82
Alicante tintorio (skin)	Mandas	1.70	1.71	2.56	39.53	33.56	2.88	17.51
Alicante tintorio (pulp)	Mandas	0.17	2.58	0.26	78.23	8.87	2.84	6.70
Barbera sardo	Mandas	3.69	1.98	3.88	30.00	37.70	2.91	18.62
Bovale grande	Mandas	9.75	0.91	7.84	2.31	23.72	8.04	43.53
Bovale muristellu	Mandas	7.03	0.59	7.86	1.26	25.92	7.81	45.24
Cannonau	Alghero	2.16	1.40	2.99	17.16	52.71	2.40	19.28
Cannonau	Jerzu	6.83	1.36	8.44	12.28	55.42	1.75	13.13
Cannonau	Mandas	4.97	0.72	4.86	7.82	65.42	1.43	13.49
Cannonau	Oliena	3.78	1.76	4.40	14.14	55.16	2.73	16.67
Cannonau	Dorgali	4.24	3.29	4.50	28.88	46.12	1.42	10.59
Carignan	Alghero	6.20	0.37	6.13	1.74	42.03	1.18	40.57
Giro' rosa	Mandas	6.40	18.07	6.59	24.26	26.88	0.38	15.98
Giro' rosso	Mandas	9.19	2.79	9.26	7.40	53.26	1.85	14.75
Monica	Mandas	8.08	2.81	7.75	14.83	45.73	3.37	15.71
Montepulciano	Alghero	2.02	0.33	3.75	3.36	45.03	7.96	35.82
Pascale di Cagliari	S. Maria	4.87	1.58	5.51	5.18	50.70	2.54	27.36
Pascale di Cagliari	Alghero	4.56	3.59	5.32	18.22	44.23	1.45	20.77
Pinot noir	Alghero	2.19	1.95	3.91	27.89	64.06	0.00	0.00
Sangiovese	Alghero	11.59	24.42	12.86	16.89	32.53	0.07	1.60

For two varieties, grapes samples from different ripening stages and different training systems were included, with the aim of examining a possible influence of these variables on the characterization itself.

The data bank grouping was first tested for Cabernet-sauvignon, a variety spread all over the world and here included in the group 1a. Samples taken at different ripening stages from tunnel and espalier trained wines were considered. Among the 17 picked samples, 15 were assigned to group 1a, and 2, related to overripened grapes from the tunnel system, were classified in group 5 and in group 1b respectively *(table 8a)*.

The data of Cannonau, a variety not included in the data bank, concerning both growing systems, different ripening stages and some samples of ripe grapes from different places of Sardinia, brought out little differences in the anthocyanin profile and a not univocal distribution. These samples were situated in the multidimensional space among groups 1b, 2 and 5 *(tables 8b and 8c)*.

The samples of Cannonau were characterized by a prevalence of tri-substituted anthocyanins in comparison with the di-substituted. The most representative pigment was malvidin-3-monoglucoside. The profile variability was mostly related to the content of peonidin-3-monoglucoside and p-coumaric esters.

By processing the data about Sardinian ripe grapes — as for Pinot noir and Sangiovese — they were assigned to groups 0 and 3. That confirmed the previous assignment of the same varieties included in the reference data bank.

Considering other varieties sampled at ripeness, Barbera sardo was classified in group 1b and showed several differences if compared with the more famous Barbera, typical of Piedmont, which was assigned to group 1a in the data bank. These two varieties were characterized by a different methylation degree. The variety called Alicante revealed a similarity with the variety Cannonau; the analyses of anthocyanins classified Alicante in group 1b as they did for most of the samples of Cannonau at ripeness. Alicante tintorio, a teinturier variety, showed a different chromatographic profile between skin and pulp. These parts of the berry were assigned to groups 4 and 6 respectively, owing to their different malvidin-3-monoglucoside percentages. The anthocyanin profiles of skins and pulps are very closed to the data of Alicante Bouschet (ROGGERO *et al.*, 1986), which is the supposed corresponding variety according to Italian Official Ampelography (MINISTERO AGRICOLTURA E FORESTE, 1960). In particular, they are also practically identical to the ones of the teinturier variety Colorino, coming from the region of Lucca (Tuscany) and included in the data bank.

The two samples of Pascale di Cagliari from different areas have been assigned to two contiguous groups 1b and 2, and that of Monica or Pascale di Sassari to group 1b. The presence in group 1b also of Tuscan varieties Canaiolo and Bonamico (or Giacomino) as well, which the ampelography put in possible relationship with Monica and with Pascale di Cagliari respectively, can be emphasized.

The varieties called Bovale grande and Bovale muristellu, were both assigned to group 2, which includes varieties with a considerable content of p-coumaric anthocyanin esters.

Table 8a

Assignment of the samples inside the groups 1-7 and subgroups 1a-1b of data bank by means of two subsequent discriminant analysis: var. Cabernet-sauvignon samples from different ripening stages and training system

Date of harvesting	Highest group and relative probability of membership	2nd highest group and relative probability of membership
Tunnel training system		
22/08	1 (0.999) 1a (0.991)	
29/08	1 (0.999) 1a (0.999)	2 (0.001)
05/09	1 (0.999) 1a (1.000)	
12/09	1 (0.995) 1a (1.000)	5 (0.005)
19/09	1 (0.947) 1a (0.700)	5 (0.044)
26/09	1 (0.997) 1a (1.000)	5 (0.003)
05/10	1 (0.644) 1a (0.722)	5 (0.356)
12/10	5 (0.969)	1 (0.025)
19/10	1 (0.863) 1b (0.739)	5 (0.136)
Espalier training system		
22/08	1 (1.000) 1a (1.000)	
29/08	1 (1.000) 1a (1.000)	
05/09	1 (1.000) 1a (1.000)	
12/09	1 (1.000) 1a (0.999)	
19/09	1 (0.999) 1a (1.000)	5 (0.001)
26/09	1 (0.994) 1a (0.876)	5 (0.005)
05/10	1 (0.971) 1a (1.000)	5 (0.029)
12/10	1 (0.978) 1a (0.821)	5 (0.022)

Table 8b

Assignment of the samples inside the groups 1-7 and subgroups 1a-1b of data bank by means of two subsequent discriminant analysis: var. Cannonau samples from different ripening stages and training system

Date of harvesting	Highest group and relative probability of membership	2nd highest group and relative probability of membership
Tunnel training system		
22/08	2 (1.000)	
29/08	2 (1.000)	
05/09	2 (1.000)	
12/09	2 (1.000)	
19/09	2 (1.000)	
26/09	2 (0.763)	5 (0.236)
05/10	1 (0.962) 1b (1.000)	2 (0.036)
12/10	1 (0.904) 1b (1.000)	2 (0.084)
19/10	2 (1.000)	
Espalier training system		
22/08	2 (0.791)	5 (0.208)
29/08	2 (0.545)	5 (0.293)
05/09	1 (0.920) 1b (1.000)	2 (0.070)
12/09	5 (0.813)	1 (0.152)
19/09	1 (0.997) 1b (1.000)	2 (0.002)
26/09	1 (0.996) 1b (1.000)	2 (0.004)

Table 8c

Assignment of the samples inside the groups 1-7 and subgroups 1a-1b of data bank by means of two subsequent discriminant analysis: samples of different varieties and from different growing areas and training systems, picked at ripeness

Variety	Growing area (and training system)	Highest group and relative probability of membership	2nd highest group and relative probability of membership
Alicante	Mandas (E)	1 (0.980) 1a (0.926)	
Alicante tintorio (skin)	Mandas (E)	4 (1.000)	
Alicante tintorio (pulp)	Mandas (E)	6 (1.000)	
Barbera sardo	Mandas (E)	1 (0.939) 1b (0.998)	4 (0.054)
Bovale grande	Mandas (E)	2 (1.000)	
Bovale muristellu	Mandas (E)	2 (1.000)	
Cannonau	Jerzu (B)	1 (0.982) 1b (0.999)	5 (0.018)
Cannonau	Oliena (B)	1 (0.706) 1b (1.000)	5 (0.259)
Cannonau	Dorgali (B)	1 (0.983) 1b (1.000)	3 (0.014)
Cannonau	Alghero (E)	1 (0.637) 1b (1.000)	2 (0.346)
Cannonau	Mandas (E)	5 (1.000)	
Carignan	Alghero (T)	2 (1.000)	
Giro' rosa	Mandas (E)	3 (0.998)	1 (0.001)
Giro' rosso	Mandas (E)	1 (0.939) 1b (0.974)	2 (0.035)
Monica	Mandas (E)	1 (0.998) 1b (0.948)	2 (0.002)
Montepulciano	Alghero (E)	2 (1.000)	
Pascale di Cagliari	Alghero (E)	2 (1.000)	
Pascale di Cagliari	Alghero (T)	1 (0.842) 1b (0.998)	2 (0.158)
Sangiovese	Alghero (T)	3 (1.000)	

Training systems: (T) = tunnel; (E) = espalier; (B) = bush pruning (goblet pruning).

On the contrary, Girò rosso and Girò rosa were so different in their profiles, that they were assigned to groups very far from each other, i.e. Girò rosa to group 3 and Girò rosso to group 1b. This difference was ascribed to the prevalence in Girò rosso of the tri-substituted anthocyanins on the di-substituted ones (about 7:1), whereas Girò rosa had a slight prevalence of di-substituted forms.

Carignan grown in Sardinia was assigned to group 2. Also this variety was not present in the reference bank, but its anthocyanin profile agreed with the data reported by ROGGERO *et al.* (1988) so confirming the correspondence with the French variety.

4 - CONCLUSIONS

The applicability of a chemometric method based on anthocyanin profile which aims at obtaining the characterization of different *Vitis vinifera* varieties harvested in Sardinia, confirmed its effectiveness as a complementary tool to the traditional amplelographic ones. The samples of Cabernet-sauvignon, picked at different ripening stages and from two different training systems, were mostly assigned to the same group in agreement with the previous attribution in the data bank. The great differences in climate, geomorphology and cultivation technique between Sardinia and the other areas, and even the different ripening stages, have not significantly influenced the anthocyanic profile, confirming its prevalently genotypic character, as it happened for Pinot noir and Sangiovese picked at ripeness.

The variety Cannonau, not previously studied, shows the tendency to form an autonomous group. The anthocyanin characteristics of this cultivar confirm the ampelographic observations which identify Cannonau as a clone of Grenache.

A possible origine of Pascale di Cagliari and Pascale di Sassari from Tuscan varieties is in accordance with these results, as well as with the official ampelography. The varieties Bovale grande and Bovale muristellu seem to belong to the same family. On the contrary, an anthocyanin profile similarity was not found between Alicante and Alicante tintorio, Girò rosso and Girò rosa, and Barbera sardo and Barbera.

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