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Prediction of Wine Color Attributes from the Phenolic Profiles of Red Grapes (*Vitis vinifera*)

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Knowledge about the relation between grape and wine phenolics is of key interest for the wine industry with respect to being able to predict wine quality from analyses of grapes. Prediction of the phenolic composition and color of experimentally produced red wines from the detailed phenolic composition of the corresponding grapes was investigated using a multivariate approach. Grape extracts and wines were produced from 55 different grape samples, covering 8 different *Vitis vinifera* cultivars: Alicante, Merlot, Syrah, Cinsault, Grenache, Carignan, Cabernet Sauvignon, and Mourvedre. The phenolic composition of the grapes and wines showed that the average ratios between wine and grape phenolics ranged from 0.25 to 7.9 for the different phenolic compounds. Most interestingly, the average ratios were low for anthocyanins (0.31) and tannins (0.32), intermediate for (+)-catechin (0.75) and polymeric pigments (0.98), and high for gallic acid (7.9). Individual wine phenolics in general correlated well with several grape phenolics, indicating that a multivariate approach might be advantageous for prediction of wine phenolics from grape phenolics analysis. However the use of multivariate prediction of individual wine phenolics from the complete grape phenolic composition only improved the prediction of wine polymeric pigments, whereas wine anthocyanins were predicted with the same precision as from the direct relation with grape anthocyanins. Prediction of color attributes of pH normalized experimental wines from the phenolic profiles of grapes was accomplished using a multivariate approach. The correlation between predicted and measured total wine color was high ($r = 0.958$) but was very similar to the correlation coefficient obtained for the direct relation between grape anthocyanins and total wine color ($r = 0.961$). Color due to copigmentation, color due to anthocyanins, and color intensity were also predicted well.

KEYWORDS: Polyphenols; red grapes; red wine; wine color; correlation; prediction.

INTRODUCTION

It has long been recognized that the color intensity of young red wines to some extent correlates positively with the overall wine quality (*1, 2*). It is also known that the color of red wine, to a large degree, depends on its phenolic composition, notably the level of anthocyanins, anthocyanin derivatives, and polymeric pigments (*3–5*). The polyphenols of red wines also impact the taste and mouth-feel properties (*6*). During the red winemaking process the polyphenols are mainly extracted from the grapes during the 5−14 days of maceration, during which the gradually increasing ethanol concentration, resulting from the fermentation, progressively enhances the extraction (*7*). However, even with prolonged maceration, the extraction of polyphenols rarely exceeds 50% of the total grape phenolic content (*8*). In addition, the extraction of polyphenols from grapes is affected by the winemaking conditions, including, in particular, the fermentation temperature, must freezing, skin to juice ratio, maceration time, and enzyme additions (*9*). All of this complicates the establishment of a direct relationship between grape and wine polyphenols. Even though polyphenols undergo several changes and enter into different types of reactions during winemaking — in particular during the fermentation and maturation steps — the main premise of our current research work on understanding quality parameters of red wine is that the polyphenols present in the grapes have a significant influence on the color of the finished wines. In turn, this has led to the hypothesis that it may be possible to predict the wine color from the levels and the profile of the grape polyphenols.

The two most abundant classes of polyphenols found in grapes are anthocyanins and condensed tannins (**Figure 1**). Anthocyanins are almost exclusively located in the outer layers of the grape skin and, under acidic conditions, are highly colored compounds, which are responsible for the color of red grapes (*10*). Tannins are located in the grape seeds and skin and are highly associated with the mouth-feel properties of wine but have also been reported to affect the color development during wine maturation (*3*). Despite these known associations between certain grape polyphenols and wine color attributes, surprisingly

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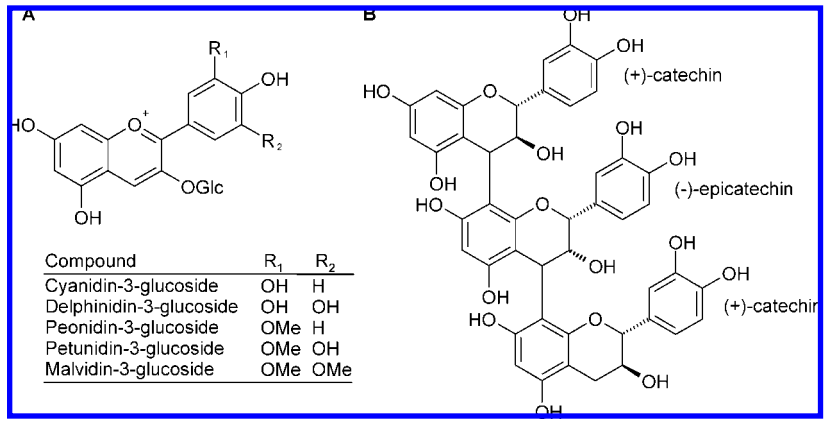

Figure 1. Chemical structures of the unacylated anthocyanins found in *Vitis vinfera* and a hypothetical trimeric procyanidin (tannin) molecule.

Table 1. Grape Sugar Content and Wine Alcohol Levels for the Studied Cultivars[a,b,c]

	grape sugar (°Brix)			wine alcohol (% v/v)		
cultivar	range	mean	SD	range	mean	SD
all samples	18.5–25.6	22.8	1.8	10.4–15.4	13.6	1.2
Alicante	18.6–21.1	19.6 ab	1.1	10.4–12.9	11.5 ab	1.0
Cabernet Sauvignon	21.3–24.9	22.8 bcdef	1.8	12.5–14.4	13.3 bcde	0.9
Carignan	20.4–22.3	21.4 abcd	0.8	12.0–13.2	12.8 abcd	0.5
Cinsault	18.5–24.6	21.8 bcde	2.7	10.5–14.8	13.1 bcd	1.8
Grenache	20.7–23.3	22.5 bcdef	1.2	12.3–14.2	13.6 bcde	0.9
Merlot	21.2–25.6	23.7 def	1.1	12.1–15.4	14.2 de	0.8
Mourvedre	19.8–22.2	21.3 abcd	1.1	11.9–13.3	12.9 bcd	0.7
Syrah	20.9–25.2	23.3 cdef	1.8	11.8–15.0	13.5 bcde	1.4

[a] Four different samples were analyzed for each cultivar, except Merlot with 27 different samples. [b] ANOVA showed significant differences ($p < 0.05$) between cultivars for both grape sugar and wine alcohol levels. [c] Values in the same column followed by the same letter are not significantly different ($p < 0.05$) from a LSD test.

few studies have systematically investigated the overall relation between grape and wine polyphenols. Through the use of an extensive extraction protocol, Iland found a direct linear relation ($R^2 = 0.82$) between grape anthocyanins and wine color density (*11*). González-Neves et al. found that the correlation coefficients between wine color intensity and grape anthocyanins were of similar magnitude irrespective of extracting at pH 1 or at a typical pH of red wine (*12*). The data reported by Romero-Cascales et al. also indicated that anthocyanins extracted at a typical pH of red wine correlated to wine color (*13*). However their results, obtained using five grape samples, also indicated that the extractability of anthocyanins from grapes affected the significance of the correlation (*13*).

Because wine color not only relates to the levels of anthocyanins but also to the level of other phenolic compounds (*3, 5, 14*), the use of a multivariate approach on several grape phenolic parameters could lead to a better understanding of the relation between grape phenolics and wine color. The objective of this study was to investigate the relationship between the polyphenols in grapes and those in corresponding young wines and their color attributes at the end of the alcoholic fermentation. We here report the identification of such a relationship and thus demonstrate that at least some wine color attributes can be predicted from the phenolic composition of grapes.

MATERIALS AND METHODS

Chemicals. Technical grade 96% v/v ethanol (V&S Distillers, Aalborg, Denmark) and analytical grade hydrochloric acid (Merck, Darmstadt, Germany) were used for preparing solvents for grape extractions. Acetonitrile, *o*-phosphoric acid, gallic acid, (+)-catechin hydrate, (−)-epicatechin, rutin hydrate, and caffeic acid were all of high-performance liquid chromatography (HPLC) grade and were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade malvidin-3-glucoside hydrochloride was purchased from Extrasynthese (Genay, France). Chemicals for color analysis and protein precipitation: Bovine serum albumin (BSA, fraction V powder), tartaric acid, potassium tartrate, sodium dodecyl sulfate (SDS), triethanolamine (TEA), ferric chloride hexahydrate, potassium disulfite, and acetaldehyde were all of analytical grade and were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Instrumentation. HPLC analysis was carried out on an 1100 series HPLC instrument (Agilent, Santa Clara, CA, USA) equipped with a vacuum degasser, a quaternary pump, an autosampler, a thermostatted column compartment, and a diode array detector. Ultraviolet-visible (UV/vis) absorbance readings were measured on a Lambda2 spectrophotometer (PerkinElmer, Waltham, MA, USA). Infrared spectra in the mid-infrared range (926−5012 cm^{-1}) were measured by Fourier transform interferometry on a Winescan FT120 spectrometer (FOSS, Hillerød, Denmark) equipped with a liquid flow system and a 37 μm calcium fluoride cuvette, thermostatted at 40 °C.

Grape Material. Fifty-five different grape samples covering eight different red cultivars (Alicante, Merlot, Syrah, Cinsault, Grenache, Carignan, Cabernet Sauvignon, and Mourvedre) of *Vitis vinifera* were collected from different fields in the south of France in August and September, 2005 and 2006. For each sample, mature grapes were manually picked and stored immediately at −30 °C.

Each sample was manually destemmed and mixed well while frozen. Sample aliquots of 100−250 g were taken from the same lot of frozen grapes for determination of grape sugar (100 g), grape extractions (150 g), and for microscale wine making (250 g). For sugar determination, the grapes were thawed and manually squeezed to obtain a juice. The grape juice was centrifuged (15 000*g*, 10 min) and filtered through a Whatman grade 4 cellulose filter, and the infrared spectra were recorded on the Winescan. The sugar levels (see **Table 1**) of the grapes were then determined from the infrared spectra via a calibration model for grape juice (FOSS, Hillerød, Denmark).

Grape Extraction Procedure. The grapes were extracted using a fast extraction protocol, found to extract a high proportion of the grape polyphenols (*15*). Briefly, the grapes were thawed and homogenized

thoroughly with an Ultra-Turrax T25 high-speed homogenizer (IKA-Werke & Co. GmbH KG, Janke & Kunkel, Staufen, Germany). Extraction was conducted by mixing a 1:1 (w/v) ratio of grape homogenate and acidic (0.1 M HCl) aqueous ethanol (50% v/v) at 40 °C, followed by neutralization of the added hydrochloric acid with a stoichiometric amount of sodium hydroxide (5M). The sample was centrifuged (15 000*g*, 10 min), filtered through a Whatman grade 4 cellulose filter, and filtrates were frozen for later analyses (HPLC and protein precipitation assay). Total phenols and anthocyanins of the unfrozen filtrates were measured as outlined below. The phenolic contents per grape mass unit were calculated from the diluted extracts using an experimentally determined average volume of extracted sample (V_s) at 1.891 mL of extract per g of grape.

Measurement of Total Phenols and Anthocyanins by Spectroscopy. Samples were centrifuged for 5 min at 23 000*g*, diluted in 1 M HCl, and after one hour the absorbances at 280 and 520 nm were measured in 10 mm quartz cuvettes. The anthocyanin content (abbreviated Anth-spec) was expressed in mg of malvidin-3-glucoside equivalents (ME) per kg of grape from the absorbance at 520 nm (*16, 17*) via use of an extinction coefficient $\epsilon = 58.3$ mL/(mg · cm), found from a standard curve of malvidin-3-glucoside, using equation 1. The content of total phenols per kg of grape was calculated and expressed as 0.01 absorbance units at 280 nm (*16, 17*), from equation 2.

$$\text{Anthocyanins (mg/kg)} = 1000V_s\text{DF}\cdot\text{abs}(520\text{ nm})\cdot 1/\epsilon \quad (1)$$

$$\text{Total phenols (0.01abs)} = 1000V_s\text{DF}\cdot\text{abs}(280\text{ nm})/100 \quad (2)$$

where DF is the dilution factor of the extract in 1 M HCl, V_s is the volume of extracted sample per g of grape, and 1 is the cuvette path length in cm.

Wine Making Procedure. Wines were produced in microscale by the following protocol: approximately 250 g of grapes were weighed and thawed overnight at 5 °C, supplemented with 69 mg/L potassium disulfite (corresponding to 40 mg/L SO_2) and gently crushed for 1 min in a Stomacher laboratory-blender (Seward, Thetford, UK), without crushing the grape seeds. The crushed grapes were transferred to a 500 mL glass bottle, sealed with an airlock, and heated to 25 °C in a water bath. The crushed grapes were supplemented with diammonium hydrogenphosphate (100 mg/L) and inoculated with approximately 0.2 g/L *Saccharomyces cerevisiae* dry yeast (Vinoflora Ruby.ferm, Chr. Hansen, Hoersholm, Denmark), from a yeast starter culture prepared the previous day and kept at 25 °C. The wines were fermented in the dark for a total of 14 days in a thermostatted water bath at 25 °C. Two days after inoculation, the headspace of each fermenting wine sample was carefully replaced with air, and the bottle was shaken to ensure sufficient oxygen for the yeast. During the entire period, the cap was broken twice a day by manually shaking the bottles. The conversion of sugars to ethanol was monitored during the fermentation for a few selected fermentations and was determined for all wines after 14 days of fermentation by measuring the infrared spectra on the Winescan and predicting the level of ethanol and sum of glucose and fructose via a calibration model for fermenting must samples (FOSS, Hillerød, Denmark). After 14 days of fermentation the wines were all fermented to dryness (less than 4 g/L of glucose + fructose, except one Syrah wine sample having 11 g/L glucose + fructose) and had alcohol levels ranging from 10.4 to 15.4% v/v (**Table 1**). The wines were weighed and separated from the pomace by centrifugation (15 000*g*, 10 min) and filtered through a Whatman grade 4 cellulose filter. The wines were then flushed with nitrogen and allowed to settle at 8 °C for one week in airtight flasks, and wine color attributes were measured as described below. In addition, total phenols and anthocyanins of the wines were estimated by spectroscopy (eqs 1 and 2). Samples were frozen for later phenolic analyses (HPLC and protein precipitation). An average yield of sample volume (V_s) at 0.861 mL of wine per g of grape was found and used to report phenolic content based on the original grape mass.

Analysis of Phenolic Compounds by HPLC. Phenolic compounds of both extracts and wines were determined by HPLC using a newly developed method (*15*). Briefly, the separation of the phenolics was conducted on a Gemini C18 column (150 mm × 4.6 mm, 3 μm particle size, 110 Å pore size) from Phenomonex (Phenomenex, Torrance, CA, USA) with a 4 × 3 mm guard column of the same material used as stationary phase at 40 °C. The solvents were: solvent A (water with 0.20 M *o*-phosphoric acid and 3% v/v acetonitrile, adjusted to pH 1.5 with aqueous sodium hydroxide) and solvent B (a 1:1 v/v mixture of solvent A and acetonitrile). A constant flow of 0.5 mL/min was applied with a linear gradient elution profile of: 0 min (11% solvent B), 40 min (40% solvent B), 50 min (60% solvent B), 53 min (100% solvent B), 60 min (100% solvent B), 61 min (11% solvent B), and 66 min (11% solvent B). Prior to injection, each sample was centrifuged at 23 000*g* for 5 min, filtered through a Phenex 0.45 μm nylon syringe filter (Phenomenex, Torrance, CA, USA), and stored under nitrogen until analysis. The injection volume was 10 μL. The compounds were identified according to their retention times and spectral properties. Gallic acid, (+)-catechin, and (–)-epicatechin were quantified at 280 nm from external standard curves of authentic standards. On the basis of spectral identification and external standard curves, hydroxycinnamates (abbreviated hydroxycin) were quantified at 316 nm as caffeic acid equivalents (CFAE), flavonols were quantified as rutin equivalents (RUE) at 365 nm, and anthocyanins (abbreviated Anth-HPLC) were quantified as malvidin-3-glucoside equivalents (ME) at 520 nm (*15, 18*).

Protein Precipitation Assay. Monomeric pigments (MP), polymeric pigments (PP), small polymeric pigments (SPP), large polymeric pigments (LPP), and tannins were measured using a slightly modified method of Harbertson et al. (*19*). Briefly, the method relies on that tannins are precipitated with bovine serum albumin, redissolved, and measured by a color reaction with ferric chloride. The polymeric pigments are measured by bleaching with sulfite and SPP and are defined as the fraction of the polymeric pigments that is not precipitated with bovine serum albumin. Prior to analysis, wine or grape extracts were filtered through Phenex 0.45 μm nylon syringe filters and diluted in a model wine solution of 12% v/v ethanol containing 5 g/L of tartaric acid, which had been adjusted to a pH value of 3.3 with NaOH. The modifications to the original method were as follows. The precipitation step was conducted for 30 min instead of 15 min, the centrifugation speed for forming the tannin−protein pellet was increased from 13 500*g* to 14 000*g*, and finally, the SDS/TEA buffer volume for redissolving the tannin−protein pellet was increased from 0.875 to 1.5 mL to allow background measurement (A^{BG}) on a 1 mL sample, which was then reacted with 0.125 mL of iron chloride (11.4 mM $FeCl_3$ in 11.4 mM aqueous HCl), and the absorbance measured after 10 min (A^{FeCl_3}). Dilution of the samples in the model wine solutions was carried out to give a tannin response (calculated as $1.125A^{FeCl_3} - A^{BG}$) between 0.3 and 0.75, which was defined as the valid range of the assay. Accounting for the dilutions MP, PP, SPP, and LPP were expressed as absorbance units, and tannins were expressed as mg catechin equivalents (CE)/mL from a standard curve of the color reaction between catechin and ferric chloride.

Wine Color Measurements. Prior to all color measurements, wines were normalized to pH 3.6, by adjusting with a minimum volume of aqueous NaOH or HCl and filtered through a Phenex 0.45 μm nylon syringe filter (Phenomenex, Torrance, CA, USA). Boulton's color assay was used to determine the total wine color and wine color due to copigmentation, anthocyanins, and polymeric pigments, respectively (*20*). Full UV/vis transmission spectra (250–750 nm) of the pH adjusted and filtered wines were measured in 1 mm quartz cuvettes. The absorbance values at 420 and 520 nm were used to calculate the color intensity and tonality (*21*).

Repeatability. To asses the experimental error, triplicate grape extractions and wines were produced using the described protocols and were analyzed for three different samples (Cinsault, Merlot, and Alicante). The repeatability (Rep) for each measured variable was calculated as the average standard deviation, obtained from the pooled average variance of the three samples (*22*), divided by the average value (eq 3).

$$\text{Repeatability (in \%)} = \frac{100}{\text{average}(y)}\sqrt{\frac{1}{n(J-1)}\sum_{i=1}^{n}\sum_{j=1}^{J}(y_{ij} - \text{average}(y_i))^2} \quad (3)$$

where n is the number of samples, J is the number of replicate measurements, i is the sample number, j is the replicate measurement number, and y is the value of the measured variable.

Table 2. Mean Values of the Phenolic Composition of Grape Extracts (per kg of Grape) for the Studied Cultivars[a,b,c]

phenolic compound	all samples[d]	Alicante	Cabernet Sauvignon	Carignan	Cinsault	Grenache	Merlot	Mourvedre	Syrah
total phenols (0.01 abs)	1518 (±23%)	2064 c	1585 b	1210 a	876 a	1183 a	1585 b	1621 b	1638 b
anth-spec (mg ME/kg)	1258 (±43%)	2622 f	1381 cde	1265 cde	608 ab	800 abc	1142 bcd	1398 cde	1514 de
MP (abs)	3.4 (±51%)	8.3 f	3.6 cde	3.4 cde	1.7 ab	2.0 abc	2.9 bcd	3.8 cde	4.3 de
SPP (abs)	0.45 (±37%)	0.87 e	0.54 cd	0.33 ab	0.30 ab	0.36 abc	0.41 abc	0.49 bcd	0.53 cd
LPP (abs)	0.53 (±50%)	0.63 bcd	0.65 bcd	0.32 abc	0.23 ab	0.32 abc	0.56 bcd	0.68 cd	0.69 cd
tannins (mg CE/kg)	2662 (±28%)	1826 abc	3492 fg	1923 abc	1303 ab	2204 bcd	2934 def	3347 efg	2701 cde
PP (abs)	0.98 (±38%)	1.5 de	1.2 cde	0.66 abc	0.53 ab	0.68 abc	0.97 bcd	1.2 cde	1.2 cde
gallic acid (mg/kg)	3.4 (±52%)	2.2 abcd	3.3 bcd	1.1 ab	2.5 abcd	1.6 ab	4.7 de	1.1 ab	3.6 bcde
(+)-catechin (mg/kg)	127 (±47%)	101 cd	159 e	40 ab	46 ab	104 cd	170 e	54 abc	93 bcd
(−)-epicatechin (mg/kg)	114 (±51%)	129 cde	97 bcd	23 ab	61 abc	60 abc	157 de	32 ab	105 bcd
hydroxycin. (mg CFAE/kg)	54 (±57%)	122 b	40 a	41 a	38 a	98 b	48 a	37 a	43 a
flavonols (mg RUE/kg)	254 (±34%)	350 cd	295 bcd	244 bc	105 a	230 bc	247 bc	297 bcd	307 bcd
anth-HPLC (mg ME/kg)	1267 (±42%)	2607 e	1339 bcd	1371 bcd	576 a	778 a	1160 bc	1389 bcd	1521 cd

[a] Four different samples were analyzed for each cultivar, except Merlot with 27 different samples. [b] ANOVA showed significant differences ($p < 0.05$) between cultivars for all 13 phenolic compounds. [c] Values in the same row followed by the same letter are not significantly different ($p < 0.05$) from a LSD test. [d] The mean value (±relative SD) for all 55 samples.

Table 3. Mean Values of the Phenolic Composition of Wines (per kg of Grapes Used for Winemaking) for the Studied Cultivars[a,b,c]

phenolic compound	all samples[d]	Alicante	Cabernet Sauvignon	Carignan	Cinsault	Grenache	Merlot	Mourvedre	Syrah
total phenols (0.01 abs)	665 (±27%)	887 f	636 bcd	491 abc	347 ab	391 ab	754 de	596 bcd	711 cde
anth-spec (mg ME/kg)	518 (±38%)	944 e	568 bcd	515 bc	239 a	278 a	497 bc	524 bcd	692 cd
MP (abs)	2.2 (±46%)	4.7 f	2.1 bcde	2.0 bcd	0.96 a	1.1 a	2.2 bcd	2.1 bcde	2.9 ce
SPP (abs)	0.67 (±38%)	1.1 g	0.79 cdef	0.48 abd	0.31 ab	0.32 ab	0.68 cde	0.69 bcdef	0.86 def
LPP (abs)	0.28 (±50%)	0.55 d	0.27 bc	0.21 abc	0.10 ab	0.12 ab	0.30 bc	0.24 abc	0.31 bc
tannins (mg CE/kg)	860 (±38%)	681 abcde	807 cde	547 abcd	422 abc	426 abc	1121 f	675 abcde	702 bcde
PP (abs)	0.95 (±40%)	1.7 e	1.1 cd	0.70 abc	0.41 ab	0.44 ab	0.98 cd	0.93 bcd	1.2 cd
gallic acid (mg/kg)	23 (±42%)	19 abcde	25 cdef	9.5 abc	13 abcd	23 bcdef	29 def	14 abcd	22 bcdef
(+)-catechin (mg/kg)	94 (±49%)	66 cde	107 f	29 abc	32 abcd	63 cde	132 g	48 abcde	59 bcde
(−)-epicatechin (mg/kg)	77 (±57%)	59 bcd	60 bcd	12 ab	37 abc	31 abc	114 e	24 ab	71 cd
hydroxycin. (mg CFAE/kg)	12 (±79%)	35 d	5.4 ab	11 abc	11 abc	17 bc	10 abc	6.1 ab	9.7 abc
flavonols (mg RUE/kg)	86 (±45%)	94 bc	75 abc	90 bc	37 ab	39 ab	94 bc	99 bc	108 bc
anth-HPLC (mg ME/kg)	392 (±39%)	725 e	411 bcd	409 bcd	187 a	233 a	378 bc	384 bcd	491 cd

[a] Four different samples were analyzed for each cultivar, except Merlot with 27 different samples. [b] ANOVA showed significant differences ($p < 0.05$) between cultivars for all 13 phenolic compounds. [c] Values in the same row followed by the same letter are not significantly different ($p < 0.05$) from a LSD test. [d] The mean value (±relative SD) for all 55 samples.

Statistical and Multivariate Data Analysis. Analysis of variance (ANOVA) and the least significant differences (LSD) test (*23*) was carried out to detect differences in the phenolic contents between the grape cultivars and to categorize the significant differences ($p < 0.05$), using MATLAB R14 (MathWorks, Natick, MA, USA) and the Statistics Toolbox 5.0.2 (MathWorks, Natick, MA, USA). Multivariate data analysis was carried out in MATLAB using the PLS toolbox 4.02 (Eigenvector Research, Natick, MA, USA). Principal component analysis (PCA) was performed to visualize the main variations between samples, groupings of samples, and the relation between samples and the phenolic composition. Calibration models were developed with partial least-squares (PLS) regression using leave-one-out cross validation. The optimal number of factors in the model (termed latent variables) was determined by minimizing the root-mean-square error of cross validation (RMSECV). Other model statistics included the correlation coefficient (r) between the actual and predicted values, the root-mean-square error of calibration (RMSEC), and the residual predictive deviation (RPD), defined as the standard deviation of the sample population divided by the standard error in cross validation (SECV).

RESULTS AND DISCUSSION

Phenolics in Grapes and Wines. The determination of the phenolic composition of grapes is strongly dependent upon the employed extraction method. Most reported extraction methods require long extraction times, use of different organic solvents, or multistep sample preparation (*16, 24, 25*). In this study, we have used a newly developed extraction method, by which a high degree of extraction from the grapes has been obtained using acidified aqueous ethanol and short solvent contact time (*15*).

To allow direct comparisons of the phenolic levels in grapes and wines, the phenolic levels were reported in per kg of grape used for grape extraction and winemaking, respectively. The average level of tannins amounted to 2662 mg CE/kg of grape in the grapes (**Table 2**) and 860 mg CE/kg of grape in the wines (**Table 3**). On average, anthocyanins determined by HPLC amounted to 1267 mg ME/kg of grape in the grapes (**Table 2**) and 392 mg ME/kg of grape in the wines (**Table 3**). Considerable amounts of flavonols, (−)-epicatechin, (+)-catechin, hydroxycinnamates, and gallic acid (mostly in wines) were also detected in both grapes and wines (**Tables 2** and **3**). The average levels of small polymeric pigments (0.67 abs) and large polymeric pigments (0.28 abs) in wines (**Tables 2** and **3**) were at least two times lower than the levels reported in commercial wines (*19*). These relatively low levels may be a result of these polymeric pigments being primarily formed during the maturation process (*5*), which was not included in this study.

For both grapes and wines the differences between the eight cultivars were rather complex, and typically, the phenolic levels overlapped between several cultivars. Interestingly, the tannin levels of Merlot wines were found to be significantly higher than those in the wines produced from other cultivars (**Table 3**), whereas tannins in Merlot grapes were only significantly higher than the levels found in Cinsault, Carignan, Alicante, and Grenache, but significantly lower than the tannin levels in

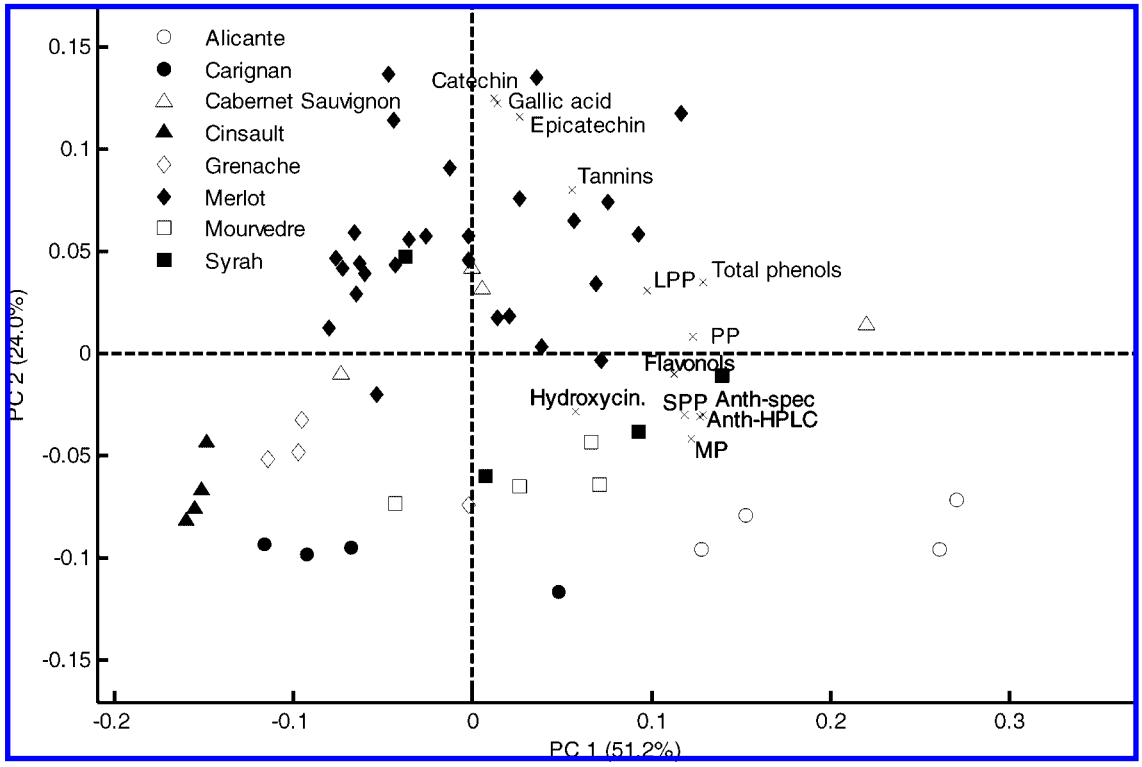

Figure 2. Biplot of scores and loadings from the PCA of the phenolic composition of the grapes.

Mourvedre and Cabernet Sauvignon grapes (**Table 2**). This difference in groupings from grape to wine may be a result of both chemical and physiological differences between the cultivars. Anthocyanin levels in the wines, as determined by HPLC, were consistently the highest in Alicante wines, lowest in Cinsault and Grenache wines and almost similar between the other cultivars (**Table 3**). The same grouping pattern for anthocyanins was found in the grape extracts (**Table 2**), which indicated some similarities between the anthocyanin levels in grapes and wines. The levels of total phenols in grapes seemed to categorize the grapes in three significantly different groups with Alicante having the highest levels; Cabernet Sauvignon, Merlot, Mourvedre, and Syrah having intermediate levels; and Carignan, Cinsault, and Grenache having the lowest levels (**Table 2**). The pattern for total phenols was slightly altered in the wines, in which a bigger overlap between cultivars caused less sharp groupings of the cultivars, which, as for grapes, signified Alicante wines to have high levels; Cabernet Sauvignon, Merlot, Mourvedre, and Syrah wines to have intermediate total phenols levels; and the wines made from Carignan, Cinsault, and Grenache to have low levels of total phenols (**Table 3**).

Sample Characterization by Principal Component Analysis of Phenolics. Principal component analysis (PCA) of the phenolic compositions was used to identify the most important differences between the samples and to relate this to both the phenolic compositions and the cultivar. For grape extracts, the first principal component explained 51% of the variation and was associated with anthocyanins, polymeric pigments, flavonols, and hydroxycinnamates (**Figure 2**). The second principal component explained another 24% of the variation and was associated with tannins, catechins, and gallic acid. Some cultivar differences were observed from the two first principal components (**Figure 2**). Merlot grape samples were found to have quite high levels of tannins, catechins, and gallic acid and intermediate levels of anthocyanins and other pigments. Alicante samples had very high levels of anthocyanins and pigments but had intermediate levels of tannins and catechins. Grenache, Cinsault, and Carignan samples were characterized by low levels of all the phenolics. Mourvedre, Syrah, and Cabernet Sauvignon grapes were generally characterized by intermediate levels of phenolics, although considerable sample differences were recorded for Cabernet Sauvignon and Syrah, in particular. In addition, the first two principal components did not capture the actual high tannin levels of Mourvedre extracts (**Figure 2**), probably due to this cultivar simultaneously having low levels of catechins and gallic acid (**Table 2**). PCA on the phenolic composition of wines (**Figure 3**) gave a slightly higher explained variation (57 and 24%), but gave in general similar groupings as found in the PCA of the phenolic composition of grapes. The largest difference between the two PCA plots were that the position of LPPs moved from the first to fourth quadrant from grape to wine, indicating that the relations between LPP levels and the cultivars were slightly different from grapes to wine.

Ratios between Grape and Wine Phenolics. The magnitude of the ratio between the phenolic contents of wines to grapes (**Table 4**) described how large a proportion of the grape phenolics that was recovered in the wine. The average ratio of 0.44 for total phenols was in good accordance with the general observation that extraction of phenols during wine making rarely exceeds 50% (*8*). However, large differences in the wine/grape ratios were observed among the different phenolic compounds. The most striking observation was that the levels of gallic acid were found to be much higher in wines than in grapes, with an average ratio of 7.9 (**Table 4**). Elevated levels in wines versus the corresponding grapes have also been found by others and are suggested to be caused by a release of gallic acid by hydrolysis of gallate esters during wine manufacturing (*26*). Interestingly, there was a difference between the ratios for small and large polymeric pigments (on average 1.5 and 0.57, respectively), which could reflect differences in formation and/or extraction kinetics. Average ratios for (+)-catechin and (−)-epicatechin were 0.75 and 0.66, respectively, which indicated

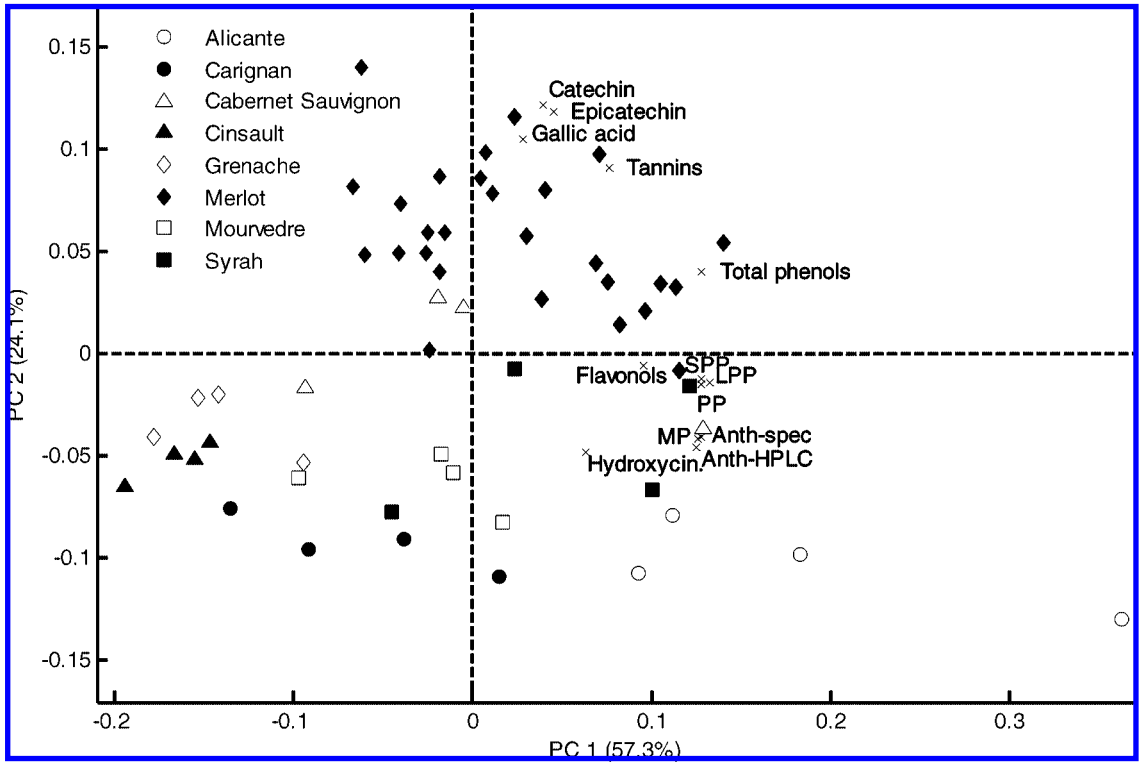

Figure 3. Biplot of scores and loadings from the PCA of the phenolic composition of the wines.

Table 4. Ratio between Phenolic Levels in Grapes and Wines for the Studied Cultivars[a,b,c]

	all samples[d]	Alicante	Cabernet Sauvignon	Carignan	Cinsault	Grenache	Merlot	Mourvedre	Syrah
total phenols	0.44 (±13%)	0.43 cd	0.41 bcd	0.41 bcd	0.40 bcd	0.33 ab	0.48 e	0.37 abc	0.43 cd
anth-spec	0.42 (±12%)	0.36 abcd	0.42 bcdef	0.41 bcdef	0.39 abcde	0.35 abc	0.44 cdef	0.38 abcd	0.47 def
MP	0.66 (±17%)	0.56 ab	0.60 abc	0.61 abc	0.56 ab	0.54 ab	0.74 cd	0.56 ab	0.71 bcd
SPP	1.5 (±24%)	1.3 bcd	1.5 cde	1.5 cde	1.0 abc	0.89 ab	1.7 de	1.4 cde	1.7 de
LPP	0.57 (±43%)	0.86 cde	0.42 abcd	0.66 abcde	0.46 abcd	0.39 abd	0.63 bcde	0.39 abd	0.45 abcd
tannins	0.32 (±27%)	0.37 def	0.22 abc	0.28 bcd	0.33 cde	0.19 ab	0.38 ef	0.20 ab	0.25 abc
PP	0.98 (±24%)	1.1 defgh	0.93 abcdefgh	1.1 cdefgh	0.79 abcde	0.64 abcd	1.1 defgh	0.81 abcdeg	0.97 bcdefgh
gallic acid	7.9 (±47%)	8.7 a	7.6 a	8.8 a	6.0 a	14 b	6.5 a	14 b	5.9 a
(+)-catechin	0.75 (±16%)	0.66 ab	0.67 ab	0.74 abc	0.72 abc	0.60 ab	0.79 bcd	0.90 cd	0.69 abc
(−)-epicatechin	0.66 (±22%)	0.45 abc	0.61 abcdf	0.51 abcd	0.64 bcdef	0.51 abcd	0.74 def	0.74 cdef	0.65 bcdef
hydroxycin.	0.25 (±79%)	0.27 abcde	0.15 abc	0.42 bcde	0.48 cde	0.17 abcd	0.23 abcd	0.18 abcd	0.24 abcde
flavonols	0.34 (±28%)	0.27 abcde	0.24 abcd	0.36 cdef	0.35 cdef	0.17 abc	0.37 def	0.31 bcdef	0.38 def
anth-HPLC	0.31 (±12%)	0.28 abd	0.31 abcd	0.30 abcd	0.33 abcd	0.30 abcd	0.33 bcd	0.28 abd	0.33 abcd

[a] Four different samples were analyzed for each cultivar, except Merlot with 27 different samples. [b] ANOVA showed significant differences ($p < 0.05$) between cultivars for the ratios of all phenolic compounds, except hydroxycinnamates ($p = 0.161$) and anthocyanins HPLC ($p = 0.131$). [c] Values in the same row followed by the same letter are not significantly different ($p < 0.05$) from a LSD test. [d] The mean value (± relative SD) of the ratios for all 55 samples.

that the majority of these compounds were recovered in the wines (**Table 4**). An average ratio of 0.32 for tannins showed that tannins were only partly recovered in the wines, which is in accordance with the known slow extraction of tannins from grapes during winemaking (*9*). Ratios for the tannins showed some differences between the cultivars, with notable high ratios for Merlot and Alicante and low ratios for Grenache, Mourvedre, and Cabernet Sauvignon (**Table 4**). On the other hand, for anthocyanins determined by HPLC, the low average ratio of 0.31 was likely caused by the mixed effect of incomplete extraction and chemical transformations of the anthocyanins during wine making. It is well-known that anthocyanins are simultaneously extracted and transformed during the fermentation (*3*). From the ANOVA we were not able to significantly detect cultivar differences in the ratios for anthocyanins ($p = 0.161$) and pairwise LSD tests showed large overlapping of the anthocyanin levels between the cultivars (**Table 4**). Also, considering the relatively small sample variation in the ratios for anthocyanins (CV = 12%, **Table 4**), it seemed that anthocyanins were recovered to a similar extent in the different cultivars during wine making. The average ratio for MP was more than twice as high as anthocyanins determined by HPLC, which showed that MP was not an accurate measure of anthocyanins (**Table 4**). For grapes, the anthocyanin levels determined by spectroscopy (anth-spec) were in good accordance with levels measured by HPLC (**Table 2**) but not for wines (**Table 3**).

Relation between Grape and Wine Phenolics. To investigate how the phenolic composition of grapes and wines correlated, the correlation coefficients between grapes and wines for individual phenolic groups were calculated (**Table 5**). In general, the level of each wine phenolic was best correlated with the level of the same corresponding phenolic compound in grape, with only a few exceptions. Wine tannins were not very well related to any of the phenolics in grapes (**Table 5**), and it was noticed that the best correlations were found with grape tannins ($r = 0.68$), (+)-catechin ($r = 0.67$), (−)-epicatechin ($r = 0.62$), and gallic acid ($r = 0.61$) (**Table 5**). In

Table 5. Correlation Coefficients (r) between the Content of Phenolics in Grape Extracts and Wines for All Samples ($N = 55$)[a]

	wine content												
grape content	total phenols	anth-spec	MP	SPP	LPP	tannins	PP	gallic acid	(+)-catechin	(−)-epicatechin	hydroxycin.	flavonols	anth-HPLC
total phenols	**0.89**	0.85	0.84	0.87	0.80	0.53	0.88	0.30	0.29	0.28	0.35	0.59	0.83
anth-spec	0.68	**0.95**	0.94	0.88	0.81	0.14	0.88	−0.04	−0.12	−0.09	0.51	0.47	0.94
MP	0.61	0.92	**0.92**	0.84	0.77	0.04	0.84	−0.10	−0.19	−0.13	0.53	0.41	0.91
SPP	0.58	0.83	0.82	**0.82**	0.70	0.08	0.80	−0.04	−0.12	−0.07	0.40	0.35	0.81
LPP	0.61	0.57	0.52	0.62	**0.48**	0.38	0.59	0.16	0.16	0.10	0.05	0.54	0.54
tannins	0.54	0.26	0.18	0.37	0.32	**0.68**	0.37	0.41	0.48	0.33	−0.31	0.40	0.21
PP	0.70	0.79	0.74	0.81	0.66	0.31	**0.78**	0.09	0.06	0.04	0.21	0.54	0.75
gallic acid	0.51	0.07	0.08	0.26	0.20	0.61	0.25	**0.64**	0.80	0.90	−0.10	0.15	0.03
(+)-catechin	0.49	0.00	0.04	0.17	0.20	0.67	0.19	0.66	**0.96**	0.85	0.00	0.09	−0.01
(−)-epicatechin	0.60	0.16	0.22	0.35	0.34	0.62	0.36	0.69	0.84	**0.95**	0.12	0.09	0.14
hydroxycin.	0.22	0.36	0.43	0.28	0.35	−0.15	0.32	0.06	−0.08	−0.10	**0.81**	−0.03	0.39
flavonols	0.66	0.81	0.75	0.74	0.71	0.32	0.75	0.01	0.02	−0.09	0.29	**0.78**	0.82
anth-HPLC	0.68	0.95	0.94	0.87	0.80	0.13	0.87	−0.06	−0.12	−0.08	0.51	0.49	**0.94**

[a] Values in bold indicate the correlation coefficients between grape and wine for the same phenolic compounds.

Table 6. Direct and Multivariate Relation between Grape and Wine Phenolics for All Samples ($N = 55$)

	repeatability[a]		multivariate relation[b]				direct relation[c]	
phenolic compound	grape	wine	LV[d]	r[e]	RMSEC[f]	RMSECV[g]	r[e]	RMSECV[g]
total phenols (0.01 abs)	4%	1%	2	0.910	68	75 (11%)	0.880	86 (13%)
anth-spec (mg ME/kg)	4%	1%	5	0.932	48	61 (12%)	0.941	67 (13%)
MP (abs)	9%	4%	6	0.896	0.29	0.39 (18%)	0.897	0.45 (20%)
SPP (abs)	9%	1%	5	0.916	0.07	0.09 (13%)	0.801	0.15 (22%)
LPP (abs)	21%	10%	1	0.798	0.08	0.08 (30%)	0.384	0.13 (46%)
tannins (mg CE/kg)	6%	3%	3	0.754	189	205 (24%)	0.653	244 (28%)
PP (abs)	13%	3%	5	0.910	0.12	0.14 (15%)	0.755	0.25 (26%)
gallic acid (mg/kg)	9%	2%	2	0.671	6.8	7.2 (31%)	0.608	7.7 (33%)
(+)-catechin (mg/kg)	4%	4%	8	0.912	11	15 (16%)	0.954	14 (15%)
(−)-epicatechin (mg/kg)	5%	3%	5	0.888	12	13 (17%)	0.948	14 (18%)
hydroxycin. (mg CFAE/kg)	18%	5%	6	0.230	4.5	6.2 (52%)	0.735	6.3 (53%)
flavonols (mg RUE/kg)	3%	5%	7	0.518	19	23 (27%)	0.757	25 (29%)
anth-HPLC (mg ME/kg)	5%	2%	6	0.911	40	53 (13%)	0.934	54 (14%)

[a] The repeatability of from triplicate determinations of three samples (in % of the mean) for both grape and wine. [b] Multivariate relation was evaluated from all 13 phenolic compounds of grapes using PLS model with full cross validation. [c] The direct relation between grape and wine was evaluated using a one factor PLS model with full cross validation. [d] LV is the number of latent variables used for the PLS model. [e] The r value is the correlation coefficient between the predicted and measured color attribute. [f] RMSEC is the root-mean-square error of calibration. [g] RMSECV is the cross validated root-mean-square error of prediction, with the % of the mean given in the brackets.

the study of Romero-Cascales et al. it was demonstrated that seed tannins correlated very well ($r = 0.90$) with wine tannins (*13*), however the increased number of samples in our study seemed to scatter the expected relationship between grape and wine tannins. Wine anthocyanins (by HPLC) were highly correlated with grape anthocyanins ($r = 0.94$), but also with grape total phenols ($r = 0.83$), flavonols ($r = 0.82$), SPP ($r = 0.81$), and polymeric pigments ($r = 0.75$) (**Table 5**). Interestingly, wine polymeric pigments (PP, SPP, and LPP) were all slightly better correlated with grape anthocyanins and total phenols, than grape polymeric pigments, which is in good accordance with the central role of anthocyanins in the formation of polymeric pigments (*3, 4*). Despite the low levels of gallic acid determined in grapes, a good correlation from grape gallic acid to wine (+)-catechin ($r = 0.80$) and (−)-epicatechin ($r = 0.90$) was found. In contrast, the correlation between grape and wine contents of gallic acid was lower ($r = 0.64$). This result may be a consequence of the release of gallic acid from different hydrolysis reactions during winemaking.

Because many wine phenolics correlated well with more than one group of phenolics in the grapes (and vice versa), multivariate PLS models using the complete phenolic profiles of grapes to model the levels of individual wine phenolic compounds were developed and compared with models of the direct relationship from grape to wine for each individual phenolic compound (**Table 6**). In general, the RMSECV values of the multivariate models were only slightly smaller than the RMSECV values for the direct relation between the individual grape and wine phenolics. Apparently, the biggest improvement using multivariate models was obtained for the polymeric pigments (SPP, LLP, and PP), with RMSECV values about 40% lower than for the direct linear relations. The observed minor improvements using multivariate models could be because only small evolutions in the phenolic composition of the wines had occurred at the moment of analysis. The repeatability estimates (**Table 6**) of especially the grape determinations (describing the combined sampling, extraction, and measurements errors) in many cases amounted to a considerable proportion of the model errors (RMSECV in %). The highest proportions were found for MP, SPP, LPP, PP, gallic acid, and hydroxycinnamates.

To exclude any potential variation caused by varietal differences between the grape cultivars, the direct and multivariate relations between grape and wine phenolics were analyzed for only the 27 Merlot samples (**Table 7**). For all phenolic compounds, except gallic acid, the RMSECV values of the direct relation between grape and wine phenolics improved (i.e., both the absolute and the relative percent values of the RMSECV data were lower) when only the Merlot samples were studied, as compared to the analyses done on all the grape samples (cf. **Table 6** with **Table 7**). For the Merlot grapes, the RMSECV values of the multivariate relation between grape and wine phenolics as compared to the direct models were slightly

Table 7. Direct and Multivariate Relation between Grape and Wine Phenolics for Merlot Samples ($N = 27$)

	repeatability[a]		multivariate relation[b]				direct relation[c]	
phenolic compound	grape	wine	LV[d]	r^e	RMSEC[f]	RMSECV[g]	r^e	RMSECV[g]
total phenols (0.01 abs)	5%	2%	2	0.857	41	50 (7%)	0.834	54 (7%)
anth-spec (mg ME/kg)	3%	2%	4	0.903	31	41 (8%)	0.920	46 (9%)
MP (abs)	4%	5%	1	0.886	0.20	0.24 (11%)	0.906	0.23 (10%)
SPP (abs)	10%	2%	4	0.782	0.06	0.08 (11%)	0.617	0.13 (18%)
LPP (abs)	7%	10%	1	0.434	0.07	0.08 (27%)	−0.135	0.11 (35%)
tannins (mg CE/kg)	10%	4%	1	0.076	153	170 (15%)	0.641	130 (12%)
PP (abs)	4%	4%	1	0.756	0.13	0.15 (15%)	0.405	0.22 (22%)
gallic acid (mg/kg)	15%	3%	2	0.228	8.4	9.2 (32%)	0.155	9.3 (32%)
(+)-catechin (mg/kg)	7%	2%	2	0.675	16	18 (14%)	0.845	13 (10%)
(−)-epicatechin (mg/kg)	5%	3%	4	0.761	10	13 (11%)	0.865	12 (11%)
hydroxycin. (mg CFAE/kg)	11%	14%	2	0.149	3.6	4.2 (41%)	0.521	3.5 (34%)
flavonols (mg RUE/kg)	7%	10%	2	0.695	22	25 (27%)	0.891	16 (17%)
anth-HPLC (mg ME/kg)	8%	5%	3	0.897	30	38 (10%)	0.888	44 (12%)

[a] The repeatability of from triplicate determinations of one Merlot sample (in % of the mean) for both grape and wine. [b] Multivariate relation was evaluated from all 13 phenolic compounds of grapes using PLS model with full cross validation. [c] The direct relation between grape and wine was evaluated using a one factor PLS model with full cross validation. [d] LV is the number of latent variables used for the PLS model. [e] The r value is the correlation coefficient between the predicted and measured color attribute. [f] RMSEC is the root-mean-square error of calibration. [g] RMSECV is the cross validated root-mean-square error of prediction, with the % of the mean given in the brackets.

Table 8. Relation between Grape Sugar Content (° Brix) and Individual Wine Phenols (Both Total Levels and Ratios) and Modeling of Wine Phenols from Both Grape Phenol and Sugar Levels

	relation between °Brix and wine phenols[a]		relation between °Brix and phenol ratios (wine/grape)[b]		modeling of wine phenols (Merlot) from grape phenols and °Brix[c]		
phenolic cmpound	$r_{\text{all samples}}$	r_{Merlot}	$r_{\text{all samples}}$	r_{Merlot}	LV	RMSECV[d]	r^e
total phenols (0.01 abs)	0.28	0.24	0.62	0.49	1	44	0.89
anth-spec (mg ME/kg)	−0.08	0.37	0.65	0.55	3	40	0.94
MP (abs)	−0.10	0.38	0.59	0.29	3	0.23	0.90
SPP (abs)	0.06	0.52	0.37	−0.07	1	0.12	0.64
LPP (abs)	0.09	0.48	0.08	0.20	2	0.09	0.30
tannins (mg CE/kg)	0.55	0.16	0.42	0.41	1	123	0.69
PP (abs)	0.07	0.56	0.23	0.22	1	0.18	0.61
gallic acid (mg/kg)	0.35	−0.38	−0.32	−0.34	2	9.5	0.21
(+)-catechin (mg/kg)	0.52	−0.28	0.21	0.12	3	13	0.85
(−)-epicatechin (mg/kg)	0.54	−0.05	0.49	0.07	3	12	0.87
hydroxycin. (mg CFAE/kg)	−0.26	0.12	0.04	0.12	1	3.5	0.52
flavonols (mg RUE/kg)	0.22	0.22	0.36	0.38	1	15	0.91
anth-HPLC (mg ME/kg)	−0.12	0.24	0.50	0.20	3	42	0.90

[a] Direct relation between grape sugar content and total level of wine phenols. [b] Direct relation between grape sugar content and the ratio between wine and grape phenols (**Table 4**). [c] The levels of individual wine phenols was modeled from three variables: the level of the grape phenolic compound, the grape sugar content, and the product between grape sugar and phenol content using PLS with full cross validation and up to three latent variables (LV). [d] RMSECV is the cross validated root-mean-square error of prediction, with the % of the mean given in the brackets. [e] The r value is the correlation coefficient between the predicted and measured levels of the individual wine phenol.

improved for total phenols, anthocyanins, and the various polymeric pigments, but the relation deteriorated somewhat for tannins, flavonols, and (+)-catechin (**Table 7**). These cases of poorer multivariate models — as compared to the direct models — of the relation between grape and wine phenolics may be related to the halving of the number of samples, when analyzing only the Merlot samples.

The evaluation of any eventual impact of the variation in grape sugar content on the extraction of phenolics during fermentation (due to the increased ethanol levels) showed that there was only a weak relation between the grape sugar levels and the total levels of the individual wine phenols (**Table 8**). The correlation coefficients between grape sugar and wine phenolics were also not consistent for all samples as compared to only Merlot samples. This could be a result of cultivar differences skewing the relations between the sugar contents and the phenolic levels. A more consistent relation was found between the grape sugar content and the phenolic ratios (wine/grape), indicating that the grape sugar content slightly impacted the extraction kinetics of the phenols (**Table 8**). To test if the grape sugar could improve the prediction of the levels of wine phenolics, a PLS model was developed from the grape sugar content, the levels of the individual phenolic compound, and the interaction term between these two for only the Merlot samples (**Table 8**). In most cases it was only possible to slightly improve the prediction of wine phenols, as compared to the direct relation between grape and wine phenols (**Table 7**). This indicated that wine phenols primarily correlated with the levels of phenols in the grapes and only to a lesser extent with sugar levels.

Prediction of Wine Color Attributes from Phenolic Profiles. Wine color attributes for all samples were determined after pH normalization (pH = 3.6), allowing comparison of the color attributes without interference from the potential influence of pH on the equilibria between the differently colored forms of anthocyanins. Good correlation to total wine color (i.e., the color after adjustment of pH to 3.6) was found for both wine anthocyanins ($r = 0.986$) and grape anthocyanins ($r = 0.961$), which clearly showed the importance of anthocyanins for the color intensity of young wines. It has been shown that grape anthocyanins can be used for predictive purposes for wine color (*11*). However, molecular associations between pigments and

Table 9. Prediction of Color Attributes of pH Normalized Wines from the Phenolic Profiles of Grapes (see **Table** 2) by PLS Regression

color attribute	mean (±relSD)[a]	Rep[b]	LV[c]	r[d]	RMSEC[e]	RMSECV[f]	RPD[g]
total wine color	11.4 (±49%)	1%	5	0.958	1.15	1.60 (14%)	3.5
wine color due to copigmentation	4.0 (±61%)	3%	5	0.962	0.49	0.66 (16%)	3.7
wine color due to polymeric pigments	1.7 (±36%)	1%	4	0.932	0.18	0.22 (13%)	2.7
wine color due to anthocyanins	5.7 (±47%)	1%	5	0.943	0.61	0.87 (15%)	3.0
tonality	0.47 (±8%)	1%	7	0.713	0.02	0.03 (6%)	1.4
color intensity	1.64 (±47%)	1%	5	0.957	0.16	0.23 (14%)	3.4

[a] Mean values (±relative SD) for the 55 samples. [b] Rep is the estimated repeatability from triplicate determinations of three samples (in % of the mean). [c] LV is the number of latent variables used for the PLS model. [d] The *r* value is the correlation coefficient between the predicted and measured color attribute. [e] RMSEC is the root-mean-square error of calibration. [f] RMSECV is the cross validated root-mean-square error of prediction, with the % of the mean given in the brackets. [g] RPD is the residual predictive deviation calculated as SD/SECV.

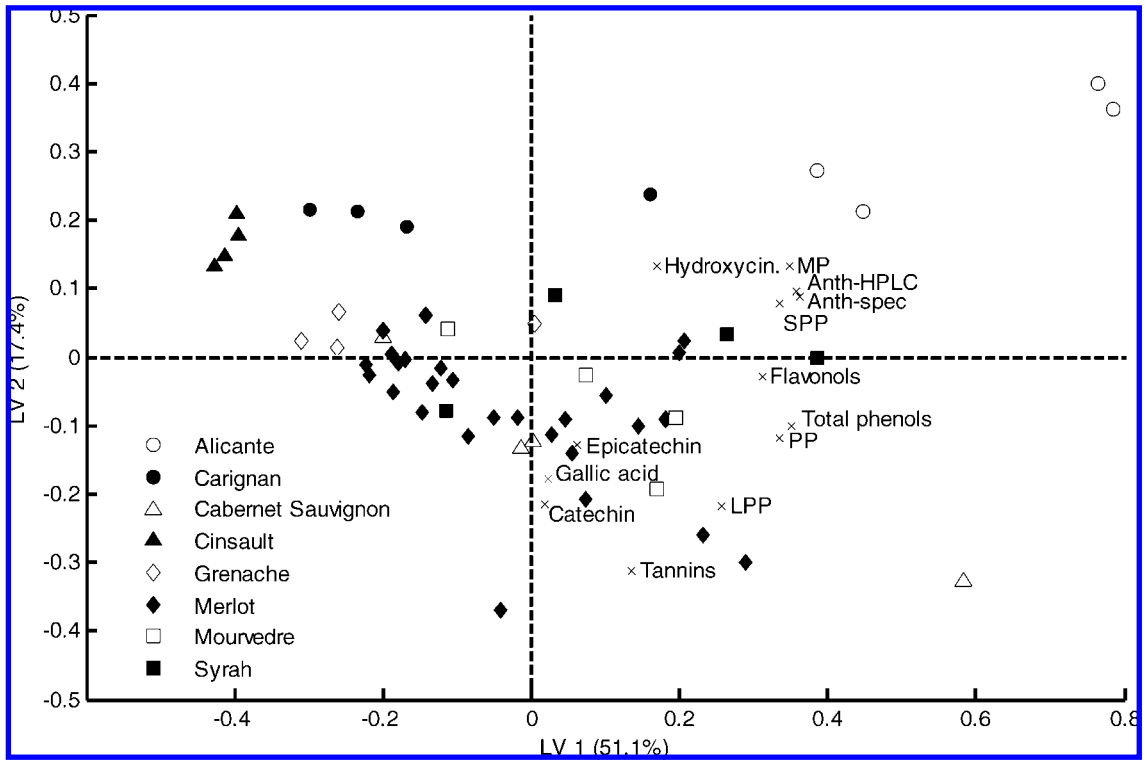

Figure 4. Biplot of the scores and loadings from the partial least-squares regression of total wine color from the detailed phenolic composition of grapes (as in **Table 2**).

noncolored compounds (copigmentation cofactors) are known to strongly increase the red wine color intensity, in some cases up to 50% (*14*). Whereas the color of grapes and young wines is dominated by anthocyanins, these compounds are not very stable, and their color impact moreover varies with pH. As the wine ages, anthocyanins both degrade and condense with other compounds, in particular tannins, producing more stable pigments (*3*). Therefore, red wine color depends not only on the actual concentration of the anthocyanins and the pH, but also on the levels polymeric pigments and copigmentation cofactors, in particular other phenolic compounds.

Color analysis with Boulton's assay (*20*) made it possible to quantify the average percentage of color due to anthocyanins (51%), polymeric pigments (16%), and copigmentation (34%) in the wines. Realizing that the wine color is not only a product of the concentration of anthocyanins, we investigated if using detailed phenolic profiles of grapes would improve the prediction of total wine color and allow prediction of other wine color attributes. The residual predictive deviation (RPD) is a good tool for evaluating model performance, and in general, calibrations with RPD values greater than three are considered to be very good for prediction purposes (*27*). Total wine color (RPD = 3.5), color due to copigmentation (RPD = 3.7), color due to anthocyanins (RPD = 3.0), and color intensity (RPD = 3.4) were predicted very well from the phenolic profiles of the grapes (**Table 9**). Probably due to a lower variation between the samples (relative SD = 36%), color due to polymeric pigments was slightly more difficult to predict (RPD = 2.7). Likewise, color tonality was poorly predicted (RPD = 1.4); this might be ascribed to a very low variation between samples (relative SD = 8%). The repeatability estimates for all the color attributes (**Table 9**) were much lower than the RMSECV percentages and were likely to have a smaller effect on the model errors than the repeatability of the grape measurements (**Table 6**).

The biplot for the PLS regression model for total wine color (**Figure 4**) was very similar (with an opposite sign on the second latent variable) to the PCA of the phenolic composition of grapes (**Figure 2**) and showed that the first latent variable, which was the most important for wine color, once again was associated with the variation on anthocyanins, polymeric pigments, total phenols, and flavonols.

The predicted total wine color correlated well with the measured total wine color (i.e., the color measured after normalization of the pH of the wines to 3.6) ($r = 0.958$; **Figure 5**). This confirmed that prediction of the total wine color from the phenolic composition of grapes could be accomplished by

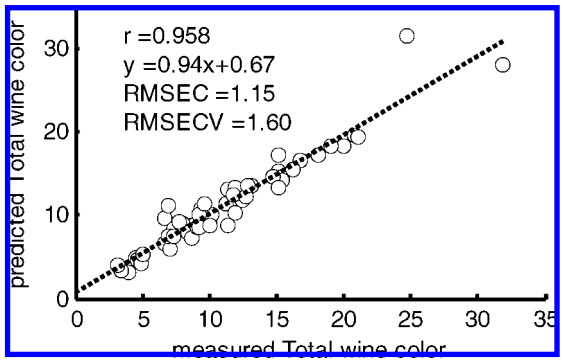

Figure 5. Relationship between the measured and predicted values (by cross validation) of the PLS regression model of total wine color.

multivariate regression, at least when wines were normalized to the same pH, thereby avoiding confoundings from the influence of pH on the color response by anthocyanins. The data obtained is a first step in providing a prediction of wine color from grape phenolic profile analysis. However, the direct relation between grape anthocyanins and total wine color (r = 0.961) was just as good as the relation between the measured and predicted total wine color found in the multivariate model (r = 0.958; **Figure 5**). Hence, determination of only grape anthocyanins is sufficient to obtain a satisfactory prediction of total wine color in very young wines. The relation between the grape anthocyanins and total wine color found in this study was in good accordance with the reported correlation of R^2 = 0.82 by Iland (*11*).

In the present study, the wines were produced in a laboratory scale setup, and the evolution of the phenolic profiles — and the putative alterations in wine color attributes — during maturation, aging, and prolonged storage, were not examined. The average total color in the present study (11.4 absorbance units, **Table 9**) was slightly higher than the average reported total color of young commercially produced Cabernet Sauvignon wines as measured by the same method (8.2 absorbance units) (*20*). The color value obtained was also higher than the reported average total color (approximately 4.5 absorbance units) of commercial wines — also measured by the same method — covering a wide range of cultivars (*28*). The higher color values in the present study were probably a result of the fact that only freshly fermented wines were examined. For practical and comparative (precision) purposes, frozen grape material was used as the starting material in the present work. The extraction of phenolic compounds from frozen grapes might therefore have been higher than for fresh grapes (*9*). Also, wine phenolics and color attributes do change during extended maturation and storage of wines. It is worth noting, however, that the color values obtained were nevertheless of the same order of magnitude as those reported previously for commercial wines. The data obtained signify that it is possible to predict the color quality of fresh wines from grape measurements and they thus provide an important starting point for further identification and prediction of wine quality parameters from grape measurements. The integration of the current data with data obtained in large-scale commercial wine making will be an important next step in the prediction of wine color from grapes.

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