COLOR AND ANTHOCYANIN EVALUATION OF RED WINEGRAPES BY CIE L*, a*, b* PARAMETERS

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Abstract

Aims: The aim of this work was to study the color of the wine grapes using the CIELAB parameters and to develop colorimetric indexes which can be used as a rapid method to assess the anthocyanin content of the wine grapes in the vineyard.

Methods and results: L*, a*, b* parameters were measured by a reflectance spectrophotometer on eighteen red winegrapes at harvest. Two indexes were calculated: CIRG2, used as colorimetric index for table grapes and CIRWG (Color Index for Red Wine Grapes). These indexes showed effective potentialities for descriptive, taxonomic and ampelografic studies. Particularly the CIRWG index is more correlated to the total anthocyanin amount, while the CIRG2 is more correlated to the different forms of the anthocyanins.

Conclusion: The colorimetric indexes applied directly in the vineyard permit to assess the total anthocyanin content and to give further quickly information on grape quality and maturity.

Significance and impact of study: The development of analytical methods for the evaluation of anthocyanin immediates, easily applicable, at low cost in the field by grape-growers and winemakers, can be very useful in deciding the harvest date.

Key words: CIELAB, anthocyanins, *Vitis vinifera* cultivars, color indexes, classification

Résumé

Objectif : Le but de cette expérimentation était d'étudier la couleur des raisins de cuve en utilisant les paramètres CIELAB et de développer des indices colorimétriques qui puissent être employés pour l'évaluation rapide de la teneur en anthocyanes des raisins de cuve au vignoble.

Méthodes et résultats : Les paramètres L*, a*, b* ont été mesurés en utilisant un spectrophotomètre à réflectivité sur dix-huit échantillons à la vendange. Deux indices ont été calculés : le CIRG2, employé comme indice colorimétrique pour les raisins de table et le CIRWG (Color Index for Red Wine Grape) proposé pour les raisins de cuve à baie colorée. Ces indices ont démontré des bonnes potentialités pour les études de type descriptif, taxonomique et ampélographique. Le CIRWG en particulier s'est montré mieux corrélé avec la teneur en anthocyanes totaux, tandis que le CIRG2 apparaît plus corrélé avec les différentes formes d'anthocyanes.

Conclusion : L'utilisation des indices colorimétriques directement au vignoble permet d'évaluer la teneur en anthocyanes totaux et fournit une information rapide sur la qualité et le degré de maturité des raisins.

Signification et impact de l'étude : Le développement de méthodes analytiques visant l'évaluation immédiate des anthocyanes, méthodes faciles à appliquer au vignoble par les viticulteurs et par les œnologues à un coût limité, serait très utile pour choisir la date optimale de récolte.

Mots clés : CIELAB, anthocyanes, cépages de *Vitis vinifera*, classification, indices colorimétriques

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INTRODUCTION

The anthocyanin content of wine grapes is of considerable importance in evaluating their oenological potentiality. The profile and the total amount of anthocyanins in grapes, as well as their degree of extractability, determine, in fact, the chromatic characteristics of wines (SAINT-CRIQ *et al.*, 1998; ROMERO-CASCALES *et al.*, 2005). Knowing the grapes anthocyanin characteristics permits the rationalization of maceration and winemaking processes thus allowing winemakers to best exploit the potential of the grape reached in the vineyard (CHEYNIER *et al.*, 1990; CHEYNIER *et al.*, 1997; GERBI *et al.*, 2002; GONZALEZ-NEVES *et al.*, 2004).

The total amount of anthocyanin accumulated in berry skins depends on the variety (i.e. pink, red or black skin variety), but many other factors may influence skin color intensity at harvest. As reported by several authors, environmental parameters and agronomical practices can influence the anthocyanin accumulation in berry skins and the anthocyanin amount at harvest (JACKSON and LOMBARD, 1993, DOKOOZLIAN and KLIEWER, 1996; KELLER and HRAZDINA, 1998; GUIDONI *et al.*, 1997; YOKOTSUKA *et al.*, 1999; ESTEBAN *et al.*, 2001; VIVAS DE GAULEJAC *et al.*., 2001; OJEDA *et al.*, 2002; ROBY *et al.*, 2004; DE LA HERA ORTS *et al.*, 2005; MORI *et al.*, 2005; ORTEGA-REGULES *et al.*, 2006; YAMANE *et al.*, 2006).

The methods proposed and generally used for the qualitative and quantitative analysis of anthocyanin in wine grapes are multivarious: UV-Vis spectrophotometry, near infrared (NIR) and mid-infrared wavelength regions (MIR) spectroscopy and chromatography (HPLC, LC-MS) (DI STEFANO and CRAVERO, 1991; GISHEN *et al.*, 2005; DAMBERGS *et al.*, 2006). However, such methods are not of immediate use in the vineyard as they include sample preparation and preferably the close proximity of an analytical laboratory.

Prompt analysis of the anthocyanins is possible thanks to the relationship between the color and anthocyanins in the grape skin demonstrated in several reports (CARRENO *et al.*, 1995; CARRENO *et al.*, 1997; FERNADEZ LOPEZ *et al.*, 1998; KITAMURA *et al.*, 2005).

In such studies, the evaluation of the color is based on the use of the CIELAB color system (COMMISSION INTERNATIONAL DE L'ÉCLAIRAGE, 1986) in which the L* a* b* values, detected by reflectance spectrophotometry, describe a three-dimensional color space, where L is the vertical axis and defines the lightness, from completely opaque (0) to completely transparent (100); a* and b* are the horizontal axes and define, respectively, the redness (or - a* of greenness), and the yellowness (or - b* of blueness) (BAKKER *et al.*, 1986). In particular, for the table grapes, an index has been already proposed derived from the CIELAB values (CIRG, Color Index for Red Grape), applicable to the determination of the color and to the classification of grapevines (CARRENO *et al.*, 1995).

CIELAB parameters have also been used in order to evaluate and to define the color of flowers (BIOLLEY and JAY, 1993; IMAYAMA and YABUYA, 2003), fruits (DELWICHE and BAUMGARDNER, 1985) and vegetables (DODDS *et al.*, 1991).

CIELAB parameters are also widely used for the characterization and evolution of the color of drinks and wines (PIRACCI, 1994; GIL-MUNOZ *et al.*, 1997; HUERTAS *et al.*, 2003; PEREZ-CABALLERO *et al.*, 2003). Furthermore, they have been used in order to characterize single anthocyanins in model solutions, using colorimetry (HEREDIA *et al.*, 1998; MATTIVI *et al.*, 2001; TORSKANGERPOLL and ANDERSEN, 2005) and for taxonomical studies, based on their correlation with the anthocyanin profile of grapes (CARRENO *et al.*, 1997).

The grape color can also be described through the use of the O.I.V. (ORGANISATION INTERNATIONALE DE LA VIGNE ET DU VIN) descriptive cards (O.I.V., 1983); however they do not allow a quantitative evaluation of the anthocyanins to be made.

To our knowledge, information relating to the application of CIELAB parameters for the color study of wine grapes is scarse.

The aim of this work was therefore to study the color of the wine grapes using the CIELAB parameters and to develop colorimetric indexes usable for descriptive, taxonomic and ampelografic studies about the wine grape cultivar according to what has already been proposed for the table grapes (CARRENO *et al.*, 1997).

Moreover, we aimed at evaluating the use of these indexes as a rapid method to appraise the anthocyanin content of the wine grapes in the vineyard and to assess the potentiality of the colorimetric parameters in assessing the anthocyanin varietal profile.

MATERIALS AND METHODS

The study was carried out on eighteen red winegrape cultivars of 2006 vintage belonging to autochthonous and international germplasm (*Vitis vinifera* L.) grown in Piedmont (North-west Italy).

Furthermore, in the 2007 vintage, two new cultivars of red winegrape and ten samples of Nebbiolo grapes, harvested at technological ripeness in several vineyards of the Langhe area (province of Cuneo), were analyzed.

1. Color measurement and indexes

For color measurement, thirty berries, three replicates of ten berries, were chosen randomly from different parts of the bunch at technological ripening and directly analyzed in the vineyard. The bloom was removed from the skin by paper tissue. The color values, L*, a*, b* (CIE, 1986) were determined by a reflectance spectrophotometer Minolta CR-410 Chroma Meter (Minolta Corp., Osaka, Japan). Standard illuminant C was used as reference. Three measurements were made around the equatorial belt of each berry. The chroma value (C) was calculated as $C = [(a^{*2}+b^{*2})]^{0.5}$ and hue (h) value as h = arctang b*/a*.

On the bases of the variables, three indexes for each variety were calculated: CIRG = (180-H) / (L*+C), proposed as colorimetric index for the table grapes, CIRG2 = (180-H) / (L*x C) (CARRENO *et al*., 1995) where H (hue) was calculated considering the hue values included between 360° and 270° as negative (BAKKER *et al.*, 1986), and a proposed new index CIRWG (Color Index for Red Wine Grape) as [h/(L*x b*)x100], where h was expressed as radians.

2. Anthocyanin extraction and analysis

For chemical analysis, three replicates of ten berries, the same ones used for color measurement, were picked and prepared according to DI STEFANO and CRAVERO (1991). The berry skins, removed manually from the pulp and dried with absorbent paper, were quickly immersed in 25 mL of hydro-alcoholic buffer at pH 3.2, containing 2 g/L of $Na_2S_2O_5$, as extractant, and 12 % of ethanol. They were subsequently homogenised with an Ultraturrax T25 (IKA Labortechnik, Staufen, Denmark) and centrifuged for 10 min at 3,000 rpm at 20 °C. The supernatant was then used for analysis.

A spectrophotometric method was used to evaluate the total anthocyanin index (TAI) in the berry skins (DI STEFANO and CRAVERO, 1991). Analysis of individual anthocyanins was performed after application of berry skins extract to a SEP-PAK C18 cartridge (Waters Corporation, Milford, MA, USA) and elution with methanol. The chromatograph was a P100 equipped with a AS3000 auto-sampler (Spectra Physics Analytical, Inc, San Jose, CA, USA) and a 20 mL Reodyne sample loop. A LiChroCART column (25 cm x 0.4 cm i.d.) from Merck (Darmstadt, Germany) packed with LiChrosphere 100 RP-18 5-µm particles by Alltech (Deerfield, IL, USA). A Spectra Focus Diode Array Detector (Spectra Physics Analytical, Inc, San Jose, CA, USA) operating at 520 nm was employed. The following conditions were used: solvent A = 10 % formic acid in water. Solvent B = 10 % formic acid with 50 % methyl alcohol in water. These solvents were filtered through a 0.20 µm filter. The solvent flow rate was 1 mL/min. The solvent program used was 72 % A to 55 % A over 15 min; to 30 % A over 20 min; to 10 % A over 10 min; to 1 % A over 5 min; to 72 % A over 3 min. An equilibrium time of 10 min was used (ZEPPA *et al*., 2001). Data treatment was carried out using the ChromQuest™ chromatography data system (ThermoQuest, Inc, San Jose, CA, USA).

The identification of the free form of anthocyanins, in the berry skin extract, was performed in comparison with external standards (Delphinidin-3-O-glucoside chloride, Malvidin-3-O-glucoside chloride, Peonidin-3-O-glucoside chloride, Petunidin chloride, Cyanidin chloride; Extrasynthèse, Genay, France); the acylated forms of anthocyanins were identified comparing the retention time of each chromatographic peak with available data in literature (Di Stefano *et al*., 1995). The percentages of individual anthocyanins were determined by comparing the area of the individual peak with the total peak area.

3. Statistical analysis

The means of different parameters were studied by analysis of variance (ANOVA) and by Cluster analysis (Ward method). Means submitted to analysis of variance were separated with the Duncan test and relationship between anthocyanin content and color indexes was studied by correlation index.

All statistical analyses were carried out with the statistical packages SAS (SAS Institute Inc., Cari, NC, USA).

RESULTS AND DISCUSSION

Grape CIELAB parameters and the calculated colorimetric indexes highlight the relevant differences between the studied cultivars (table 1).

The CIRG2 and the CIRWG (Color Index for Red Wine Grapes) showed the best potential for evaluation of the color of the wine cultivars (table 2). The CIRG2 index showed a variability between 1.5 and 13.7, while the CIRWG was between 0.9 and 10.6, respectively for the Grignolino and Neretta cuneese cultivars (table 1).

The CIRG index (Color Index for Red Grapes), has been preferred to others, among those the CIRG2 tested at the same time, because it showed the best correlation with the skin color identified with O.I.V. codes (CARRENO *et al*., 1995). This index did not appear adapted to the differentiation of the wine grapes because it did not show a sufficient variability among the cultivars.

Table 1 - Studied cultivars, abbreviations used in the figure 1, CIELAB variables and CIRG2 and CIRWG indexes.

Mean values of 30 replicates ± standard error (s.e.).

Cultivar	cod.	L*		a*		b*		C*		h (rad) arctang (b*/a*)		CIRG2		CIRWG	
		means	s.e.	means	s.e.	means	s.e.	means	s.e.	means	s.e.	means	s.e.	means	s.e.
Avanà	Avana	29,3	± 0,07	1,88	± 0,13	-0,05	± 0,07	1,91	± 0,13	-0,08	± 0,04	3,74	± 0,27	2,01	± 0,13
Avarengo	Avar	28,8	± 0,07	0,99	± 0,08	0,23	± 0,03	1,03	± 0,08	0,24	± 0,04	6,45	± 0,73	3,93	± 0,37
Barbera	BRB	28,3	± 0,09	0,73	± 0,06	0,24	± 0,03	0,77	± 0,06	0,32	± 0,04	8,87	± 0,79	5,56	± 0,45
Bonarda	BNRD	28,5	± 0,09	0,68	± 0,03	0,02	± 0,05	0,72	± 0,03	0,03	± 0,07	8,87	± 0,31	5,11	± 0,18
Cabernet sauvignon	CS	29,3	± 0,11	0,48	± 0,03	-0,33	± 0,05	0,62	± 0,04	-0,56	± 0,07	12,41	± 0,55	6,28	± 0,27
Chatus	Chatus	27,9	± 0,08	0,78	± 0,07	0,22	± 0,03	0,83	± 0,06	0,32	± 0,05	7,98	± 0,59	5,17	± 0,46
Croatina	Croat	29,3	± 0,07	0,52	± 0,03	-0,01	± 0,03	0,54	± 0,03	-0,04	± 0,04	12,51	± 0,61	6,85	± 0,32
Dolcetto	DLC	30,0	± 0,20	0,33	± 0,06	-1,19	± 0,14	1,35	± 0,11	-0,67	± 0,19	6,44	± 0,94	3,15	± 0,57
Freisa	FRE	29,2	± 0,19	1,06	± 0,07	-0,53	± 0,07	1,26	± 0,06	-0,48	± 0,06	6,09	± 0,35	3,11	± 0,17
Gamay	GMY	29,0	± 0,11	0,97	± 0,07	0,06	± 0,04	0,99	± 0,07	0,07	± 0,04	6,77	± 0,37	3,89	± 0,22
Grignolino	GRI	28,4	± 0,18	4,42	± 0,27	0,78	± 0,11	4,52	± 0,29	0,16	± 0,01	1,49	± 0,09	0,87	± 0,05
Grisa nera	GrNE	29,3	± 0,09	1,25	± 0,12	0,09	± 0,06	1,28	± 0,12	0,05	± 0,04	5,96	± 0,57	3,36	± 0,31
Mondeuse	Mndse	28,2	± 0,13	0,76	± 0,05	0,11	± 0,07	0,82	± 0,06	0,13	± 0,08	8,23	± 0,52	4,87	± 0,29
Nebbiolo	NE	28,7	± 0,08	2,68	± 0,16	0,40	± 0,03	2,71	± 0,16	0,15	± 0,01	2,39	± 0,12	1,40	± 0,07
Neretta cuneese	NerCun	28,4	± 0,09	0,27	± 0,02	0,27	± 0,03	0,41	± 0,02	0,72	± 0,08	13,65	± 1,37	10,55	± 0,79
Petit rouge	PetRo	29,0	± 0,10	0,63	± 0,05	0,18	± 0,03	0,67	± 0,05	0,31	± 0,04	6,44	± 0,58	6,03	± 0,38
Pinot nero	Pinot	28,4	± 0,08	1,33	± 0,07	0,21	± 0,02	1,35	± 0,06	0,17	± 0,02	4,70	± 0,21	2,78	± 0,13
Plassa	Plassa	30,0	± 0,11	1,12	± 0,08	-0,31	± 0,07	1,22	± 0,07	-0,31	± 0,06	6,00	± 0,37	3,11	± 0,18

Table 4 - Anthocyanin profile of each cultivar (%) listed according to the group identified by the cluster analysis carried out on the CIELAB variables.

Each variable represents the sum of the free and derivative forms of each compound. Means of three replicates ± error standard (s.e.).

Cultivar	Groups	delphinidin - 3 gluc		cyanidin - 3 gluc		petunidin - 3 gluc		peonidin - 3 gluc		malvidin - 3 gluc	
		means	s.e.	means	s.e.	means	s.e.	means	s.e.	means	s.e.
Avarengo	1	8,14	± 0,15	5,36	± 0,08	10,07	± 0,19	20,94	± 0,83	55,49	± 0,72
Gamay	1	2,56	± 0,13	0,61	± 0,12	4,40	± 0,47	11,20	± 0,82	81,24	± 1,17
Grisa nera	1	11,14	± 0,66	32,70	± 0,70	10,26	± 0,43	20,36	± 1,18	25,54	± 0,70
Pinot nero	1	2,25	± 0,04	1,52	± 0,10	5,03	± 0,19	27,78	± 2,37	63,43	± 2,32
Barbera	2	10,87	± 0,34	8,25	± 0,54	15,38	± 0,71	11,37	± 0,34	54,13	± 1,10
Bonarda	2	4,69	± 0,50	4,49	± 1,03	7,65	± 0,64	26,27	± 1,36	56,89	± 1,57
Chatus	2	9,24	± 0,70	3,81	± 0,22	9,83	± 0,53	10,24	± 3,59	66,89	± 2,98
Mondeuse	2	6,05	± 0,03	0,52	± 0,04	6,59	± 0,08	5,45	± 0,13	81,40	± 0,17
Cabernet Sauvignon	3	14,85	± 0,89	1,45	± 0,12	8,96	± 0,40	7,46	± 0,50	67,28	± 1,15
Croatina	3	7,87	± 0,34	2,54	± 0,16	10,59	± 0,47	11,55	± 0,40	67,44	± 0,50
Petit Rouge	3	10,61	± 1,01	2,04	± 0,38	11,14	± 0,43	9,73	± 0,68	66,48	± 2,12
Avanà	4	0,91	± 0,08	35,76	± 0,94	1,93	± 0,12	54,84	± 0,55	6,56	± 0,48
Freisa	4	4,05	± 0,07	9,10	± 0,41	5,44	± 0,31	46,34	± 1,03	35,07	± 0,30
Plassa	4	10,54	± 0,47	24,70	± 1,55	10,15	± 3,25	14,93	± 0,64	39,68	± 1,03
Grignolino	5	3,12	± 0,76	12,32	± 0,74	2,55	± 0,50	62,14	± 1,93	19,88	± 1,39
Nebbiolo	5	4,76	± 0,05	17,69	± 1,15	3,83	± 0,14	53,48	± 0,67	20,24	± 1,29
Neretta cuneese	6	12,19	± 0,32	6,00	± 0,31	10,34	± 2,79	17,13	± 1,15	54,34	± 1,61
Dolcetto	7	8,38	± 0,15	1,14	± 0,01	8,22	± 0,16	11,63	± 0,20	70,64	± 0,36

Table 2 - Mean values of the colorimetric variables and of the total anthocyanin index (mg malvidin-3-glucoside chloride/kg grape), calculated for each cluster analysis identified group.

Values followed by different letters significantly differ for $p \leq 0.05$.

Groups	L*		a*		b*		CIRG		CIRG2		CIRWG		Total anthocyanins	
1	28.9	bcd	1.13	bc	0:15	abc	5.7	ab	6.0	d	3.5	d	675	de
2	28.2	d	0.74	bc	0:15	abc	5.8	ab	8.5	c	5.2	c	1194	b
3	29.2	bc	0.54	bc	-0.05	bc	6.2	a	11.5	b	6.4	b	1111	bc
4	29.5	ab	1.35	b	-0.30	c	6.3	a	5.3	d	2.7	d	702	cde
5	28.5	cd	3.55	a	0.04	a	5.3	ab	1.9	e	1.1	e	353	e
6	28.4	d	0.27	c	0.02	ab	4.8	b	13.6	a	10.6	a	1905	a
7	30.0	a	0.33	bc	-1.19	d	6.2	a	6.4	d	3.2	d	951	bcd

Table 3 - O.I.V codes and mean values of the calculate indexes for the seven cluster analysis identified groups.

Groups	CIRG	CIRG2	CIRWG	cod.OIV
1	5.7 (min 5.6, max 5.9)	6.2 (min 5.0, max 7.0)	3.5 (min 2.8, max 3.9)	5-6 dark red-violet or bleu.black
2	5.8 (min 6.1, max 5.6)	9.1 (min 8.6, max 9.8)	5.2 (min 4.9, max 5.6)	6 blue-black
3	6.2 (min 5.5, max 7.1)	11.2 (min 10.6 max 12.3)	6.4 (min 6.0, max 6.9)	7 red-black
4	6.3 (min 5.9, max 6.8)	4.8 (min 3.6, max 5.5)	2.7 (min 2.0, max 3.1)	6 blue-black
5	5.3 (min 5.2, max 5.5)	2.0 (min 1.6 max 2.5)	1.1 (min 0.9, max 1.4)	5 dark red-violet
6	4:08	17:02	10:06	7 red-black
7	6:02	5:07	3:02	6 blue-black

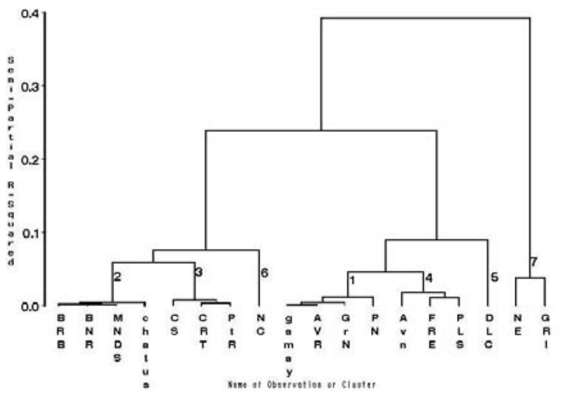

Figure 1 - Dendrogram for the examined cultivars obtained by cluster analysis of the CIELAB parameters and of the color indexes.

On the values of CIELAB parameters and CIRG2 and CIRWG indexes of each cultivar, a cluster analysis was carried out: this allowed the studied cultivars to be subdivided into seven distinguishable groups (figure 1).

For each identified group of cultivars the medium value of the variables was calculated, assessing by Duncan's test, the significance of the differences between the averages (table 2).

CIRG2 and CIRWG indexes allowed five of the seven groups identified by the cluster analysis to be distinguished meaningfully (table 2). The cultivars belonging to groups 1 and 4 did not show, in fact, significant differences, and they differed from those of the group 7 only by the L* and b* coordinates.

The use of the indexes allowed us to distinguish grapes that, based on the color O.I.V code, would be classified in the same group (table 3). In particular, the cultivars with code OIV 7 (red-black), not distinguished on the basis of this parameter, can be separated using the CIRG2 and CIRWG indexes into groups 3 (Cabernet sauvignon, Croatina and Petit rouge) and 6 (Neretta cuneese). Similarly the cultivars with O.I.V. code 5 (dark red-violet) were classified by the indexes into two groups: 1 (Grisa nera) and 5 (Nebbiolo and Grignolino). The group of 11 cultivars with O.I.V. code 6 (Blue-black), was divided among four different groups.

Indexes CIRG2 and CIRWG showed a significant correlation both with the anthocyanin amount, and with the different anthocyanin forms (tables 4 and 5). On the contrary, the CIRG index showed no correlation (R^2 = - 0,16) with the anthocyanin content of the wine grapes skins, therefore it was not taken into consideration in this work.

The indexes, in particular CIRWG, could be used to evaluate, though indirectly, the skin anthocyanin amount thanks to their high degree of correlation with the anthocyanin content. Therefore, using all cases of each variety (n = 54) acquired in the 2006 vintage, a linear regression between CIRWG index and corresponding TAI values was found.

The equation calculated, TAI = 148.03 CIRWG + 308, usable as model for estimate TAI value, showed a elevated coefficient of regression ($R^2 = 0.87$, significant at $p \leq 0.01$),

Hence, this equation was employed to estimate the TAI of samples harvest in vintage 2007. Results for the two new cultivar studied (Doux d'Henry and Pelaverga) were not acceptable (table 6). The calculated TAI, for the samples belonging to Nebbiolo cultivar, showed with respect to the analyzed TAI a variable difference, depending on the cases (from 1 to 7 in table 6), among 31 and 101 mg/kg of grapes corresponding to 5.93 % and 16.11 %, respectively. The rapidity of this method, applicable directly in vineyard, could make these differences acceptable.

Therefore, the possibility to calculate a specific curve for each cultivar was evaluated to improve the model. For this purpose, using only the seven relative Nebbiolo cases, a new regression curve for this cultivar was built (figure 2). The validity of this new curve was tested by assessing the TAI of further samples of Nebbiolo (from 8 to 10, table 6). The results showed the best performance of this model; in fact, the differences between the calculated TAI and the analyzed TAI turned out to be lower, varying from -4.61 % to +5.83 %, which therefore appears, at present, to be the limit of accuracy of the model. We conclude therefore that implementing a specific model for each cultivar which can be updated according to the acquisition of new data, can allow more definite assessments.

Increasing the total anthocyanins contents causes the CIRWG to rise, as the reflectance spectra evaluated from the veraison to the full ripening also show on the cv. Tempranillo (INARREA *et al.*, 1993). In the specific case of cv. Nebbiolo, the anthocyanins accumulation is sudden during the first 5-6 weeks after the veraison (80-90 % of total), while during the last 2-3 weeks the increase is

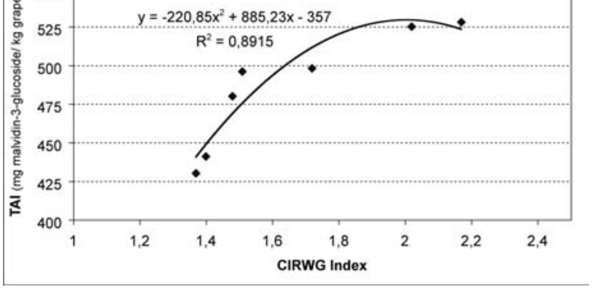

Figure 2 - Curve of regression between the TAI, expressed as mg malvidin-3-glucoside/ kg grapes, and CIRWG index calculated for cv. Nebbiolo in 2007 vintage.

Table 5 - Mean value, calculated for each group, of the total anthocyanin index (mg malvidin-3-glucoside chloride/kg grape) and of other variables anthocyanin profile related and their correlation coefficient with CIRG2 and CIRWG indexes.

The value of each group is the mean of the value of cultivar belong it. (Pn = peonidin-3-glucoside; Mv = malvidin-3-glucoside).

Groups	Total anthocyanins	delphinidin - 3 gluc	cyanidin - 3 gluc	petunidin - 3 gluc	peonidin - 3 gluc	malvidin - 3 gluc	Pn/Mv	total trisubstituted derivatives	total disubdstituted derivatives
1	675	6,0	10,0	7,4	20,1	56,5	0,36	69,9	30,1
2	1194	7,7	4,3	9,9	13,3	64,8	0,21	82,4	17,6
3	1111	11,1	2,0	10,2	9,6	67,1	0,14	88,4	11,6
4	702	5,2	23,2	5,8	38,7	27,1	1,43	38,1	61,9
5	353	3,9	15,0	3,2	57,8	20,1	2,88	27,2	72,8
6	1905	12,2	6,0	10,3	17,1	54,4	0,31	76,9	23,1
7	951	8,4	1,1	8,2	11,7	70,6	0.17	87,2	12,8
CIRWG	0.965 ***	0.908 ***	-0.472 *	0.813 ***	-0.589 **	0.465 **	-0.579 **	0.578 **	-0.578 **
CIRG2	0.934 ***	0.963 ***	-0.577 **	0.914 ***	-0.739 **	0.619**	-0.671 **	0.721 **	-0.721 **

Table 6 - CIRWG, TAI analyzed, TAI calculated and relative difference for samples of Doux d'Henry, Pelaverga and Nebbiolo grapes in 2007 vintage.

Samples*	CIRWG	TAI (I) analyzed	TAI (II) calculated	Difference TAI (II-I) (mg malvidin-3-glucoside chloride/ kg grape)	Difference TAI (%)
Doux d'Henry	1,64	440	551	111	20,14
Pelaverga	2,70	556	708	152	21,43
Nebbiolo 1	1,48	496	527	31	5,93
Nebbiolo 2	1,72	498	563	65	11,51
Nebbiolo 3	2,17	528	629	101	16,11
Nebbiolo 4	1,51	480	532	52	9,72
Nebbiolo 5	1,37	441	511	70	13,69
Nebbiolo 6	1,56	506	539	33	6,14
Nebbiolo 7	1,95	520	597	77	12,87
Samples**	CIRWG	TAI (I) analyzed	TAI (II) calculated	Difference TAI (II-I) (mg malvidin-3-glucoside chloride/ kg grape)	Difference TAI (%)
Nebbiolo 8	1,89	524	556	32	5,83
Nebbiolo 9	1,60	497	475	-22	-4,61
Nebbiolo 10	1,52	485	509	24	4,76

* samples 1-7 TAI calculated as 148,03*CIRWG+ 308; ** samples 8-10 TAI calculated as -220,85 CIRWG2 +885,23 CIRWG-357

limited (10-20 %) (GUIDONI *et al*., 2008). In the first phases of accumulation, the CIRWG could allow a good assessment of the TAI, but the evolution of the more limited variations in the last phases of maturation could be difficult. Furthermore, it was demonstrated that during the last phases of maturation the CIRG of the cv. Monastrell no longer showed a linear response with TAI (FERNANDEZ-LOPEZ *et al*., 1998). Therefore, the evaluation of the potentialities of the CIRWG during grape ripening and in overipeness must be known.

The high correlation found between the colour indexes, CIRG2 in particular, and the different anthocyanin forms agrees with previous studies which showed that CIELAB parameters, even if studied in model solutions with different pH, offer a good chromatic characterization of the five free anthocyanins (HEREDIA *et al.*, 1998). In particular, it was found that, in the CIELAB space, the peonidin-3-glucoside and the cyanidin-3-glucoside (di-substituted anthocyanin in the B ring) are localized in the area of the orange hues, while petunidin-3-glucoside, delphinidin-3-glucoside and malvidin-3-glucoside (molecules three-substituted in the B ring) are localized in the red-purple area. For this reason, probably, CIELAB indexes applied *in vivo*, will reflect a significant change depending on the varietal anthocyanin profile.

That would further justify the necessity of developing a specific model for each cultivar.

CONCLUSIONS

Differences among the chromatic characteristics of anthocyanins are both qualitative and quantitative. This allows colorimetric parameters to be applied to the study of the anthocyanin pigments of red wine grapes.

The colorimetrical indexes applied to the study of the color of the red wine grapes (CIRG2 and CIRWG), showed high potential since they are strongly correlated to the total anthocyanin amount and to the anthocyanin profile of the skins.

They were also interesting for the study of color and for variety classification as they present high variability according to the color of the grapes.

These indexes originate from non-destructive analysis of the samples and are easily obtainable using handy and small instrumentation directly in the vineyard. Therefore, it will be possible to quickly evaluate the total anthocyanin content of the grape at low cost in the field.

It would be useful to implement the proposed model by studying a single model, with a greater number of cases, for each cultivar and to evaluate the model application during the ripening.

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