Chemical and Sensory Characterization of DOC Red Wines from Marche (Italy) Related to Vintage and Grape Cultivars

EMANUELE BOSELLI,*,† ROGER B. BOULTON,§ JOHN H. THORNGATE,§ AND
NATALE G. FREGA†

Dipartimento di Scienze degli Alimenti, Università Politecnica delle Marche, Via Brecce Bianche,
I-60131 Ancona, Italy, and Department of Viticulture and Enology, University of California,
Davis, California 95616-8749

Monomeric phenols, color and copigmentation parameters, pigments with different chemical structure, tannin, glucose, fructose, glycerol, ethanol, and organic acids were determined in DOC red wines from Marche (Italy), obtained during three different vintages ranging from 1996 to 2000. The intensity of the bitter and astringent tastes of the wines was determined with panel tastings. Lacrima di Morro and Vernaccia di Serrapetrona (obtained from local cultivars) were different from Rosso Piceno, Rosso Piceno Superiore, and Rosso Conero (produced from different percentages of Sangiovese and Montepulciano). Vernaccia, a red, sweet, "spumante" wine, was an outlier. Lacrima showed a low tannin content, a high content of small pigments and phenols, and a high ratio of copigmented color, which persisted after 3 years of aging. The chemical determinations accounted for a high percentage of variability of measured panel astringency, copigmented color, and measured wine absorbance at 520 nm. It was not possible to create a predictive model for bitterness.

INTRODUCTION

The relationship between the sensory evaluation and the chemical composition of wine is a critical subject in current enological research (*1*−*7*). The aim is to understand which components influence the final sensory properties of wines and to what extent they affect it (*8*). Moreover, the determination of the minor (quantitatively) chemical components is a promising approach to assess the stability of the wine (*9*), its origin (*10*) and authenticity (*11*, *12*), and thus its commercial quality. Since the mid-1970s, the development of rigorous procedures for the determination of the sensory properties (*13*−*15*) together with the evolution of the analytical tools in chemistry has provided a large amount of data on the characterization of wine phenolics (*9*, *16*), which are responsible for the color (*17*), bitter taste, and astringency of wine (*18*−*20*). However, it is known that not only chemical composition but also molecular interactions among the wine components play a determinant role in the chemical stability of wine and can affect the sensory properties (*6*), copigmentation providing a significant example (*21*). Finally, once the experimental data have been collected, the multivariate statistical analysis of a large number of determinations has proven to be an irreplaceable procedure for analyzing the results (*11*). Principal component analysis (PCA)

has been used for the determination of the parameters explaining the variability of biological samples (*22*) and wine (*23*), and partial least-squares (PLS) regression analysis has been used to evaluate the reliability of the prediction (*24*).

Much information on the chemical and sensory attributes has been collected on the red wines produced with the "international" grape cultivars, such as Cabernet Sauvignon, Merlot, Pinot noir, and Syrah (*13*, *25*). However, the study and characterization of musts and wines produced with local, autochthon, or unusual cultivars can be also of interest because of their possible commercial exploitation (*26*).

The aim of the current study is the characterization of the sensory aspects and the related chemical components of five minor Italian Denominazione di Origine Controllata (DOC) red wines from the region of Marche, a hilly area facing the Adriatic coast in the central part of Italy, with an old tradition of winemaking for domestic use. The initial approach of this work was the characterization of monomeric phenols, color and copigmentation indices, tannin fractions (monomers and small and large polymeric pigments), sugars, alcohols, organic acids, and pH. The bitter taste and astringency of the wines were determined with panel tasting. The extent to which the variance in the sensory measures could be explained by the phenol composition and other components was determined.

MATERIALS AND METHODS

Samples. The experimental work was performed on five DOC red wines produced in the region of Marche. The determinations were

* Author to whom correspondence should be addressed (telephone +39 071 2204923; fax +39 071 2204980; e-mail eboselli@univpm.it).
† Università Politecnica delle Marche.
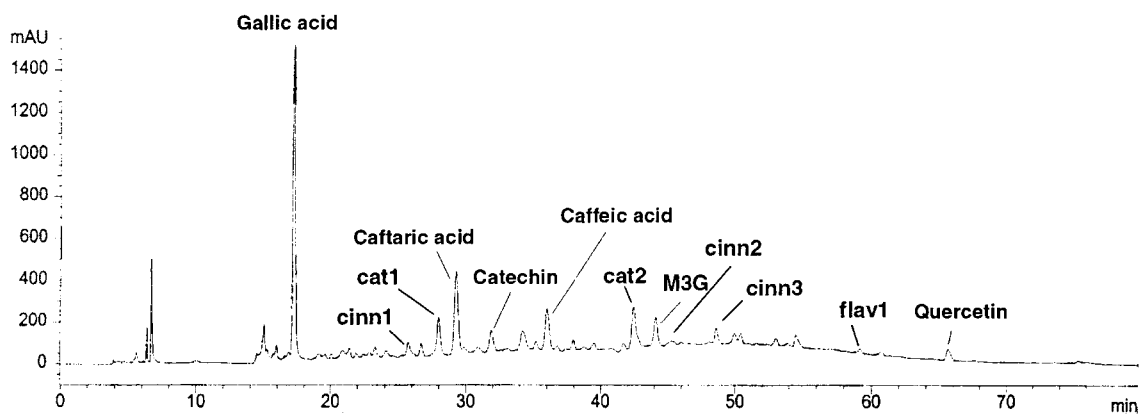§ University of California.

Figure 1. HPLC trace of monomeric phenols of Rosso Piceno Superiore, determined at a wavelength of 280 nm.

carried out on 30 commercial wine samples obtained during three different vintages: Lacrima di Morro d'Alba (L) (1998, 1999, and 2000), Rosso Conero (C) (1997, 1998, and 1999), Rosso Piceno (R) (1996, 1998, and 1999), Rosso Piceno Superiore (S) (1997, 1998, and 1999), and Vernaccia di Serrapetrona (V) (1998, 1999, and 2000). For each vintage, two samples were provided for each wine (bottles A and B). The samples were produced by five different wineries. All of the wines are dry except Vernaccia, which is a red, sweet "spumante" wine obtained with 40% raisin. Lacrima and Vernaccia are produced using the respective homonymous variety of *Vitis vinifera*. Rosso Conero, Rosso Piceno, and Rosso Piceno Superiore are produced in different areas of the region using 85, 50, and 50% Montepulciano variety, respectively, blended with Sangiovese.

Prior to all chemical determinations (except the tannin essay), the wines were adjusted to pH 3.6 and filtered through Acrodisc 13 mm (0.45 μm) PTFE syringe tip filters (Gelman Sciences, Ann Arbor, MI) into 2 mL vials (with a minimum headspace) sealed with PTFE-lined crimp caps. All of the solvents used for the determinations were of analytical grade.

High-Performance Liquid Chromatography (HPLC) of Monomeric Phenols. The determination of monomeric phenols was carried out according to the method reported by Donovan et al. (*27*). HPLC was performed using an HP (Palo Alto, CA) 1100 series HPLC with a UV−visible photodiode array detector. An HP Lichrosphere C18 column (100 RP-18), 4 mm × 250 mm, 5 μm particle size, was used as the stationary phase. The injection volume was 25 μL, and the flow rate of the mobile phase was 0.5 mL/min. Four wavelengths were monitored: 280 nm for catechins and benzoic acids, 316 nm for hydroxycinnamates, 365 nm for flavonols, and 520 nm for anthocyanins. Phenols were identified by comparing their UV−vis spectra and HPLC retention times with those obtained by injecting pure standard substances. The pure standards were purchased from Sigma (St. Louis, MO).

HPLC of Sugars, Organic Acids, Ethanol, and Glycerol. The organic acids (citric, tartaric, malic, acetic, succinic, and lactic), as well as glucose, fructose, glycerol, and ethanol, were quantified using HPLC. The system was composed of an HP 1100 with a refractive index (RI) detector (HP 1047A). A cation H+ cartridge guard column (Bio-Rad, Hercules, CA) was used to protect the separation column, consisting of two 30 cm × 7.8 mm Aminex HPX-87H columns mounted in series. The columns were thermostated at 50 °C. The mobile phase was a solution of 1 mM sulfuric acid at a flow rate of 0.6 mL/min; the run time was 45 min, and the injection volume was 20 μL. For the quantitation, a calibration curve was prepared for each substance; a stock solution containing all of the pure standards was prepared at a concentration of 10 g/L (except citric, succinic, and lactic acid, 2 g/L; ethanol, 10%, v/v). Four different dilutions of the stock solution, 1/1, 1/2, 1/3, 1/5, and 1/10, were used for calibration. The coefficient of determination (R^2) for the calibration curves ranged between 0.9783 and 0.9999.

Sensory Evaluation. The wines were evaluated with panel tastings. Seven panelists were trained to evaluate the astringency and bitterness of wines by tasting two model solutions, which were assigned the

highest score on the intensity scale (10 points). The standard solutions were prepared by dissolving 1.5 g of pure gallic acid (for astringency) or catechin (for bitterness) in 1 L of a wine model solution (12% ethanol in distilled water; 2.5 g/L potassium bitartrate). Mineral water was used as the low standard and was assigned the score of 0 points. The panelists rated the intensity of bitterness and astringency of wines successively using this 11-point scale. The panel tasting took place in an air-conditioned room (24 °C), in five different sessions, on consecutive days (one session for each wine); the session duration was ∼30 min for each judge. The judges tasted the samples in separate booths. During each of the five sessions, each judge evaluated three vintages of the same wine in three replications. Thus, every judge tasted nine glasses of wine presented randomly during each session. Each judge had access to the standard solutions when evaluating the samples and could rinse his/her mouth with water and/or eat unsalted bread ad libitum to reduce carry-over effects. All scorecards were collected at the end of each session, and the average values, given by all seven judges for bitterness and astringency of each sample, were used for the multivariate statistical analysis.

Spectrophotometric Determinations. *(a) Evaluation of Copigmented Anthocyanin Content.* The effect of copigmentation in the wines was evaluated according to the method of Boulton (*21*, *28*), as already described by Mazza et al. (*29*). The spectrophotometer was an HP 8453. The different forms of anthocyanins were expressed in absorbance units as copigmented anthocyanins (copg-ant), total anthocyanins (totA), fraction of color due to copigmented anthocyanins (copg-frct), polymeric pigment content (PPC), and its fraction with respect to the total color (PPf). The flavone content (flavc) and the total phenol content (totP) were also determined.

(b) Polymeric Pigments and Tannin Assay. The absorbance of monomeric pigments (MP), small polymeric pigments (SPP), large polymeric pigments (LPP), and tannin content were determined using the procedures developed by Harbertson et al. (*30*, *31*). The amount of tannin in the wine was calculated using a calibration curve obtained from the determination of 18 solutions of (+)-catechin ranging from 50 to 300 mg/L ($R^2 = 0.99993$).

Statistical Analysis. PCA was performed to examine differences or groupings among the Marche wines by means of all the chemical (absolute peak areas and absorbance units) and sensory data. PLS regression was used to determine the extent to which the variance in the sensory measures (astringent and bitter taste) and copigmented color could be explained by the remaining variables. The data used for the prediction of astringency and bitterness with PLS were the average value given by all of the panelists to each sample. PCA and PLS were performed using The Unscrambler v 7.6 (Camo Inc., Corvallis, OR).

RESULTS AND DISCUSSION

PCA. The data (samples and variables) used for the multivariate analysis are those reported in **Tables 1** and **2**, in addition to six unidentified components (cat1, cat2, cinn1, cinn2, cinn3, and flav1) detected with the HPLC analysis and tentatively identified by evaluating their UV−vis spectra (200−600 nm).

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Table 1. Chromatographic Values (Peak Areas) of Wines Used for the Multivariate Analysis

wine[a]	vintage	gallic acid	caffaric acid	catechin	caffeic acid	malvidin 3-glucoside	quercetin	2-S-gluta-thionyl-caftaric acid	2-S-gluta-thionyl-caffeic acid	tartaric acid	glucose	malic acid	fructose	succinic acid	lactic acid	glycerol	acetic acid	ethanol
L	1998	9775	12180	3231	11167	6981	2883	3647	4897	5944	878	1141	1933	1217	1963	14108	668	86676
L	1998	9632	12315	3174	11080	7196	2911	3835	4901	6100	874	1123	1990	1212	2000	14298	651	83944
L	1999	10373	12235	4014	8919	21852	1505	2148	3015	5377	1034	2259	558	1015	2886	14461	740	83401
L	1999	10650	12179	4070	8921	22329	1662	2300	3144	5592	975	1887	581	921	2779	14179	706	81278
L	2000	8474	13615	4036	11961	49011	2329	1984	2493	6380	1184	2637	770	1877	3167	14279	584	86529
L	2000	9253	13895	3944	11050	46890	2333	1860	2497	5426	680	1903	474	1224	2542	13596	628	86478
C	1997	23299	19715	2459	7588	1795	1316	564	897	6066	561	1914	1714	1323	1308	14198	589	86121
C	1997	22333	19491	2461	6818	1306	1216	531	844	6202	543	1663	283	1279	1281	14500	600	77982
C	1998	23852	19048	2566	7403	2069	1284	413	711	6634	1087	1648	1432	1293	997	13894	565	83401
C	1998	24367	18668	2770	7926	2168	1331	494	667	6591	1039	1575	370	1167	876	13723	545	83493
C	1999	20765	9183	2442	13064	7953	1998	932	1491	6229	946	2307	1318	1668	1659	14690	509	84832
C	1999	20957	8860	2570	13048	7679	1992	955	1467	5967	832	1811	319	1561	1482	14423	449	84793
V	1998	13027	15128	3188	8073	381	787	356	475	2619	33469	1458	38843	1343	2607	11416	492	81622
V	1998	12682	15344	3292	7829	396	797	363	540	5877	33297	1457	38648	1295	2586	11348	501	82000
V	1999	7597	14045	4099	6980	2171	934	301	413	6918	19289	4263	19827	1055	3802	11783	492	79740
V	1999	7210	14162	4138	6869	2140	996	304	435	14752	16102	4171	20010	1025	3766	11681	652	79231
V	2000	8806	15685	4259	7372	4753	883	322	387	6450	20551	4277	19787	1243	3259	11881	438	83932
V	2000	8186	15761	4234	7057	4204	915	290	390	13604	19930	4173	19845	1090	3268	11770	512	82579
S	1997	16751	13304	1808	6720	488	545	507	767	5803	2011	1725	3021	1133	1453	14325	392	82482
S	1997	16672	12827	1984	6549	607	558	501	873	5993	2181	1883	3576	1631	2081	15139	1046	81364
S	1998	21239	14128	2086	7549	2424	848	894	1519	5665	1742	550	600	1204	1967	13623	445	77817
S	1998	21154	14621	2112	7494	2497	855	847	1615	5425	1656	536	536	1123	1828	12860	426	72636
S	1999	19832	16026	2041	7221	4782	1159	463	682	5880	500	1776	500	1216	2119	13221	493	78204
S	1999	19762	16494	2042	7077	4599	1197	458	781	4921	2000	1485	242	1003	1754	11054	404	66380
R	1996	22551	14680	2703	10232	481	1487	232	337	6192	1047	1488	488	1197	1718	16494	490	80938
R	1996	23129	14456	2850	9933	530	1530	246	366	5220	877	1299	420	1041	1486	13964	415	67939
R	1998	21323	12083	2157	8130	269	897	117	201	6105	1996	1583	1535	1116	1464	16132	710	80072
R	1998	22188	12935	2320	8347	464	931	288	252	4839	1700	500	513	996	1302	13868	607	67233
R	1999	19587	11687	2252	8487	2229	1364	144	266	5928	987	1610	500	979	1930	13215	671	75384
R	1999	19941	11645	2162	8264	2013	1379	171	284	4864	1000	1366	438	875	1694	11265	591	63464

[a] Lacrima di Morro (L), Rosso Conero (C), Vernaccia di Serrapetrona (V), Rosso Piceno superiore (S), and Rosso Piceno (R).

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Table 2. Estimation of Copigmentation, Color Content, Tannin, and Monomeric and Polymeric Pigments and Sensory Analysis of Wines

wine	vintage	total copigmented anthocyanins	total antho-cyanins	flavone content	total phenols at 280 nm	wine absorbance at 520 nm	fraction of color due to copigmented anthocyanins	fraction of absorbance due to polymeric pigments at 520 nm	protein-precipitated tannin[a]	large polymeric pigments	small polymeric pigments	monomeric pigments	average astringency	average bitterness
L	1998	0.8	1.9	7.8	26.8	5.18	0.16	0.47	240	0.33	1.20	0.50	6.2	5.3
L	1998	0.3	2.1	8.1	27.2	4.72	0.06	0.49	326	0.25	0.86	0.50	6.0	8.0
L	1999	2.2	1.9	6.1	26.5	5.92	0.37	0.31	108	0.10	1.32	0.53	5.7	5.2
L	1999	1.4	2.3	6.8	31.3	5.58	0.26	0.33	114	0.10	1.04	0.50	3.3	3.7
L	2000	4.3	3.0	8.1	26.3	9.26	0.46	0.21	240	0.16	1.17	0.71	5.8	6.2
L	2000	3.2	3.3	8.4	25.0	8.47	0.38	0.23	362	0.19	0.79	0.72	5.0	5.7
C	1997	0.7	2.7	6.4	25.7	5.39	0.14	0.36	1063	0.75	1.02	0.39	7.3	6.9
C	1997	0.3	1.8	6.3	25.0	4.89	0.05	0.57	1012	0.72	0.69	0.43	8.5	7.5
C	1998	0.5	3.2	6.7	25.6	5.35	0.10	0.31	1230	0.82	1.03	0.40	7.7	5.7
C	1998	0.3	1.7	6.6	25.5	4.99	0.05	0.60	1172	0.74	0.69	0.41	9.0	4.3
C	1999	0.7	2.1	7.5	29.0	5.46	0.14	0.48	1147	0.64	0.95	0.41	6.8	6.7
C	1999	0.6	2.1	7.6	30.3	5.34	0.11	0.50	1008	0.57	0.62	0.43	8.5	5.8
V	1998	0.0	0.9	5.0	34.4	2.58	0.00	0.67	699	0.49	0.82	0.18	6.7	6.0
V	1998	0.0	0.9	4.9	31.4	2.58	0.00	0.67	659	0.40	0.49	0.18	4.7	5.7
V	1999	0.0	0.9	4.4	27.4	2.22	0.00	0.61	330	0.24	0.82	0.19	5.1	4.9
V	1999	0.0	0.9	4.4	25.7	2.17	0.00	0.60	343	0.23	0.50	0.20	3.0	4.3
V	2000	0.0	1.5	5.0	28.3	3.06	0.00	0.52	403	0.32	0.89	0.31	6.3	4.7
V	2000	0.0	1.4	4.8	25.8	2.94	0.00	0.53	409	0.28	0.56	0.33	4.7	5.7
S	1997	0.1	0.7	4.6	28.3	2.18	0.07	0.63	476	0.42	0.80	0.14	6.5	5.8
S	1997	0.1	0.8	4.4	27.7	2.17	0.06	0.59	433	0.27	0.49	0.16	6.5	6.5
S	1998	0.1	1.0	5.2	27.2	2.68	0.06	0.57	600	0.41	0.85	0.19	7.4	6.4
S	1998	0.2	1.0	5.0	29.7	2.57	0.07	0.55	660	0.29	0.52	0.22	5.5	5.8
S	1999	0.3	1.4	5.6	26.5	3.21	0.10	0.46	728	0.56	0.82	0.28	8.1	5.5
S	1999	0.2	1.4	5.6	28.9	3.13	0.06	0.49	724	0.32	0.51	0.29	6.5	6.0
R	1996	0.3	0.8	6.2	30.7	3.56	0.10	0.68	928	0.65	0.85	0.25	7.1	6.2
R	1996	0.0	1.3	6.7	28.3	3.26	0.00	0.66	897	0.52	0.57	0.26	7.3	4.7
R	1998	0.1	0.7	5.4	27.5	2.74	0.05	0.70	833	0.66	0.81	0.14	7.6	6.1
R	1998	0.0	0.8	5.5	29.0	2.65	0.00	0.70	956	0.61	0.48	0.18	7.7	2.0
R	1999	0.2	0.8	4.8	29.7	2.25	0.08	0.57	681	0.34	0.74	0.17	6.5	5.7
R	1999	0.0	0.9	4.9	31.6	2.18	0.01	0.58	642	0.29	0.47	0.19	8.7	3.3

[a] Tannin is reported in mg/L catechin equivalents.

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Figure 2. Principal component analysis of the Marche wines: (**a**) factor 1 versus factor 2 score plot; (**b**) factor 1 versus factor 3 score plot.

A representative chromatogram of monomeric phenols is depicted in **Figure 1**. The 38 variables were analyzed by univariate analysis of variance (ANOVA) to determine those variables that statistically significantly differentiated among the wines; 34 of the original 38 variables were significant using an $\alpha = 0.05$ criterion (data not shown). Given that the number of variables still exceeded the number of objects, the correlation matrix for the variables was analyzed, and an additional nine variables were eliminated as they were highly correlated with other, retained variables (e.g., fructose was highly correlated with glucose, $r = 0.99397$, and was thus eliminated). The PCA of the 25 variables across the 30 samples resulted in a four-factor solution explaining 76% of the total variance. The number of factors chosen was based upon the Scree plot and taking into account the Kaiser criterion and the communality estimates. The first two principal components explained 59% of the variance, as shown in **Figure 2a**. This figure graphs the scores of the samples according to these first two components and overlays the loadings (the location in the PC space of the original variables). The first principal component is positively correlated with the organic acids, catechin, and pH and negatively correlated with tannin, perceptual astringency, and gallic acid. The second PC is positively correlated with unidentified hydroxycinnamate derivative 2, total flavone content, and caffeic acid.

Two groups of samples could readily be differentiated using the first two principal components: The first group includes the six samples (two replications of each of three vintages) of Vernaccia (V98, V99, and V00) (bottom right quadrant). The second group was formed by the six samples of Lacrima (L98, L99, and L00) (upper right quadrant). The remaining wines, namely, Rosso Conero (C98, C99, and C00), Rosso Piceno (R96, R98, and R99), and Rosso Piceno Superiore (S97, S98, and S99), were less well differentiated (left quadrants) in the first two dimensions. As stated in the Introduction and under Materials and Methods, Vernaccia and Lacrima are wines obtained from their homonymous cultivars, which are unique to their respective DOC areas. The remaining wines, however, are produced from mixtures of the cultivars Montepulciano and

Sangiovese. Rosso Conero was well differentiated from Rosso Piceno Superiore but not from Rosso Piceno. Rosso Piceno and Rosso Piceno Superiore wines were poorly differentiated even though different producers employing different technologies vinified these wines in different areas. This poor separation may be a result of leveraging of the data set due to the distinct characteristics of Vernaccia and Lacrima. With regard to the vintage effects, the samples of Lacrima were located in different areas of the upper right quadrant; Vernaccia produced in 1998 was also well separated from its two other vintages. Note that PCA cannot statistically differentiate among these groupings; for that a canonical variates analysis would normally be run, although there are too few objects here to do so.

With regard to the variable loadings, those variables of greater importance (as assessed from the magnitude of their vectors) included both chromatographic and spectrophotometric data. Gallic acid (gall), total flavone content (flavc), lactic acid (lac), and absorbance at 520 nm (A520) were among the most important variables in explaining the variability among the samples. The same figure also shows how the wine samples were related to the variables. Lacrima was the most highly colored wine, although it was poor in large polymeric pigments (LPP). Malvidin 3-glucoside (M3G), small polymeric pigments (SPP), and absorbance at 520 (A520) were all important contributors to the final color of Lacrima. This color was also strongly enhanced by a high content of copigmented anthocyanins (copg-ant and copg-frct were highly correlated with M3G content and excluded from this PCA). Lacrima was also extremely low in tannin content (**Figure 2** and **Table 2**).

Vernaccia is described as a sweet wine unlike Rosso Conero; the latter is perceived as the bitterest wine among them all. Rosso Piceno and Superiore are high in gallic acid, tannin, and large polymeric pigments (LPP) and are thus associated with a strong astringent sensation. Vernaccia is characterized by the highest concentration of sugars; the average concentration of fructose in Vernaccia is 18−40 times higher than that in the remaining wines, whereas the concentration of glucose is 13− 23 times higher. This is due to the different production, as Vernaccia is a red "spumante"-like wine. **Figure 2b** plots the

a

PC2

X-loading Weights and Y-loadings

RESULT1,X-expl: 24%,33%Y-expl: 69%,3%

b

PC2

Correlation Loadings (X and Y)
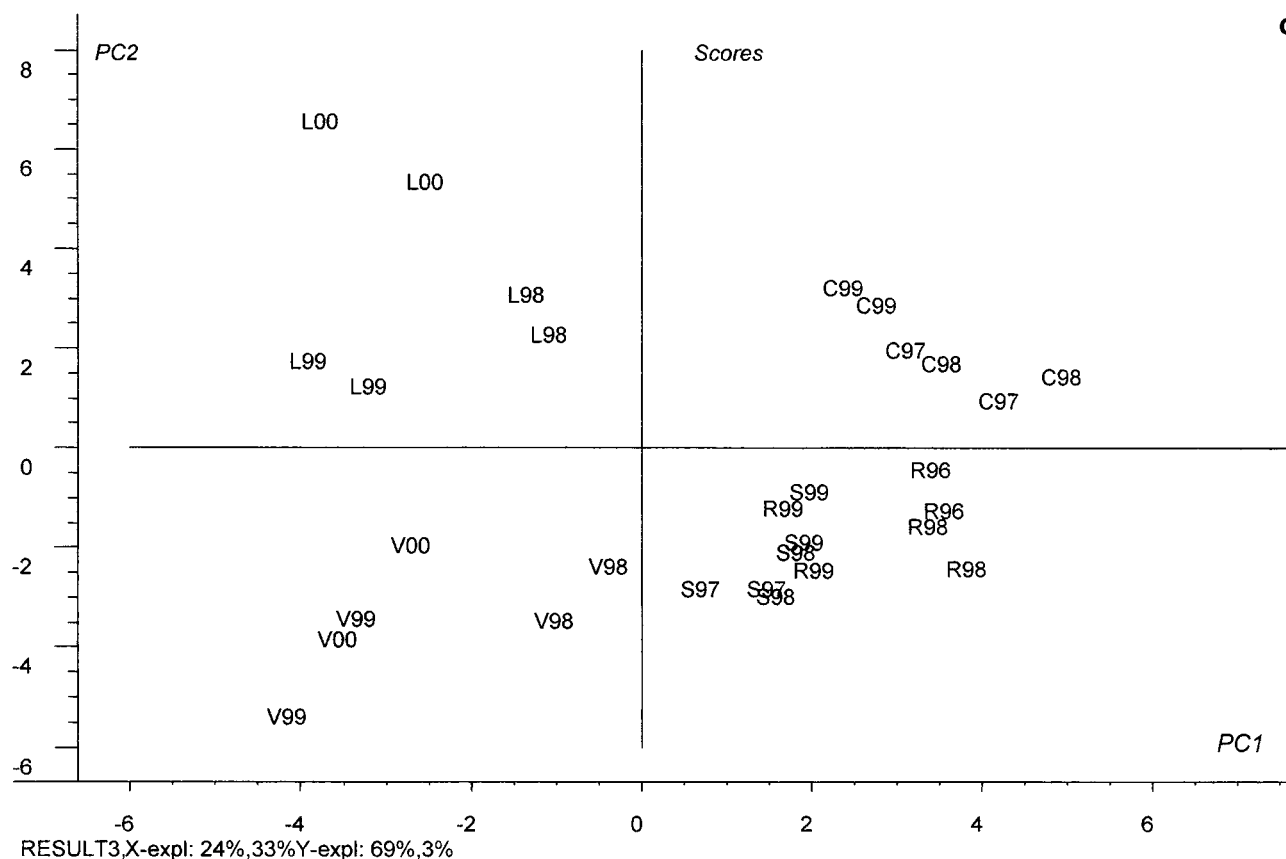
RESULT1,X-expl: 24%,33% Y-expl: 69%,3%

Figure 3. (**a**) Partial least-squares prediction of astringency (astr). (**b**) Correlation loadings for astringency (astr). (**c**) Score plot for astringency.

third principal component (explaining 11% of the variance) versus the first principal component (explaining 30% of the variance). The Lacrima wines are again localized in the right quadrants, but this time the 2000 vintage is not differentiated from the 2000 vintage of Vernaccia. The Vernaccia samples are localized to the upper right quadrant, and this time the Rosso Conero samples (upper left quadrant) are more clearly differentiated from the Rosso Piceno and Rosso Piceno Superiore samples than they were in the first two dimensions (the percentage of Montepulciano is similar in R and S). The unidentified hydroxycinnamate derivatives cinn1 and caftaric acid (caft) are highly positively loaded on PC3; the unidentified hydroxycinnamate derivative cinn2 is highly negatively loaded on PC3.

PLS Predictions of Sensory and Chemical Attributes. The chemical measurements acquired were also used to predict the astringency, the total copigmented anthocyanin content, and the total absorbance at 520 nm of the wines using PLS regression analysis; the most significant variables contributing to these parameters have been characterized. As the ANOVA of bitterness showed that it did not significantly differentiate among the wines ($p = 0.1103$, df $= 4,10$), there was no reason to create a predictive model for bitterness.

The results obtained for astringency are shown in **Figure 3a**. Astringency was positively correlated with protein-precipitable tannin (tann), large polymeric pigments (LPP), gallic acid (gall), and an unidentified catechin derivative (cat2) and negatively correlated with catechin (catech) and organic acid (particularly lactic acid, lac) content. The importance of these variables can be seen in the correlation loadings plot (**Figure 3b**); these are the variables contributing the most to the predictive model. When these six variables (and the unidentified hydroxycinnamate derivative cinn3) are used for a PLS regression, the

explained variance in *Y* remains about the same (data not shown). From this plot it is also clear that those factors indicative of the color (e.g., absorbance at 520, total anthocyanin content, etc.) are not correlated to perceptual astringency (with the exception, of course, of the large polymeric pigment, which is composed of an anthocyanin molecule bound to condensed tannin). These variables are associated with PLS2, which explains only 3% of the perceptual astringency variance. Perceptual astringency was also negatively correlated with fructose and glucose, implying that sugar content (i.e., perceptual sweetness) decreased astringent sensation. The associated scores plot (**Figure 3c**) indicates that the most astringent wines were obtained from blends of Montepulciano and Sangiovese, whereas the Lacrima and Vernaccia wines were less astringent.

It is interesting to note that both the PCA and PLS results indicate that gallic acid is an important contributor to perceptual astringency. Gallic acid, the monomeric phenolic subunit of the hydrolyzable tannins, has been used as an astringent probe by a variety of researchers (*32, 33*). The negative correlation of catechin concentration to perceptual astringency is also of interest; catechin's oral sensation has been characterized as "astringent" by a number of researchers (*34, 35*; compare ref *36*), although catechin is not a chemical astringent. Similarly, the negative correlation of the organic acids to perceptual astringency found here may indicate that their associated oral sensation is not actually astringency per se, although some researchers have characterized it as so (e.g., ref *37*).

Anthocyanin copigmentation is considered to be an important factor determining the intensity and stability of the color of red wines, and its extent has not been evaluated in the wines from Marche so far. The prediction of the content of copigmented anthocyanins is shown in **Figure 4a**. The first two factors
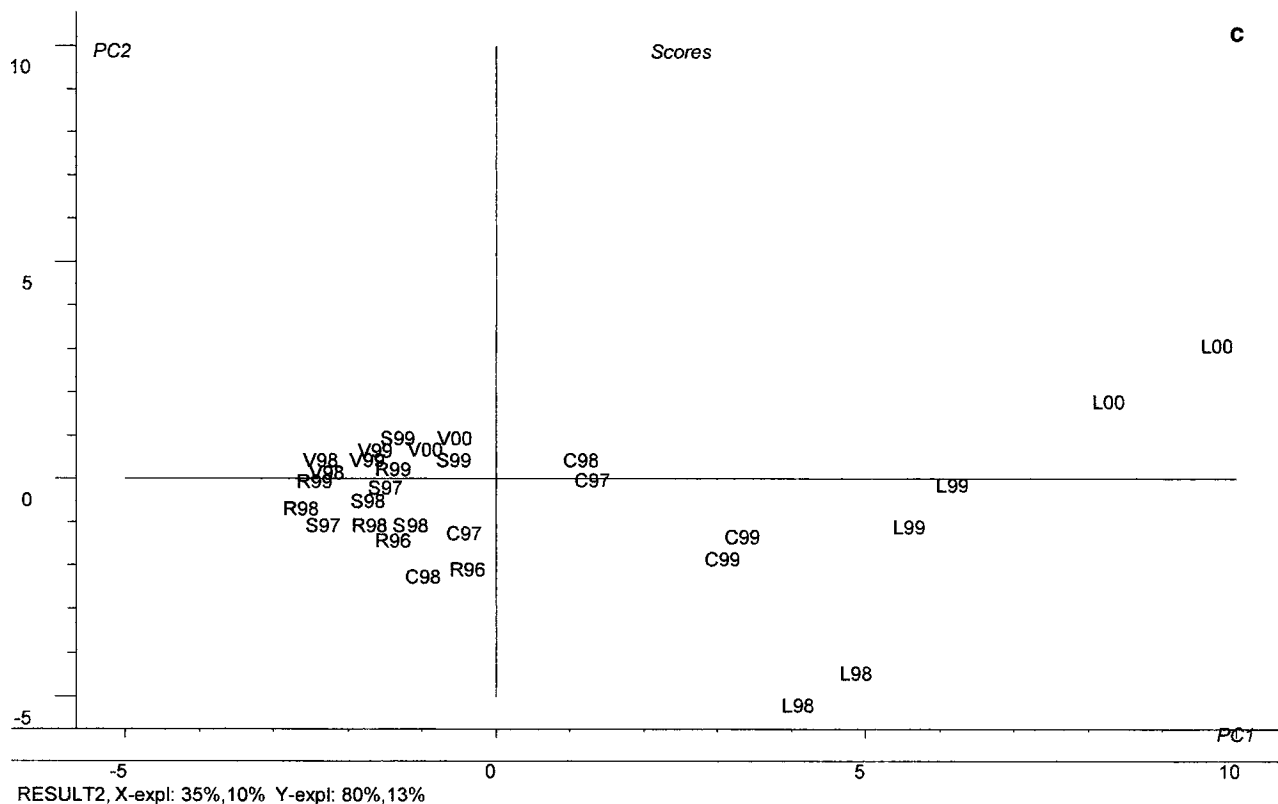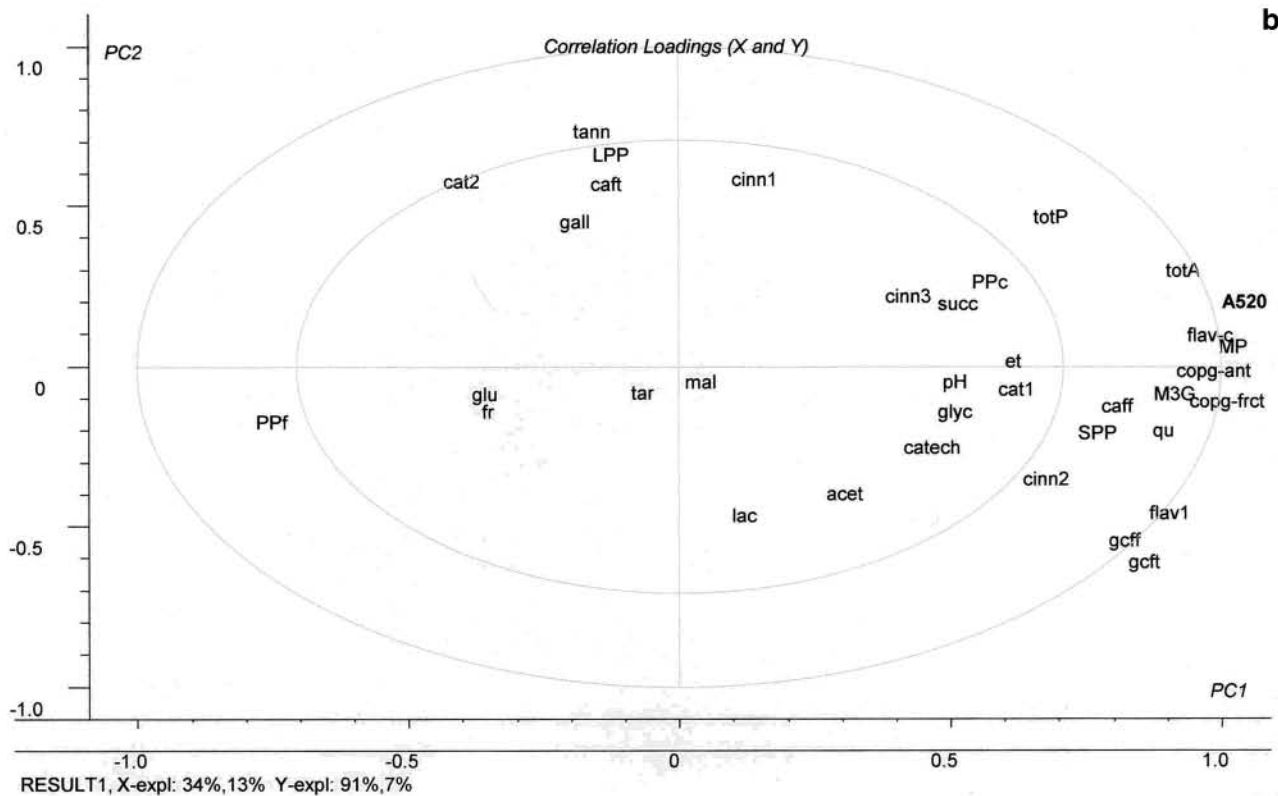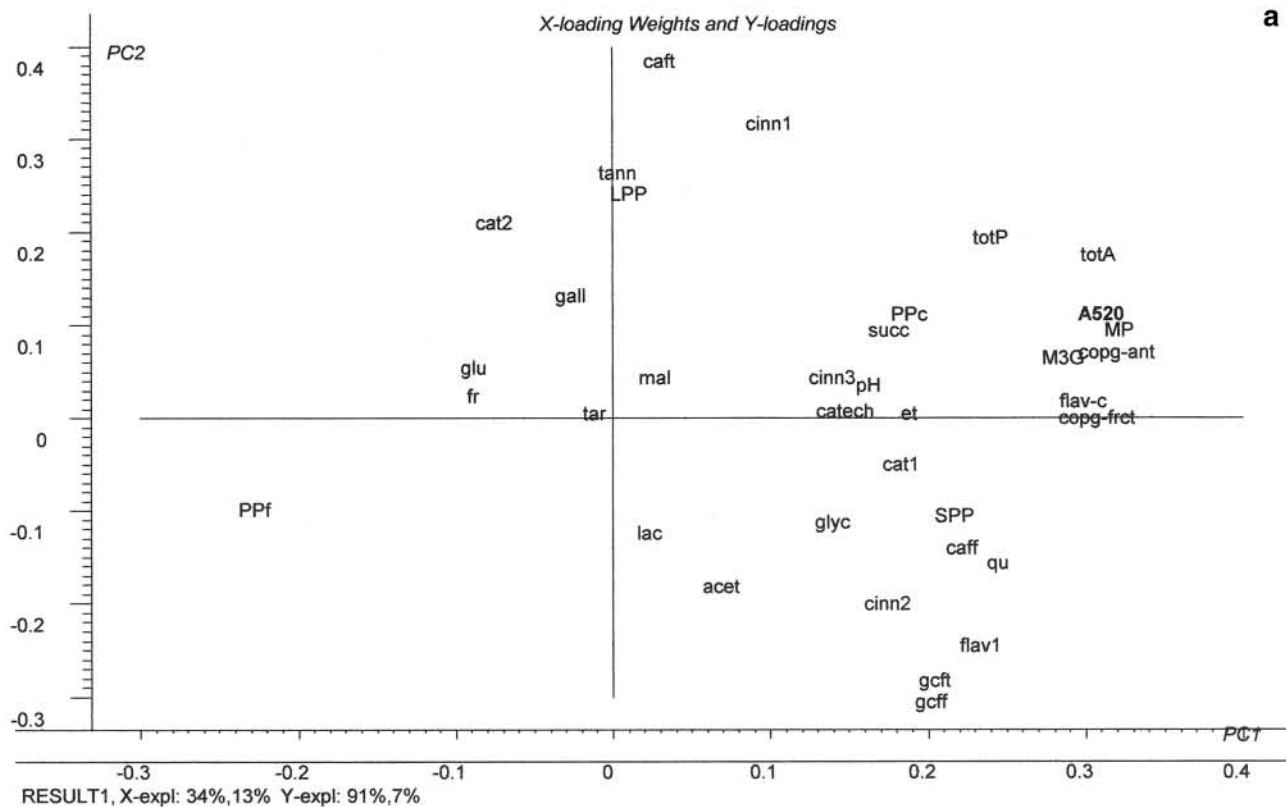
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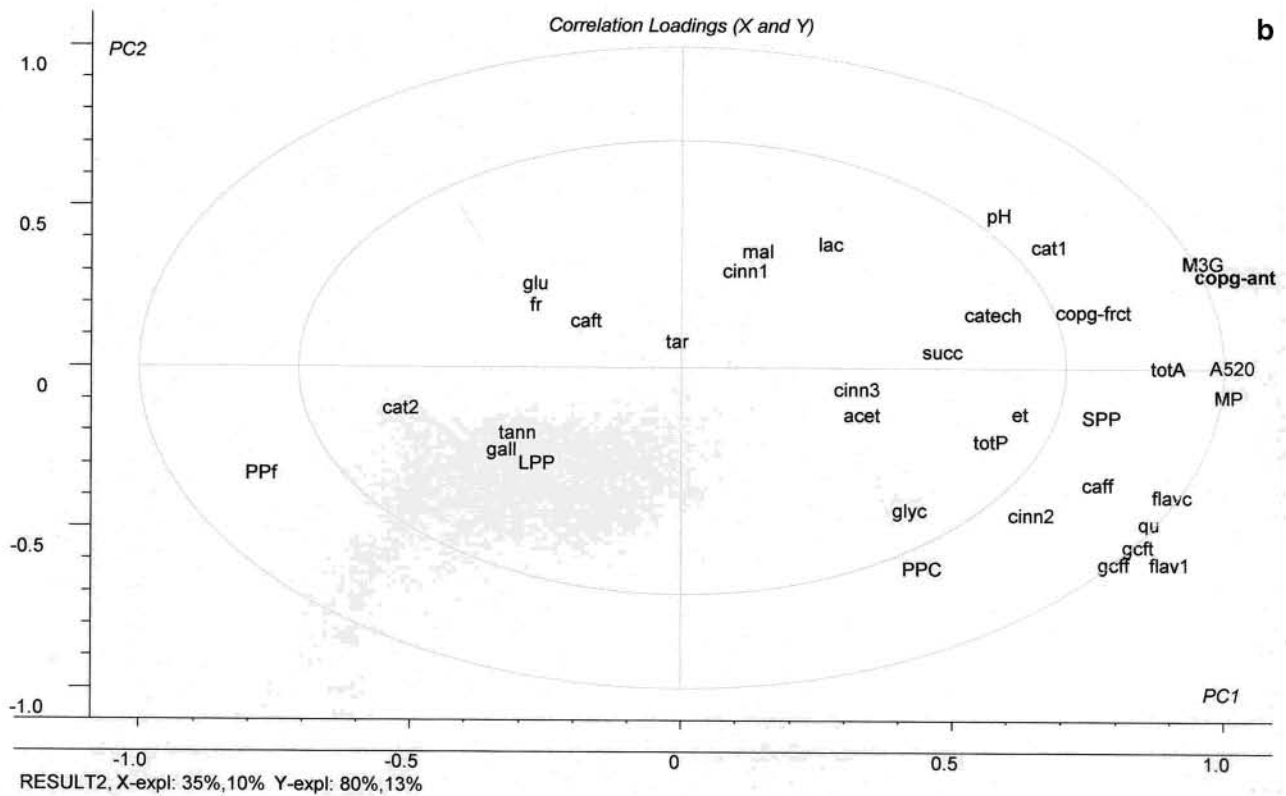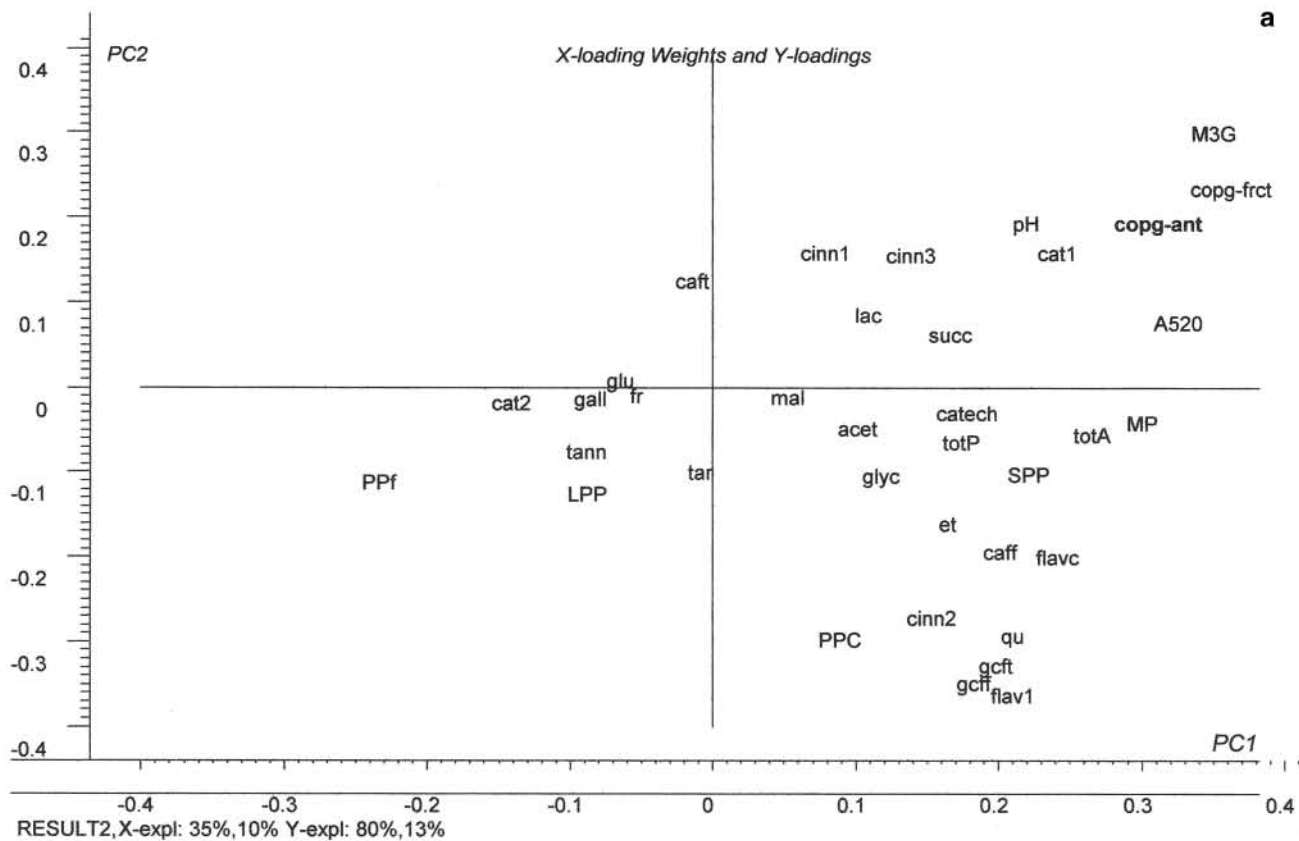
Figure 4. (**a**) Partial least-squares prediction of copigmented anthocyanin content. (**b**) Correlation loadings for copigmented anthocyanin content. (**c**) Score plot for copigmented anthocyanin content.

accounted for 93% of the variance in *Y*. The content of copigmented anthocyanins was positively correlated with malvidin 3-glucoside (M3G), pH, absorbance at 520 nm (A520), unidentified catechin derivative 1, and, of course, the fraction of color due to copigmented anthocyanins (copg-frct). The purple color of the wine (A520) was also highly correlated with the content of copigmented anthocyanins. The copigmented anthocyanins were also associated with the total phenols (totP), but inversely correlated with tannin (tann), large polymeric pigments (LPP), and the fraction of color due to polymeric pigments (PPf), showing that polymeric phenols are not involved in copigmentation.

Whereas the concentration of quercetin (qu) and total flavone content (flavc), putative important cofactors, were not closely associated with the content of copigmented anthocyanin on the loadings plot (**Figure 4b**), the correlation loadings plot indicates that both are indeed important for the predictive model. Also of importance to the predictive model were the absorbance at 520 nm (A520), the malvidin 3-glucoside content (M3G), the total anthocyanin content (totA), the content of monomeric pigments (MP), and the fraction of color due to polymeric pigments (PPf). As is evident from the scores plot (**Figure 4c**), Lacrima was the wine with the highest value of copigmented color, whereas copigmentation in Vernaccia, Rosso Piceno, and Rosso Piceno Superiore was negligible. Both Lacrima and Vernaccia were characterized by the lowest tannin content among the wines (231 and 474 mg/L catechin equiv, respectively), but Lacrima showed the highest content of monomeric pigments and flavones and the lowest content of large polymeric pigments among the wines. This peculiar composition of Lacrima produced a high copigmentation value not only in the new wine but also in the wine aged for 3 years (L98); the final color of this wine was still purple-red. The color of Vernaccia was less purple even in the most recent vintage, and its fraction

of copigmented color was negligible, probably due to its low flavone content or anthocyanin structure. Rosso Conero showed the highest concentration of tannin and a high content of LPP, SPP, and monomeric pigments. Rosso Piceno was characterized by a low content of total and copigmented anthocyanins; thus, the color was due to polymeric pigments (the fraction of color due to polymeric pigments was the highest among the wines). With regard to the color of wines produced in different vintage years, the most important evidence is the lower anthocyanin content in the older wines. These data were confirmed by the HPLC determination of malvidin 3-glucoside. This trend is exhibited in all of the wines except Rosso Conero, possibly because of its high concentration of polymeric pigments and tannins that prevented oxidation of its pigments. The polymerization of anthocyanins into tannin affected copigmentation; the fraction of copigmented anthocyanins decreased, whereas the fraction of color due to the polymeric pigments usually increased (except in Rosso Conero, which showed a constant value regardless of age). With regard to the flavones, they showed different behaviors according to the type of wine: their concentration was lower in Superiore of older vintages; in Rosso Piceno, however, flavones were higher in older vintages; in all of the other wines flavones were variable, probably due to the weather and climatic conditions during ripening of grapes. Polymeric pigments were higher in the older vintages of Lacrima and Rosso Piceno, demonstrating anthocyanin polymerization, but were lower in Superiore. The changes in the concentration of total phenols remain very difficult to interpret.

The main wavelength contributing to the final red color intensity of wines is ~520 nm. Thus, the wine absorbance at 520 nm was modeled using the instrumental data; see **Figure 5a**. Not surprisingly, A520 was positively correlated with total anthocyanin content (totA), monomeric pigment content (MP), malvidin 3-glucoside content (M3G), and copigmented antho-
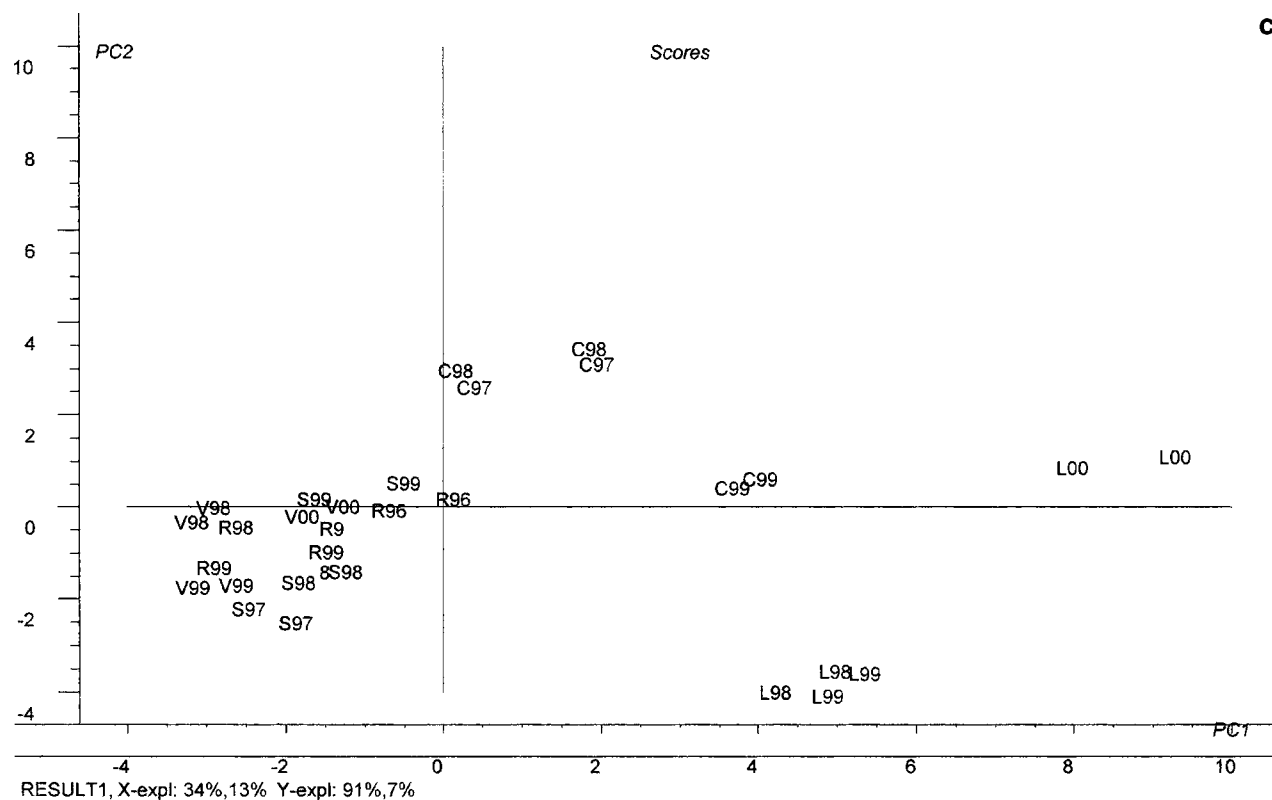
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Figure 5. (**a**) Partial least-squares prediction of wine absorbance at 520 nm (A520). (**b**) Correlation loadings for wine absorbance at 520 nm (A520). (**c**) Score plot for wine absorbance at 520 nm.

cyanins (copg-ant). Interestingly, the fraction of the 520 nm absorbance due to polymeric pigments (PPf) was negatively correlated to A520. **Figure 5b**, the correlation loadings plot, shows those variables of greatest importance to the predictive model; note that the flavones (quercetin, unidentified flavone 1, and total flavone content) are all important variables, in addition to those identified from the loadings plot (**Figure 5a**). The scores plot (**Figure 5c**) confirmed that the Lacrima wines had the greatest absorbance at 520 nm, followed by the Rosso Conero wines.

These results showed that the five DOC wines from Marche could be distinguished by several analytical components. The influence of the cultivar (or blend of cultivars) used for the winemaking was more important than the vintage year. The different phenolic compositions of the wines and the age of the wine had a deterministic influence on the extent of copigmentation. The PLS analysis relating analytical components to perceptual attributes could account for 72% of the measured panel astringency. Ninety-three percent of the variability of the measured copigmented color and 98% of the wine absorbance at 520 nm could be accounted for by the chemical determinations.

ABBREVIATIONS USED

A520, wine absorbance at 520 nm; acet, acetic acid; astr, astringency, caff, caffeic acid; caft, caffeoyltartaric acid; cat1, cat2, unidentified catechin derivatives with maximum absorbance at 280 nm; catech, catechin; cinn1, cinn2, cinn3, unidentified hydroxycinnamic derivatives with maximum absorbance at 315 nm; copg-ant, total copigmented anthocyanins; copg-frct, fraction of color due to copigmented anthocyanins; et, ethanol; flavc, flavone content; flav1, unidentified flavonol derivative with maximum absorbance at 365 nm; fr, fructose;

gall, gallic acid; gcff, 2-*S*-glutathionylcaffeic acid; gcft, 2-*S*-glutathionylcaftaric acid; glu, glucose; glyc, glycerol; lac, lactic acid; LPP, large polymeric pigments; M3G, malvidin 3-glucoside, mal, malic acid; MP, monomeric pigments; PPC, absolute absorbance due to polymeric pigments at 520 nm; PPf, fraction of absorbance due to polymeric pigments at 520 nm; qu, quercetin; SPP, small polymeric pigments; succ, succinic acid; tann, protein-precipitated tannin; tar, tartaric acid; totA, total anthocyanins; totP, total phenols at 280 nm.

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