# TITLE: Colorimetric characteristics of the phenolic fractions obtained from Tempranillo and Graciano wines through the use of different instrumental techniques.

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**Abstract**

The aim of the present work was to determine whether there is any relationship between measured by transmission and reflection (in the latter case, with and without contact with the sample). We also evaluated which methodology used would offer a better interpretation of the results in visual terms. For this purpose, different colorimetric techniques such as transmission spectrophotometry, diffuse reflectance spectrophotometry and spectroradiometry were applied. The samples consisted of increasing dilutions (0, 20, 40, 60, 80 and 100%) of the phenolic fractions obtained from 4 wines: Tempranillo (T) and Graciano (G) monovarietal wines, and 80:20 mixtures: M (wine elaborated by blending grapes) and W (a blend of the T and G wines) (9 fractions per wine). Fractionation was performed using gel permeation chromatography with a Toyopearl HW-40S column, and the dilutions of the fractions were performed with synthetic wine (pH=3.6). The spectroradiometric measurements permitted the differences due to the dilution effect on the fractions to be established more clearly than with the results obtained using diffuse reflectance and spectrophotometry. Thus, this technology is very suitable for use in comparative interpretations by the human eye. In turn, we assessed the changes in colour due to the effect of dilution on the fractions, observing that the effect of dilution led to an increase in the values of lightness (L*), while the chroma values ($C^*_{ab}$) followed the opposite trend, in agreement with its role as a variable related to chromatic intensity or vividness of the sample. In contrast, hue ($h_{ab}$) did not seem to be affected by dilution of the fractions, in consonance with the qualitative nature of this parameter.

*Keywords*

Wine colour; Anthocyanins; Tempranillo; Graciano; Colorimetric techniques; CIELAB

## 1. Introduction

In the field of oenology, the visual analysis of wine colour is included in the tasting; this is also known as organoleptic examination, which consists of appreciation by sight, smell and taste of the qualities of a wine. Sampling or tasting involves subjecting the wine to our sensory skills with a view to becoming familiar with it and determining its sensory characteristics, eventually appreciating it (or not) [1]. In red wines, colour represents the first sensory characteristic perceived by the taster. Moreover, strong correlations have been found between the colour and overall quality of wines [2,3], although colour provides not only information about possible defects or the type or state of the evolution of the wine, but also has a strong influence on its acceptability [4] and price [5].

The initial violet-red colour of young red wines is the net result of all the monomer, oligomer and polymer anthocyanins extracted from the grape skins, together with their copigmented forms and intensity and hue, dependent upon factors such as the nature and concentration of the individual anthocyanins and their degree of degradation, temperature, pH, the nature of the solvent, the presence of $SO_2$, oxygen, enzymes, copigments, sugars, etc.[6-12]. However, during the ageing process of wines this colour evolves to reddish-orange hues, mainly due to the progressive structural changes undergone by the anthocyanins. These changes occur through different mechanisms [7,13-18].

In the winery, the parameters traditionally used to described the variation in colour of the anthocyanin solutions have mainly been the changes in $\lambda_{max}$ in the visible part of the spectrum as a measurement of variations in hue, together with changes in absorbance for the variation in colour intensity [19-20]. Both indices are easy to calculate and interpret and are those most frequently used in winery [21]. Nevertheless, Gonnet [22]

reported that an adequate description of variations in the colour of anthocyanin solutions, originated -for example- by pH, require the following: (a) that the spectral variations considered should be those affecting the whole spectral curve, not only its visible $\lambda_{max}$; (b) that it would be appropriate to use the three colour attributes (hue, saturation and lightness) for its description, and (c) that these should refer to the conditions of the observer and of the light source.

The CIE has proposed different systems for colour representation in an attempt to find one that will reflect the visual sensation perceived by observers in an appropriate way. When the determination of a colour is carried out it is necessary to determine the position of the observer, the light source, and the interval of data acquisition (for example, every 3 nm).

The colour of objects can be expressed through the colour coordinates of the different colour spaces (CIEXYZ, CIELAB, CIELUV, etc.).

The CIELAB space is a Cartesian coordinate system defined by three colorimetric coordinates L*, a*, and b*, where L* represents lightness. This can only take values between 0 and 100, 0 corresponding to a black sample and 100 to a white one. The colorimetric coordinates a* and b* form a perpendicular plane with lightness. The a* coordinate defines the deviation from the achromatic point corresponding to lightness; towards the red if a*>0 and towards the green when a*<0. Accordingly, it is called the red-green component. Likewise, the b* coordinate defines the deviation towards the yellow if b*>0, and towards the blue if b*<0, thus being referred to as the yellow-blue component. Chroma ($C^*_{ab}$) is the distance between the lightness axis and the stimulus in question. The farther away it is from the L* axis, the more colour there is. It has a value of 0 for achromatic stimuli and, in general, it does not surpass 150, although it may reach higher values for monochromatic stimuli. $h_{ab}$ is hue: this is the angle of the

circumference and lies between 0º and 360º, and for achromatic stimuli (a*=b*=0) it is an undefined magnitude. Although the CIELAB space is currently the one most used, both CIELUV and CIELAB are recommended by the CIE, despite certain anomalies and limitations that may discourage its use in some cases. The CIELAB space is also adapted as a UNE norm, in which colorimetric magnitudes that can be considered to be a response of standard observers to a light stimulus are defined [23].

Many authors have addressed the colour of pure anthocyanins solutions by using the CIELAB colour space parameters [22,24-29]. Generally, in these studies the colour of wines has been measured by transmission spectrophotometry. However, recently other methodologies based on reflectance measurements have been applied to characterize the colour of orange juices and honeys [30,31]. These techniques aim at obtaining the radiometric measurement of the spectral distribution of a source of radiation (primary or secondary), with the same components as the spectrophotometer except for the light source, which in this case is external to the instrument. Accordingly there is influence from external factors. Regarding colour, this device serves to determine the distribution of the spectral radiant energy from any source so that its colour coordinates can be calculated from that distribution.

The aim of this work was to study the influence of the dilution effect on the colour of the fractions and to evaluate the relationship between the colour of the phenolic fractions of red wines containing pigments, measured by transmission and reflection techniques, and to determine which of these techniques allows a better interpretation of the colour to be obtained.

## 2. Material and methods

### *2.1. Winemaking and samples*

Three wines were elaborated separately from red grapes *Vitis vinifera* L. in Bodegas Roda S.A. (La Rioja, Spain): T from the Tempranillo variety, G from the Graciano variety, and M from a 80:20 mixture of Tempranillo and Graciano grapes. A fourth wine W was elaborated by blending (80:20 v/v) the T and G wines after post-fermentative maceration.

### *2.2. Sample fractionation*

After three months of ageing in a barrel, 180 mL aliquots of each wine sample (T, G, M and W wines) were collected and fractioned with a Toyopearl HW-40(s) gel column (Tosoh, Japan) [32]. The elution solvents were ethanol/$H_2O$ (80:20 v/v) and methanol/$H_2O$ (80:20 v/v). The different coloured bands formed during elution as well as the bleaching eluates were collected separately. Thus, nine fractions were obtained, dependent upon the change in colour produced inside the chromatographic column, each considered as a different family of pigments according to the major compounds present (Fig 1.). All fractions were acidified to pH=1 in order to reverse the existing bisulphite-anthocyanin adducts, concentrated under vacuum, re-dissolved in water, and freeze-dried. Solutions of the freeze-dried fractions were prepared to have similar contents as in the wines. Thus, depending on the fraction different amounts (mg) were dissolved in 5 mL of synthetic wine (pH 3.6, 0.2M).

### *2.3. HPLC-DAD-MS analysis*

The solutions of the fractions were acidified with 0.1N HCl (Panreac® Barcelona, Spain) and injected into the chromatographic system after filtration through a 0.45 μm Millex® syringe-driven filter unit (Millipore Corporation).

HPLC-DAD analysis was performed with a Hewlett-Packard 1100 series liquid chromatograph. The LC system was connected to the probe of the mass spectrometer via the UV cell outlet. The mass analyses were performed using a Finnigan$^{TM}$ LCQ ion trap detector (Thermoquest, San Jose, CA, USA) equipped with an API source, using an electrospray ionisation (ESI) interface. The HPLC-DAD-MS analysis of red pigments was carried out in accordance with García-Marino et al. [33].

### *2.4. Quantification*

For quantitative analyses, calibration curves were obtained using standards of anthocyanin 3-*O*-glucosides (delphinidin 3-*O*-glucoside, cyanidin 3-*O*-glucoside, petunidin 3-*O*-glucoside, peonidin 3-*O*-glucoside and malvidin 3-*O*-glucoside). Anthocyanins were purchased from Polyphenols Labs., Sandnes, Norway.

All pigments were quantified from the areas of their chromatographic peaks at 520 nm, and the results were expressed in $mgL^{-1}$ of wine. The total content of the different groups of pigments studied was calculated from the sum of the individual concentrations obtained for each individual compound.

### *2.5. Colorimetric measurements*

Prior to spectrophotometric analysis, the fractions were filtered through Millipore-AP20 filters (Millipore Corporation, Bedford, MA, USA). Plastic cuvettes (475×350×10 mm) were used for the measurements.

Transmission measurements were made with a UV/Visible HP8452 (Hewlett-Packard, Palo Alto, CA, USA) spectrophotometer diode-array. The whole visible spectra were recorded (380-780 nm, $\Delta\lambda$=2 nm). The CIE-1964 10º standard observer and CIE D65 standard illuminant (corresponding to day light) were taken as references to calculate

recommended by the "Comission Internationale de l'Éclairage" (CIE, 2004), by applying CromaLab software [34].

The reflectance measurements were performed spectroradiometrically, with the spectroradiometer connected to a TOP 100 telescopic optical probe (Instrument Systems, Munich, Germany) and a Tamron SP 23A zoom (Tamron USA, Inc., Commack, NY, USA), and with diffuse reflectance spectrophotometry, with a ISP80 integration sphere (Instrument Systems, Munich, Germany), both coupled to the CAS 140B (Instrument Systems, Munich, Germany). Samples were measured against white backing (pressed barium sulphate) and the whole visible reflectance spectra were recorded (380-780 nm, $\Delta\lambda$=2 nm). In this case, the CIELAB parameters were calculated using IS-Specwin v.1.8.1.6 (Instrument Systems, Munich, Germany) software.

*2.6. Dilution assays pigment fractions*

Dilutions of fractions were assayed in order to study the influence of each dilution on the colour of the fractions and to generate a greater number of samples to allow the different colorimetric techniques to be compared. To accomplish this, increasing volumes of the target fraction were obtained (9 fractions obtained from the fractionation of each of the 4 wines studied) and were diluted in synthetic wine, pH 3.6, finally obtaining mixtures with different percentages (0, 20, 40, 60, 80 and 100%), as shown in Fig 2. The total number of fraction samples was 216; i.e., 54 per fractionated wine.

The colour changes due to the dilution effect were evaluated by the three techniques described above.

*2.6. Statistical analyses*

Data are presented as means ± standard deviations (±S.D.) of three experiments performed in triplicate. Significant differences were determined by one-way analysis of

variance (ANOVA) using an SPSS Program, version 13.0, for Windows software package (SPSS, Inc., Chicago, IL).

## 3. Results and discussion

### *3.1. Pigments in fractions*

The use of mass spectrometry coupled to HPLC-DAD allowed the detection of thirty-seven anthocyanins and anthocyanin-derived pigments in the fraction samples: anthocyanidin-monoglucosides, anthocyanidin-diglucosides, acylated anthocyanins, pyranoanthocyanidins, acetaldehyde-mediated flavanol-anthocyanidin condensation products, and direct flavanol-anthocyanin condensation products. All the pigments identified in the fractions analyzed have been described previously in samples of wines (García-Marino et al., 2010).

Table 1 shows the mean concentration of the different pigment families of the fraction solutions of the T, G, M and W wines. It may be observed that the presence of anthocyanidin-monoglucosides was widespread in all fractions. The pigment contents were low in fractions 1, 8 and 9. Furthermore, the anthocyanidin-monoglucoside compounds were the most abundant pigment family of the sum of total pigments in the nine fractions studied, followed by the acylated compounds, pyranoanthocyanidins and pigments derived from condensation between anthocyanins and flavanols (direct-linked and ethyl-linked compounds), although these concentrations were different among the wines studied.

Analysis of variance (ANOVA) was carried out with the total pigment contents (obtained from the sum of the different fractions for each wine) to check for differences among the wines (Table 1). The results revealed that the G wine had significantly ($p<0.05$) higher total pigment contents (~874.48 $mgL^{-1}$) than the T wine (~693.77 $mgL^{-}$

212 [1]). Total pigment contents in the M and W wines were similar (~746.03 $mgL^{-1}$ and
213 ~749.76 $mgL^{-1}$ respectively), and these values were significantly different from the total
214 pigment contents in the T and G wines. These results are consistent with those obtained
215 in previous studies carried out in wines [33].

216 ***3.2. Effect of dilution on the colour of pigment fractions****. Application of different*
217 *colorimetric techniques*

218 We performed colour measurements by applying the three colorimetric techniques
219 described above (transmission spectrophotometry, diffuse reflectance
220 spectrophotometry and spectroradiometry) on the different dilutions generated from the
221 fractions obtained from the T, G, M and W wines.

222 Fig. 3 shows the values obtained using transmission spectrophotometry of the L*, $C^*_{ab}$
223 and $h_{ab}$ colour parameters of the fractions of of the T, G, W and M wines. As expected,
224 with dilution lightness, L*, increased in all the fractions of the T (Fig. 3 (A.1)), G (Fig.
225 3 (B.1)), M (Fig. 3 (C.1)) and W (Fig. 3 (D.1)) wines, with the exception of fractions
226 that without dilution (100% of fraction) already showed values of lightness close to 100,
227 as F1, ie, almost colourless fractions.

228 By contrast, the values of $C^*_{ab}$ (Fig. 3 (A.2, B.2, C.2, D.2)) decreased, this decrease
229 being greater in the fractions with the higher pigment contents (F4, F5 and F6).
230 Regarding the values of $h_{ab}$ (Fig. 3 (A.3, B.3, C.3, D.3)), in general these were not seen
231 to be affected by the dilution effect of the fractions, in agreement with the qualitative
232 nature of this parameter. This shows that no modifications occurred in the anthocyanin
233 equilbria upon diluting under fixed pH conditions.

234 Regarding the colorimetric parameters obtained by diffuse reflectance (Fig. 4), it may
235 be seen that, likewise, the dilution effect led to an increase in the values of L* (Fig. 4

(A.1, B.1, C.1, D.1)). Additionally, as with the spectrophotometer, the values of $h_{ab}$ (Fig. 4 (A.3, B.3, C.3, D.3)) remained constant while the values of $C^*_{ab}$ (Fig. 4 (A.2, B.2, C.2, D.2)) mainly showed the opposite trend with dilution, in agreement with its role as a variable related to the chromatic intensity of vividness of the sample.

The variations in the values of the colour parameters were less marked with the measurements performed with diffuse reflectance (Fig. 4) than those observed when using transmittance (Fig. 3); However, with this latter technique it was possible to differentiate the fractions with high dilutions percentages better.

With respect to the measurements of colour using spectroradiometry, the results are shown in Fig. 5. The trend of the L* (Fig. 5 (A.1, B.1, C.1, D.1)), $C^*_{ab}$ (Fig. 5 (A.2, B.2, C.2, D.2)) and $h_{ab}$ (Fig. 5 (A.3, B.3, C.3, D.3)) colour parameters are similar to those observed with the other two techniques. However spectroradiometry allowed us to note the differences in the dilution effect on the fractions more clearly than diffuse reflectance (very similar) and transmittance.

The differences among colorimetric techniques with different measurement geometries (reflectance vs. transmittance) are well known in Tristimulus Colorimetry and correspond to the differences in visual appreciation, which depend on the observation geometry, such that in general they should not be interpreted as instrumental errors. Indeed, they are due to the different type of behavior shown by light according to the angle of incidence on the sample, producing phenomena of reflection, transmission/absorption and refraction that clearly differ depending on the measurement in question.

Also, with a view to corroborating which colorimetric technique (transmission spectrophotometry, spectroradiometry and diffuse reflection spectrophotometry) allowed the samples to be differentiated better, regardless of the wine fractionated, we

261 took as a reference the location on the diagram according to the a* and b* colour
262 coordinates obtained. Thus, Fig. 6 shows the location of the samples on the (a*b*)
263 plane. The samples were the dilutions obtained from each of the nine fractions from
264 four wines. The transmission measurements (plot A) allowed us to distinguish only the
265 dilutions obtained from fractions with higher contents in pigments (4, 5 and 6) (Table
266 1). However, the distribution of the other fractions was better with the reflection
267 measurements (plot B and plotC)).

268 Diffuse reflectance spectrophotometry is a highly reproducible technology owing to the
269 better control of the measuring conditions, such as the environmental light or the
270 measuring geometry (illumination/detection). It is a "reflection with contact" method, in
271 which the sample is in direct contact with the analytical probe and blocks the
272 measurement orifice, preventing the incidence of environmental light. Therefore, the
273 sample is illuminated only with the instrument's lamp, and hence this technique is more
274 adequate for analytical objectives.

275 Spectroradiometry reproduces the colour evaluation like it does the human eye. A
276 certain distance exists between the measurement probe and the sample ("reflection
277 without contact"). Accordingly, the sample receives environmental light, which means
278 that this methodology better reproduces the differences in colour (such as the human
279 eye would do) of fractions 4, 5 and 6 and the rest of fractions.

## 280 4. Conclusions

281 The dilution effect led to an increase in L*, while the values of $C^*_{ab}$ followed the
282 opposite trend, in agreement with its role as a variable related to the chromatic intensity
283 or vividness of the sample. $h_{ab}$ did not seem to be affected by the dilution effect of the
284 fractions, which is consistent with the qualitative nature of this parameter. Also, the
285 studies using spectrophotometry, spectroradiometry and diffuse reflectance

spectrophotometry confirmed the use of spectroradiometery as the measurement method that best distinguishes the colour differences between samples, although from a analytical point of view the spectrophotometer is more appropriate to evaluate the colorimetric behaviour of each blend.

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**Figure captions**

Fig. 1. Example of the colour of the redissolution pigment fractions of red wine.

Fig. 2. Scheme of the preparation of different dilutions (0, 20, 40, 60, 80 y 100%) for assays of the dilution effect on the colour of the fractions.

Fig. 3. Values of L* (1-left), $C^*_{ab}$ (2-centre) and $h_{ab}$ (3-right) obtained using spectrophotometry of the different dilutions derived from the 9 fractions (F1-F9) of the T (A), G (B), M (C) and W (D) wines.

Fig. 4. Values of L* (1-left), $C^*_{ab}$ (2-centre) and $h_{ab}$ (3-right) obtained by diffuse reflectance of the different dilutions derived from the 9 fractions (F1-F9) of the T (A), G (B), M (C) and W (D) wines.

Fig. 5. Values of L* (1-left), $C^*_{ab}$ (2-centre) and $h_{ab}$ (3-right) obtained using spectroradiometry of the different dilutions derived from the 9 fractions (F1-F9) of the T (A), G (B), M (C) and W (D) wines.

Fig. 6. Localisation area of the wine fractions on the diagram (a*,b*) measured by spectrophotometry (A), diffuse reflectance (B) and spectroradiometry (C).

**Table 1**

**Table 1.** Mean concentration ($mgL^{-1}$, ±S.D.; n=3) of different pigment families of the fraction solutions (1-9) of the T, G, M and W wines.

| *Fraction* Pigments | Total anthocyanidin-monoglucosides | | Total anthocyanidin-diglucosides | | Total acylated anthocyanins | | **Total anthocyanins** | | Total pyranoanthocyanidins | | Acetaldehyde-mediated flavanol-anthocyanidin condensation products | | Direct flavanol-anthocyanin condensation products | | **Total derived pigments** | | Total pigments | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean ± S.D. | | Mean ± S.D. | | Mean ± S.D. | | Mean ± S.D. | | Mean ± S.D. | | Mean ± S.D. | | Mean ± S.D. | | Mean ± S.D. | | Mean ± S.D. | |
| Wine: Tempranillo (T) | | | | | | | | | | | | | | | | | | |
| *1* | 4.16 ± 0.00 | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | **4.16 ± 0.00** | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | **0.00 ± 0.00** | | 4.16 ± 0.00 | |
| *2* | 18.93 ± 0.04 | | 6.67 ± 0.00 | | 6.84 ± 0.00 | | **32.44 ± 0.05** | | 0.00 ± 0.00 | | 2.47 ± 0.00 | | 5.94 ± 0.00 | | **8.41 ± 0.00** | | 40.85 ± 0.05 | |
| *3* | 32.04 ± 0.16 | | 0.00 ± 0.00 | | 34.11 ± 0.06 | | **66.14 ± 0.23** | | 8.03 ± 0.01 | | 3.08 ± 0.01 | | 0.00 ± 0.00 | | **11.11 ± 0.03** | | 77.25 ± 0.26 | |
| *4* | 38.00 ± 0.24 | | 0.00 ± 0.00 | | 33.45 ± 0.08 | | **71.45 ± 0.32** | | 9.52 ± 0.02 | | 2.79 ± 0.00 | | 0.00 ± 0.00 | | **12.31 ± 0.05** | | 83.76 ± 0.36 | |
| *5* | 110.84 ± 0.95 | | 15.22 ± 0.05 | | 46.59 ± 0.16 | | **172.65 ± 1.16** | | 18.32 ± 0.07 | | 3.45 ± 0.01 | | 0.00 ± 0.00 | | **21.77 ± 0.16** | | 194.41 ± 1.33 | |
| *6* | 95.93 ± 0.77 | | 13.03 ± 0.02 | | 60.04 ± 0.26 | | **169.01 ± 1.05** | | 37.35 ± 0.19 | | 3.83 ± 0.01 | | 10.87 ± 0.01 | | **52.73 ± 0.44** | | 221.04 ± 1.49 | |
| *7* | 17.79 ± 0.03 | | 7.77 ± 0.00 | | 18.03 ± 0.01 | | **43.59 ± 0.04** | | 8.60 ± 0.00 | | 2.55 ± 0.00 | | 14.78 ± 0.01 | | **25.93 ± 0.04** | | 69.51 ± 0.08 | |
| *8* | 0.00 ± 0.00 | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | **0.00 ± 0.00** | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | **0.00 ± 0.00** | | 0.00 ± 0.00 | |
| *9* | 2.79 ± 0.00 | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | **2.79 ± 0.00** | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | **0.00 ± 0.00** | | 2.79 ± 0.00 | |
| Total | 320.48 ± 2.19 | a/A | 42.69 ± 0.07 | b/B | 199.06 ± 0.57 | c/B | **562.23 ± 2.85** | **a/A** | 81.82 ± 0.29 | a/B | 18.17 ± 0.03 | d/D | 31.59 ± 0.02 | c/D | **132.26 ± 0.72** | **a/B** | 693.77 ± 3.57 | a/A |
| Wine: Graciano (G) | | | | | | | | | | | | | | | | | | |
| *1* | 9.74 ± 0.00 | | 0.00 ± 0.00 | | 2.46 ± 0.00 | | **12.20 ± 0.00** | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | **0.00 ± 0.00** | | 12.20 ± 0.00 | |
| *2* | 26.11 ± 0.12 | | 10.44 ± 0.01 | | 24.32 ± 0.01 | | **60.87 ± 0.13** | | 1.53 ± 0.00 | | 2.56 ± 0.00 | | 6.03 ± 0.00 | | **10.12 ± 0.01** | | 70.99 ± 0.14 | |
| *3* | 140.65 ± 1.27 | | 11.07 ± 0.01 | | 41.29 ± 0.11 | | **193.02 ± 1.40** | | 11.49 ± 0.01 | | 3.09 ± 0.01 | | 3.46 ± 0.00 | | **18.05 ± 0.04** | | 211.06 ± 1.44 | |
| *4* | 105.98 ± 0.92 | | 14.59 ± 0.05 | | 42.86 ± 0.16 | | **163.43 ± 1.13** | | 24.52 ± 0.09 | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | **24.52 ± 0.17** | | 187.95 ± 1.31 | |
| *5* | 82.06 ± 0.63 | | 15.74 ± 0.06 | | 54.58 ± 0.24 | | **152.39 ± 0.93** | | 31.26 ± 0.12 | | 3.40 ± 0.01 | | 2.53 ± 0.00 | | **37.20 ± 0.26** | | 189.59 ± 1.19 | |
| *6* | 49.53 ± 0.30 | | 12.36 ± 0.01 | | 42.38 ± 0.08 | | **104.26 ± 0.39** | | 21.98 ± 0.06 | | 2.89 ± 0.00 | | 7.16 ± 0.01 | | **32.03 ± 0.16** | | 136.31 ± 0.55 | |
| *7* | 19.51 ± 0.04 | | 4.14 ± 0.00 | | 22.59 ± 0.01 | | **46.24 ± 0.05** | | 2.57 ± 0.00 | | 2.55 ± 0.00 | | 15.02 ± 0.02 | | **20.14 ± 0.04** | | 66.38 ± 0.08 | |
| *8* | 0.00 ± 0.00 | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | **0.00 ± 0.00** | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | **0.00 ± 0.00** | | 0.00 ± 0.00 | |
| *9* | 0.00 ± 0.00 | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | **0.00 ± 0.00** | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | **0.00 ± 0.00** | | 0.00 ± 0.00 | |
| Total | 433.58 ± 3.28 | c/A | 68.34 ± 0.14 | d/D | 230.48 ± 0.61 | d/C | **732.41 ± 4.03** | **c/A** | 93.35 ± 0.28 | c/B | 14.49 ± 0.02 | c/C | 34.20 ± 0.03 | d/D | **142.06 ± 0.68** | **b/B** | 874.48 ± 4.71 | c/A |
| Wine: Blend of grapes (M) | | | | | | | | | | | | | | | | | | |
| *1* | 4.23 ± 0.00 | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | **4.23 ± 0.00** | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | **0.00 ± 0.00** | | 4.23 ± 0.00 | |
| *2* | 18.33 ± 0.04 | | 4.10 ± 0.00 | | 4.12 ± 0.00 | | **26.56 ± 0.04** | | 1.20 ± 0.00 | | 0.00 ± 0.00 | | 3.46 ± 0.00 | | **4.65 ± 0.00** | | 31.21 ± 0.04 | |
| *3* | 36.15 ± 0.22 | | 0.00 ± 0.00 | | 26.13 ± 0.05 | | **62.28 ± 0.27** | | 11.54 ± 0.01 | | 2.81 ± 0.00 | | 3.45 ± 0.00 | | **17.81 ± 0.03** | | 80.10 ± 0.30 | |
| *4* | 43.01 ± 0.29 | | 6.21 ± 0.00 | | 35.38 ± 0.10 | | **84.60 ± 0.39** | | 12.41 ± 0.01 | | 2.60 ± 0.00 | | 0.00 ± 0.00 | | **15.02 ± 0.03** | | 99.62 ± 0.42 | |
| *5* | 233.42 ± 2.16 | | 25.14 ± 0.11 | | 65.68 ± 0.38 | | **324.24 ± 2.65** | | 46.77 ± 0.30 | | 3.84 ± 0.01 | | 2.68 ± 0.00 | | **53.29 ± 0.63** | | 377.53 ± 3.29 | |
| *6* | 28.27 ± 0.14 | | 15.12 ± 0.01 | | 24.50 ± 0.02 | | **67.89 ± 0.17** | | 14.74 ± 0.01 | | 2.49 ± 0.00 | | 10.42 ± 0.01 | | **27.63 ± 0.05** | | 95.52 ± 0.22 | |
| *7* | 14.51 ± 0.04 | | 6.65 ± 0.00 | | 16.13 ± 0.00 | | **37.29 ± 0.04** | | 2.50 ± 0.00 | | 2.48 ± 0.00 | | 10.34 ± 0.01 | | **15.32 ± 0.02** | | 52.61 ± 0.06 | |
| *8* | 2.65 ± 0.00 | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | **2.65 ± 0.00** | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | **0.00 ± 0.00** | | 2.65 ± 0.00 | |
| *9* | 2.56 ± 0.00 | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | **2.56 ± 0.00** | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | **0.00 ± 0.00** | | 2.56 ± 0.00 | |
| Total | 383.13 ± 2.89 | b/A | 57.22 ± 0.12 | c/C | 171.94 ± 0.55 | a/A | **612.30 ± 3.56** | **b/B** | 89.16 ± 0.33 | b/D | 14.22 ± 0.01 | b/D | 30.35 ± 0.02 | b/C | **133.72 ± 0.76** | **a/D** | 746.03 ± 4.33 | b/B |
| Wine: Blend of wines (W) | | | | | | | | | | | | | | | | | | |
| *1* | 0.00 ± 0.00 | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | **0.00 ± 0.00** | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | **0.00 ± 0.00** | | 0.00 ± 0.00 | |
| *2* | 21.24 ± 0.07 | | 4.19 ± 0.00 | | 17.86 ± 0.01 | | **43.29 ± 0.08** | | 18.19 ± 0.02 | | 2.51 ± 0.00 | | 3.56 ± 0.00 | | **24.26 ± 0.05** | | 67.55 ± 0.13 | |
| *3* | 19.97 ± 0.05 | | 0.00 ± 0.00 | | 23.35 ± 0.01 | | **43.32 ± 0.07** | | 9.25 ± 0.00 | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | **9.24 ± 0.01** | | 52.57 ± 0.08 | |
| *4* | 122.00 ± 1.06 | | 12.25 ± 0.02 | | 48.48 ± 0.17 | | **182.72 ± 1.25** | | 31.89 ± 0.11 | | 3.50 ± 0.01 | | 3.50 ± 0.00 | | **38.89 ± 0.25** | | 221.63 ± 1.50 | |
| *5* | 187.35 ± 1.70 | | 19.08 ± 0.05 | | 60.44 ± 0.30 | | **266.87 ± 2.05** | | 33.89 ± 0.19 | | 3.51 ± 0.01 | | 8.74 ± 0.02 | | **46.14 ± 0.45** | | 313.01 ± 2.49 | |
| *6* | 18.71 ± 0.04 | | 0.00 ± 0.00 | | 14.80 ± 0.00 | | **33.51 ± 0.05** | | 10.13 ± 0.01 | | 2.46 ± 0.00 | | 3.85 ± 0.00 | | **16.43 ± 0.03** | | 49.94 ± 0.08 | |
| *7* | 13.19 ± 0.02 | | 4.14 ± 0.00 | | 16.02 ± 0.00 | | **33.35 ± 0.03** | | 2.48 ± 0.00 | | 0.00 ± 0.00 | | 6.54 ± 0.01 | | **9.02 ± 0.01** | | 42.37 ± 0.04 | |
| *8* | 2.69 ± 0.00 | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | **2.69 ± 0.00** | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | **0.00 ± 0.00** | | 2.69 ± 0.00 | |
| *9* | 0.00 ± 0.00 | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | **0.00 ± 0.00** | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | 0.00 ± 0.00 | | **0.00 ± 0.00** | | 0.00 ± 0.00 | |
| Total | 385.15 ± 2.94 | b/A | 39.66 ± 0.07 | a/A | 180.95 ± 0.49 | b/A | **605.75 ± 3.53** | **b/A** | 105.83 ± 0.33 | d/B | 11.98 ± 0.02 | a/B | 26.19 ± 0.02 | a/A | **143.98 ± 0.80** | **b/B** | 749.76 ± 4.32 | b/A |

±S.D.: standard deviation (n=3). Different lower case letters indicate significant ($p < 0.05$) differences between wines for each group of pigments. Different upper case letters indicate significant ($p < 0.05$) differences between families of pigments for each wine.

Figure 1
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**Fig. 1.**

Figure 2
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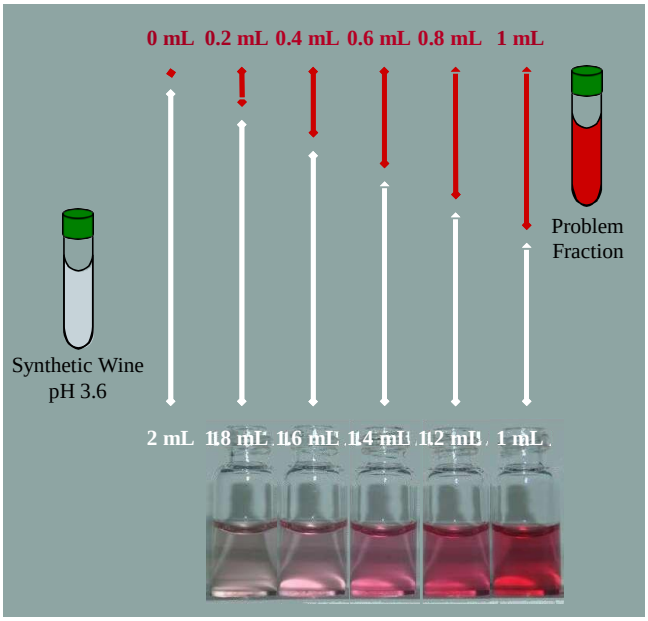


**Fig. 2.**

Figure 3

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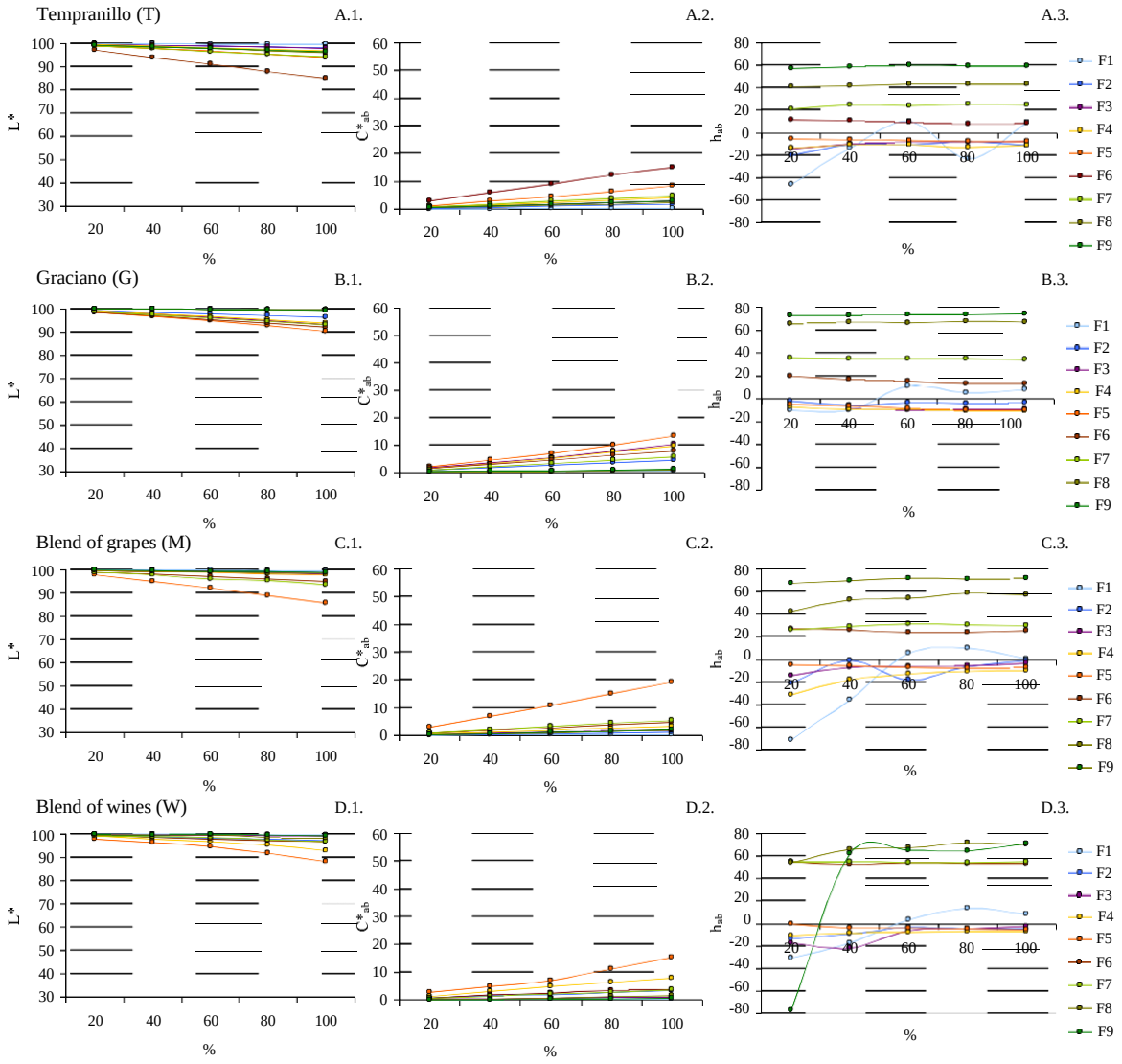


**Fig. 3.**

Figure 4

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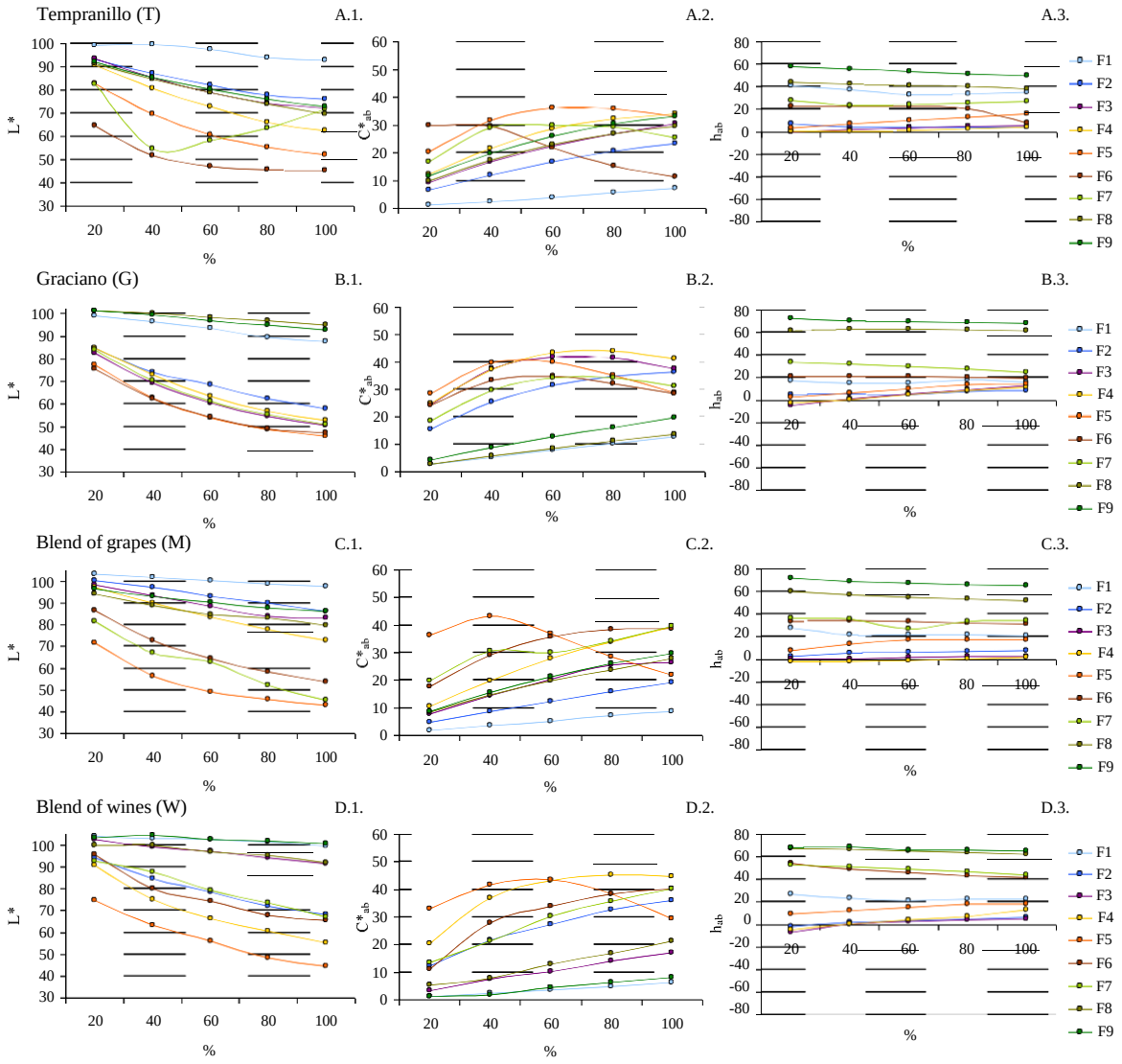


**Fig. 4.**

Figure 5

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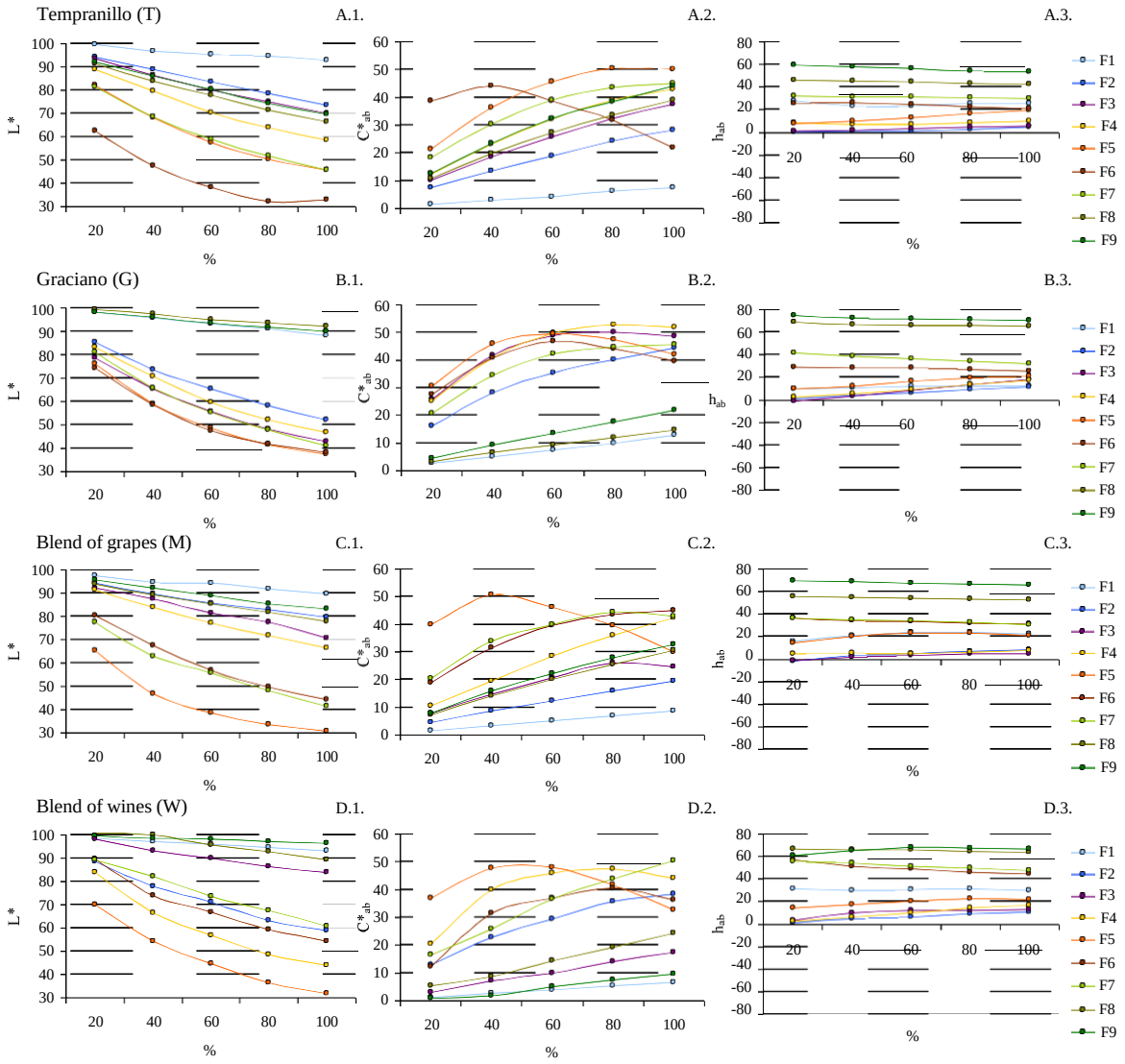


**Fig. 5.**

**Figure 6**

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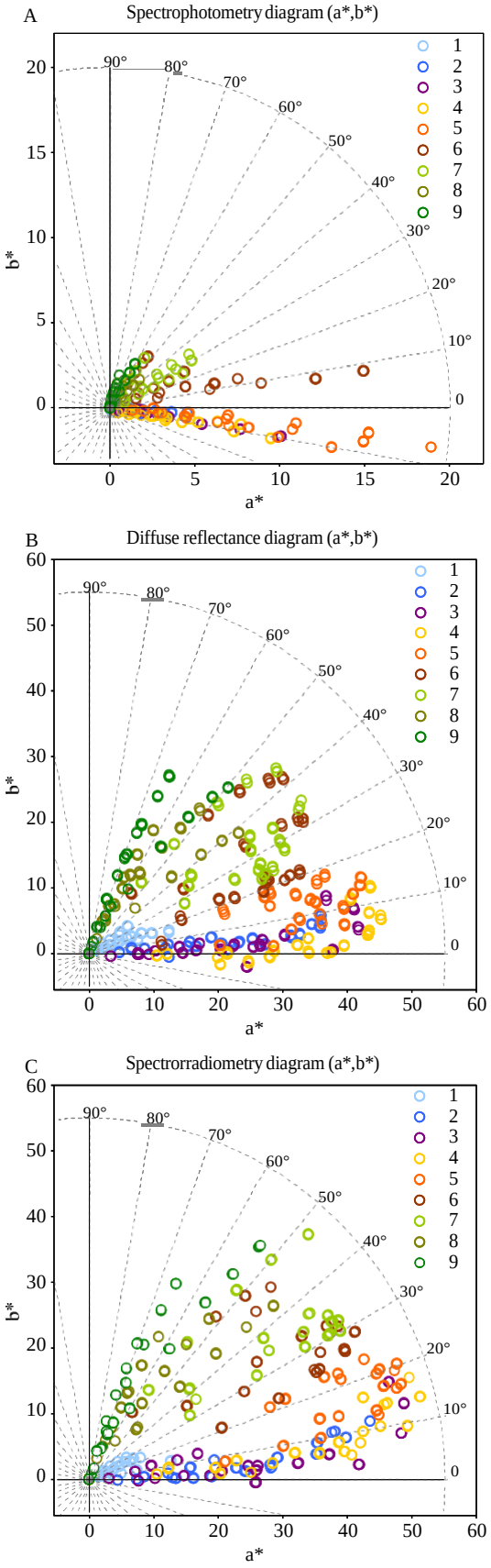


**Fig. 6.**