Chromatic characterisation of three consecutive vintages of *Vitis vinifera* red wine. Effect of dilution and iron addition

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Abstract

Both standard and CIELAB colour parameters have been evaluated for three consecutive vintages of *Vitis vinifera* (Tempranillo variety). A simple and straightforward relationship between CI and L* has been derived. Dilution effects on colour for samples under fermentation and for several commercial wines have been studied under pH controlled conditions. Disruptions of equilibria responsible for colour at the typical wavelengths upon addition of iron have been assessed.

Keywords

Wine colour; Red wine; CIELAB; Colour Intensity; Dilution study; Iron

Introduction

Colour is one of the most important organoleptic aspects of a wine. Wineries have always been aware of the importance of wine colour but recently the re-discovery of the mediterranean diet has increased the relevance of this parameter. [1]

The price of wine is assigned not only because of its alcoholic degree as before, but also because of the intensity of its colour. Thus, the interest in the knowledge and possible control of the factors affecting this parameter is maximum for enologists at the moment.

The colour of a red wine depends, mainly, on its phenolic composition, being anthocyanins the compounds responsible for the blue-red colour. These molecules are very reactive and may undergo decomposition and combination reactions throughout the vinification process, affecting the final the colour of wine [2].

Several anthocyanins have an ortho-diphenol group bound to their aromatic ring (cyanidine, delfinidin and petunidin) which will be able to chelate some metallic cations like Al^{3+}, Fe^{3+}, Cu^{2+}, Mg^{2+}, inducing a batochromic effect [3]. Tannins can also follow a similar reaction process and in both cases they produce what it is called the blue casse [4,5]. Some authors have established a relationship between the presence of anthocyanin-metal complexes with the blue colour of some native american grapes[6]. Recently [7] complexation studies have been carried out involving Cu and Zn with synthetic solutions of selected polyphenols (catechin, rutin and quercetin) as well as with wine simples, concluding that both metals chelate with the studied polyphenols.

Previous studies on the complexation of Cu, Zn and Fe in wine samples were performed by addition of different amounts of the metal of interest, which results in a dilution of the corresponding sample. Several authors [3,8-11] have faced the dilution effect on the

absorbance of the diluted samples; they have come to the conclusion that there is no proportionality between absorbance and dilution, probably due to the colloidal nature of the colouring matter present in wine.

Boutaric studied six different wines by dilution with an unbuffered 15% hydroalcoholic solution. He observed that the optical density of the diluted wines, once multiplied by the dilution factor, was different from the values found for non diluted samples, proving that Beer's law could not be applied to diluted samples. However, Negueruela [12] showed that red wines diluted in ratios up to 10-fold with an alcoholic solution at the pH of wine, do follow Beer's law and found the best correlation coefficients for those samples which absorbances were higher than 10^{-2}. Other authors [13] have described similar results and recommended 3 to 9-fold dilutions for spectrophotometric measurements of red wines.

Dilution of samples and spectrophotometric measurements are the basis for colour determination in wines. There are two widely accepted methodologies for the analysis of wine colour: the standard parameters defined by Glories [9, 10] in 1984 (colour intensity -CI-, hue –H-, brightness –B-, % Yellow, % Red and % Blue) and the chromatic coordinates (CIELAB space). Amongst all the parameters described by either of the two methodologies, CI is universally regarded as the best colorimetric quality control parameter. Accordingly, it is the most frequently measurement performed in cellars, together with the index of Sudraud[14].

On the other hand, chromatic coordinates and CIELAB space allow a much more precise definition of the colour of wine than the Glories standard parameters [1]. In fact, in 1986 the Comission Internationale de L'Eclairage (CIE) described the CIELAB space as best system for colour analysis [15].

Many authors make use of CIELAB terms -L*, a* and b*- to define the colour of the studied wines [11, 16-19]. However, such parameters are useless in wineries since they involve complex mathematical calculus, and so far there is no relationship between such chromatic coordinates and the colour quality control parameters[1].

CIELAB parameters have been calculated for various samples subjected to diverse experimental conditions. Thus, Gil-Muñoz [19] studied the evolution of such parameters during wine fermentation as well as the influence of grape temperature on colour extraction. Chicón *et al* [16] studied the composition of several red young wines in terms of anthocyanin and polyphenol contents and also chromatic characteristics, and their evolution during the first seven days of fermentation. Pérez-Magariño *et al* [20] proposed an interesting correlation between standard and CIELAB parameters, valid for young red and rosé wines. The same author has demonstrated in a recent paper [21], that is possible to obtain CIELAB parameters like L*, a* and C* from measurements at 520 nm as only wavelength.

In the present work we will focus on the chromatic analysis of three vintages -from 2002 to 2004- of *Vitis vinifera* (Tempranillo variety) grape, employing the two above described methodologies. Relationships between standard and CIELAB parameters will be sought, that might facilitate more precise measurements under real cellar conditions. Besides, dilution effects on samples undergoing vinification and on a few commercial

wines will be studied as a function of their chromatic indicators. Finally, colour modification will be examined on addition of Fe as a target metal.

Experimental

Vitis vinifera (Tempranillo variety) samples were supplied by EVENA (Station of Viticulture and Enology of Navarra) originated in an experimental supervised agricultural area located at La Jeringa (Olite, Navarra).

Vintages 2002, 2003 and 2004 were analysed along the 70 first days of fermentation. That spans from day 0 of fermentation to the day of its transfer to barrel. Besides, vintage 2002 has been thoroughly followed up to day 720. Samples were collected daily for the first two weeks of fermentation; for the following month samples were collected weekly, and thenceforth monthly.

Chromatic analysis involves a previous filtration through a Quantitative Analysis Albet DP 135 125-filter paper. Generally, dilutions of samples were performed with two different solutions: one unbuffered 15% hydroalcoholic solution and the other pH 4.0 8% ethanol buffered. All chemical reagents were analytical grade. Data are an average of at least 4 measurements.

Iron solutions were obtained from Merck (1000 ppm Certipure standard solution). Addition of Fe to studied samples was done from pH 4.0 buffered solutions containing the adequate concentration of the metal. Samples were always 5-fold diluted with either solution. When addition of Fe was required, the same dilution was done, but with a solution containing the adequate concentration of iron.

Commercially available studied wines were Tempranillo variety (Navarra Guarantee of Origin) vintages 2001 (Ochoa), 2002 (Fortius) and 2003 (Palacio de Azcona) and a young wine Monastrell variety (Jumilla Guarantee of Origin). In this case, 8 measurements were made in order to obtain the average values, for each wine.

Absorbance measurements were carried out with a Heλios Gamma UV/Visible spectrophotometer. Quartz cells from Hellma of 0.2 and 1.0 cm path length were used for undiluted and diluted samples, respectively.

Glories parameters were calculated from absorbance measurements performed at 420, 520 and 620 nm [9, 10]. CIELAB parameters were obtained from transmittance measurements at four different wavelengths, 450, 520, 570 and 630 nm [22], using de D65 illuminant and 10º observer. Data processing was performed with the MSCV® software [23] and the version 11.0 for Windows SPSS® statistical analysis software.

Results and discussion

Colorimetric analysis

Colorimetric assays carried out for three consecutive vintages along their respective vinification processes, according to Glories (standard) and CIELAB procedures, yielded the results showed in Figure 1 and Figure 2, respectively.

Colour evolution along time of vinification, expressed in terms of the respective standard parameters, adhere to a similar pattern for the three years under scrutiny. Colour intensity (CI), percentage of red (% Red) and brightness (B) increase as alcoholic fermentation takes place for ca. 20 days. Beyond that stage, their respective values slightly decrease and reach a plateau. These findings are in good agreement with previously described reports [3, 5, 24]. It is accepted that anthocyanins are favourably extracted from the skin of the grape resulting in the initial increase of CI, but later on the alcohol generated is responsible for the destruction of the partially copigmented anthocyanins. The same mechanism would explain the fate of % Red; initially, anthocyanins are favourably extracted into the alcoholic solution as alcohol is generated; then adsorption onto solid particles, complexing phenomena and even precipitation and photodecomposition reactions could be the cause for the diminishing of the free anthocyanins yielding the red colour [3]. Previous analysis of anthocyanins quantified in the very same samples of the first year confirmed that their concentration profile along vinification followed the pattern here described for % Red. [25]. Needless to say that B follow up the values of % Red, since it is calculated from data at 520 nm, as it is the case as well for % Red.

As for quantitative aspects, CI values are lower for the second and third vintages with respect to those obtained for the first one. Again, that behaviour should be related to the anthocyanins and polyphenols contents; as it happens, we have found that anthocyanins and polyphenols concentrations in those vintages are notably lower [26].

We have checked the temperatures reported for July and August 2003 [27] in which period the ripening of the second crop took place and we have found that high values between 30ºC and 35ºC were reached frequently in those months. Anthocyanins synthesis is inhibited at those temperatures [5] and accordingly lower concentrations have been experimentally found in grapes collected that year; correspondingly, % Red lowers as well. However, another relevant meteorological factor is the day-night contrast temperatures. An alternating high-low day-night temperature favours the accumulation of polyphenols in grapes. This is so because a relatively high temperature during the day stimulates the metabolic reactions, while a low temperature during the night restrains migration of formed compounds. Thus, in the third crop grapes were formed under no extremely high temperatures, what might induce a better synthesis of anthocyanins. Besides, observed smaller day-night contrast temperature intervals than in the second year, favoured migration as well, resulting a lower CI. Finally, summer 2002 recorded intermediate temperatures but a higher day-nigh contrast than in summer 2004, what yielded the best crop as for CI parameter, and anthocyanins and polyphenols contents.

When we turn our attention to the CIELAB coordinates (Figure 2), we observe that they undergo a similar evolution along the period of time studied. Lightness (L*) progressively increases from one vintage to the next, inversely to what happened with CI. On the other hand, b* values are practically null, what implies that a* and C* are numerically coincident.

It is generally accepted that CIELAB parameters allow a more precise definition of the chromatic properties of a wine. On the other hand, wine quality is mainly referred to the CI in most of wineries. A Pearson correlation matrix analysis was performed with all data obtained by both standard and CIELAB methodologies belonging to the three vintages and including all vinification stages. Good correlations were found for pairs of data of CI and L* (-0979), CI and a* (0.905), CI and C* (0.906) and CI and S* (C*/H*) (0.971). A subsequent multiple linear regression analysis by SSPS revealed that only the relationship between CI and L* could be used to precisely establish a connection between the CIELAB and the standard wine quality parameters. We have found that the equation that better describes that relationship is:

$$CI = 4.993 – 0{,}0553\ L^*$$

By using this simple formula, CIELAB precise term L* can be transformed in the better-known and appreciated standard parameter CI. Since this equation has been derived from data belonging to different stages of vinification, ranging from 0-day to young wines (ca. day-70 of vinification), we have checked its applicability to commercial wines of different varieties and ageing. We selected three Tempranillo wines (two young and one aged) from Navarra, and a young Monastrell variety wine from Jumilla.

CI values calculated from CIELAB's L* values by applying the above proposed formula, agree with experimental standard CI values within an average 2.27% for young wines. In the case of the aged wine, relative error increased up to 4.45%. Calculated CI values plotted vs. experimental CI ones, including the data of the commercial wines, give rise to a good correlation line as depicted in Figure 3.

A further check will be carried our by using the automatic CIELAB spectrophotometer provided with a photodiode array detector.

Influence of dilution on colorimetric parameters

Red wines colorimetric analysis poses a problem since usually the absorbances of their solutions are much too high, making dilution an imperative step. Several authors have referred to this question and have pointed out the disruption of the equilibria that might take place in diluted samples. In our previous works [7, 24, 28], we have always tried to preserve the naturally occurring equilibria by using pH 4 buffered conditions. In this occasion we have studied the effect of dilution on colour analysis by subjecting all samples to a 1:5 dilution with an 8% ethanol containing buffer solution. This percentage of ethanol was chosen as adequate to resemble the natural conditions in the fermentation period. In order to check whether dilution affects absorbance properties of the samples, diluted samples were measured in 1.0-cm cells, whereas undiluted samples were

introduced in 0.2-cm cells. In that way, dilution and path length should counter each other.

Figure 4 shows the experimental data obtained for diluted samples versus the undiluted ones at six different relevant wavelengths used in both standard and CIELAB methodologies. It is clearly seen that good correlation exists at wavelengths corresponding to yellow and red colours, while a poorer one is obtained for wavelengths situated in the blue zone of the visible spectrum.

Generally, the colour of grapes is intense blue, what differs from that of elaborated wines. This is due to the fact that part of the anthocyanins are copigmented in the skin of the grape. Copigmentation implies both hyperchromic and batochromic effects, displacing the wavelength to blue tonalities. Cofactors as cynamic acid, flavonols, flavones, and others, help to build up pile-like complexes with a variable number of layers. Optimum pH formation is reported to be ca. 3.5, and both oxidising conditions and presence of alcohol tend to destroy the copigments. These complexes may dissociate in aerated fermentation steps. However, in young wines copigments are able to ensemble again, and they may come to be responsible for up to 40% of the CI and the violet tone [29]. According to Somers [30] free anthocyanins are able to form polymeric structures with polyphenols, especially with tannins, giving rise to quite stable red complexes [1, 5].

Having this in mind, 8% ethanol buffered dilutions of wine would not affect greatly to either yellow or red fractions, while blue colour would be affected by the fact that ethanol would destroy copigments that are responsible for the blue fraction. If alcohol content is incremented to a 15%, as Boutaric reported in his work [8], it might be expected a larger effect of dilution on the chromatic properties for samples under vinification. On the contrary, its effect on a final wine should not be as dramatic, since relatively stable anthocyanin-tannin polymers contribute largely to the colour, as opposed to the copigments that might have undergone aeration, thus presenting a lower level in the final product.

When a further set of experiments, consisting on 1:5 dilutions with unbuffered 15% hydroalcoholic solutions, were carried out for vintage 2004 musts under vinification, larger differences with respect to undiluted samples were obtained as reflected in the following expressions:

$\lambda =$ 420 nm	$Y = 0.8243 \cdot X + 0.0217$;	$R^2 = 0.8651$
$\lambda =$ 450 nm	$Y = 0.7380 \cdot X + 0.0399$;	$R^2 = 0.8314$
$\lambda =$ 520 nm	$Y = 0.5902 \cdot X + 0.1047$;	$R^2 = 0.8336$
$\lambda =$ 570 nm	$Y = 0.6770 \cdot X + 0.0443$;	$R^2 = 0.8239$
$\lambda =$ 620 nm	$Y = 0.8254 \cdot X + 0.0008$;	$R^2 = 0.8441$
$\lambda =$ 630 nm	$Y = 0.8030 \cdot X + 0.0104$;	$R^2 = 0.7802$

where Y and X represent the absorbance of the diluted and undiluted samples, respectively.

Summarising, we may conclude that the elevated alcoholic concentration consistently disturbs the absorbing properties of samples resulting in falsely low values for CI. These experimental results confirm the above mentioned hypothesis: alcohol destroys

copigmented anthocyanins and, at the same time, measured pH 5.5 for the 15% hydroalcoholic diluted samples -higher than that of natural wines- modifies the nature of the anthocyanic complexes and their equilibria at natural pH. These findings reinforce the call for the need to dilute samples with buffered solutions.

Commercial wines

Similar tests were done for four commercial wines, in which the three types of dilutions were performed, that is to say, using 8% and 15%-ethanol buffered solutions and 15%-ethanol unbuffered hydroalcoholic solutions.

As expected, wines are less affected by dilutions and most favourably data -i.e. closer values between diluted and undiluted samples-, are obtained when pH and alcohol percentage are kept close to those naturally found (% error less than 1%, independent of the age of the wine). Main affecting factor has always been seen to be the absorbance in the blue region. One of the young wines was strikingly affected by dilution, error values rising up to 10 %; this behaviour could be ascribed to the pH of the wine, that resulted to be lower than in the other ones (3.47 *vs* 3.64).

As a conclusion we may assert that dilutions may be employed as far as pH and alcohol content are kept close to natural values.

Effect of added Fe on musts and wines

An interesting aspect of enology from a chemical point of view is to study the influence, if any, of added metals -either in the initial stages of fermentation of musts or in the final days previous to bottling or barrelling- on the quality parameters of a wine, namely CI. We intend to study the effects that may cause several metals such as Fe, Mn, Cu and others. As a first approach, we directed our attention to Fe.

Since these experiments demand dilution of samples, 8%-ethanol pH 4.0 buffered solutions were chosen as optimum for samples in vinification process. This dilution is operated in natural samples as well as in the treated with exogenous Fe, in order to assure that the only variable is the added Fe. Samples belonging to the second vintage (2003) were chosen as targets since their Fe concentration had previously been quantified in supernatant and in settlings too. This is noteworthy, since samples are filtered prior to analysis and any matter in suspension is thus irrelevant with respect to chromatic parameters.

Preliminary test carried on samples belonging to 10-day and 51-day of vinification showed that observed changes on Fe additions ceased to occur when Fe concentration doubled that of the original content in the supernatant of the sample. After the addition of Fe a period of an hour was allowed as a precaution to observe whether turbidity appeared due to blue casse that might originate from an excess of Fe. Since no turbidity surged, that extra time was shown unnecessary.

As a consequence, the study was done on eight selected samples belonging to days 1, 2, 9, 10, 25, 31, 59 and 67 of vinification. Added Fe was the same for all of them and its value guaranteed at least a 2-fold concentration.

Results showed a marked increase in % Blue, Tint and CI, the increment being larger for samples corresponding to shorter time of fermentation. Thus, ca. 30% and 50% increments are observed for % Blue and CI, respectively, for the first two samples; those parameters stabilise at values around 8% and 7%, respectively, for the last two samples. On the other hand, the red component of colour, % Red, tend to decrease upon addition of Fe, with negative variations ranging from ca. 8% to 3% depending on whether we refer to samples at the beginning or the end of the fermentation period. Finally, no significant variation for % Yellow was found along this experiment.

As for the CIELAB parameters H* and b* they are strongly influenced by the presence of Fe, and their values suffer a notable increment (over 50% in all instances) along all the period of vinification.

From this study, it is clear that exogenous Fe alters distribution of the colour components of the samples, resulting in an increment of blue colour and in a decrease of red colour. As a whole, CI increases as well. These findings are consistent with the fact that ortho diphenyl group containing anthocyanins may complex Fe^{3+} inducing a batochromic displacement to the blue region [3]. Moreover, since a larger quantity of free anthocyanins (unpolymerised) is available at the beginning of fermentation, it is only logical that this effect is more acute for the firsts samples [31]. As fermentation takes place, polymerisation of anthocyanins occurs and Fe cannot interact with them in such a smooth way, as reflected in a lower % Red increment at the end of the studied time. Furthermore, Fe could be forming blue complexes with other ligands present, that otherwise would not absorb in the red wavelength.

Although these experiments have to be checked for a larger number of samples and for a variety of metals, we have proved that cationic metal content would be a noticeable parameter to be considered in the production of final wines with a desired colour.

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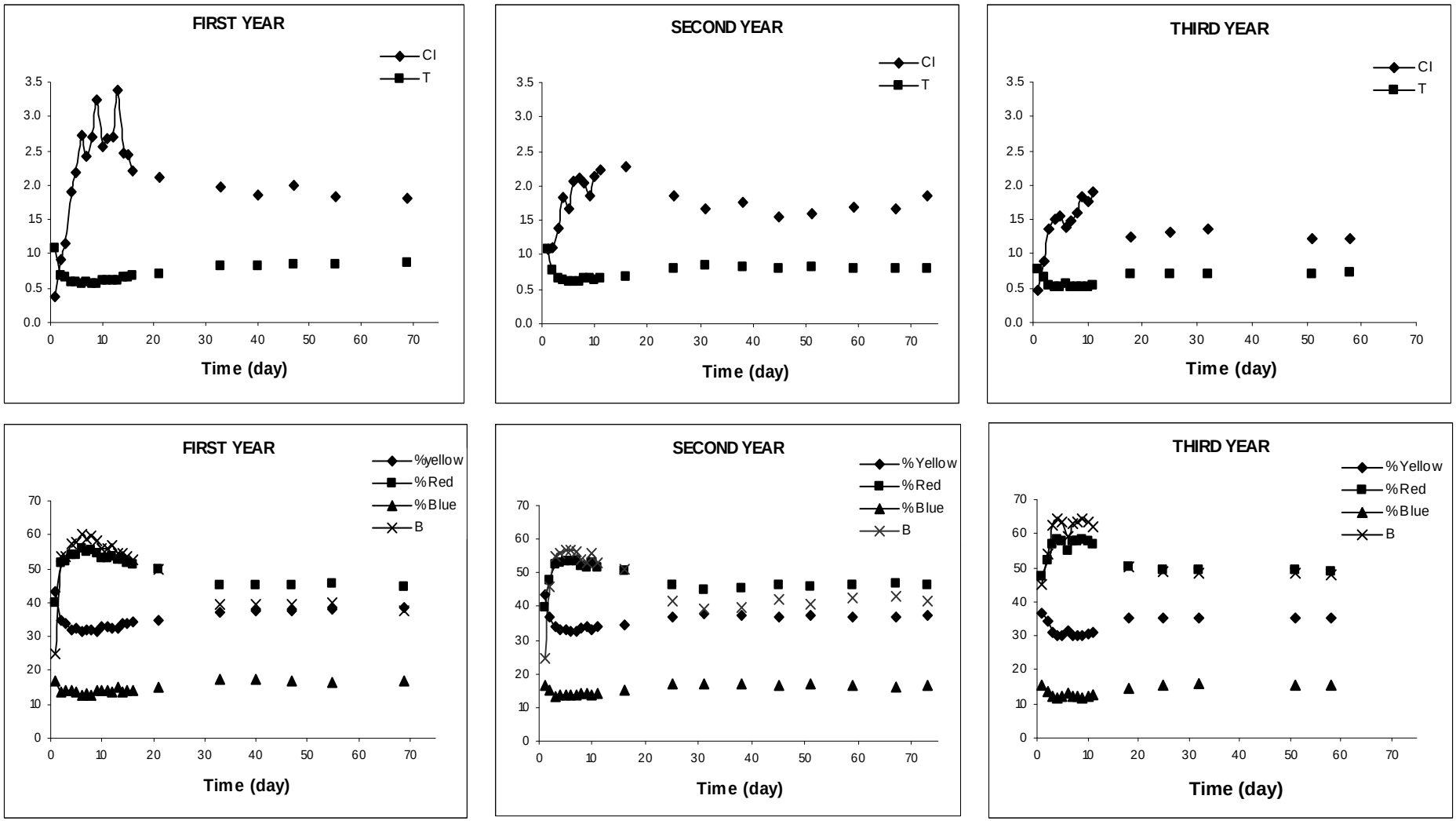


Fig.1. Evolution of Glories parameters with fermentation time for three consecutive vintages (2002 – 2004). Y axis values are in arbitrary units.

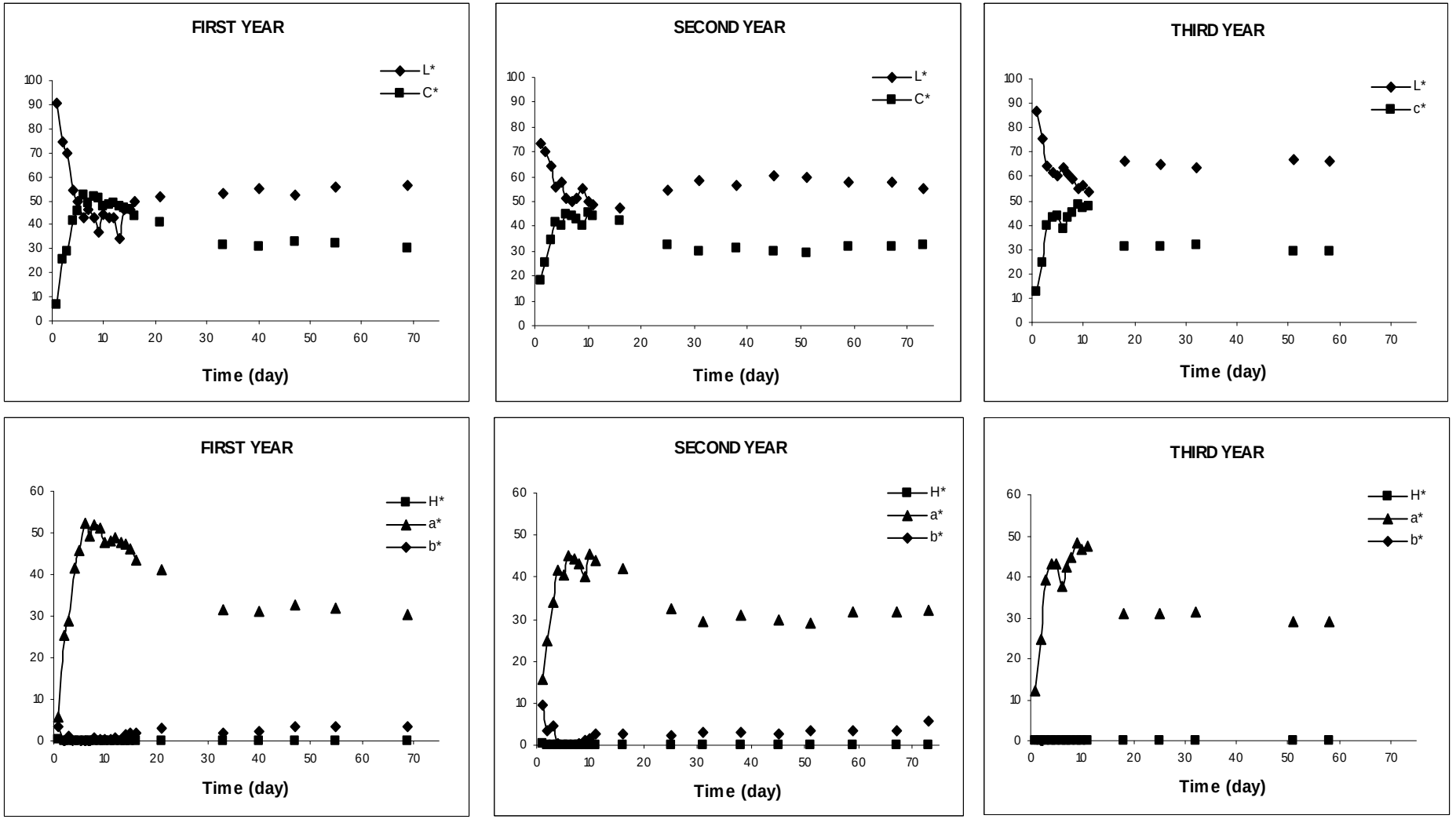
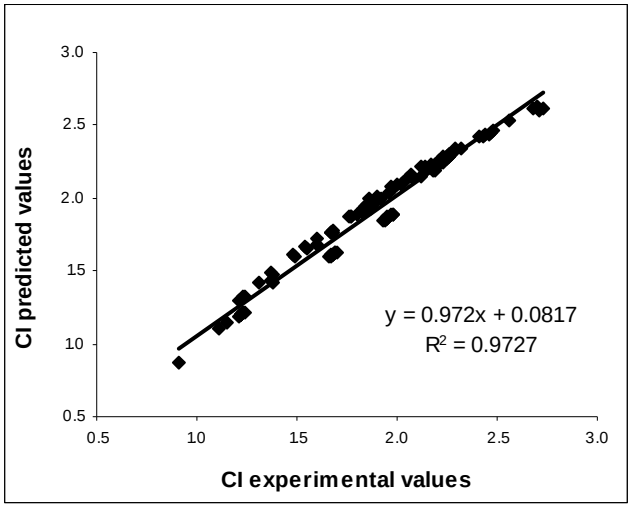


Fig. 2. Evolution of CIELAB parameters with fermentation time for three consecutive vintages (2002 – 2004). Y axis values are in arbitrary units.

Fig. 3. Comparison between predicted and experimental CI values



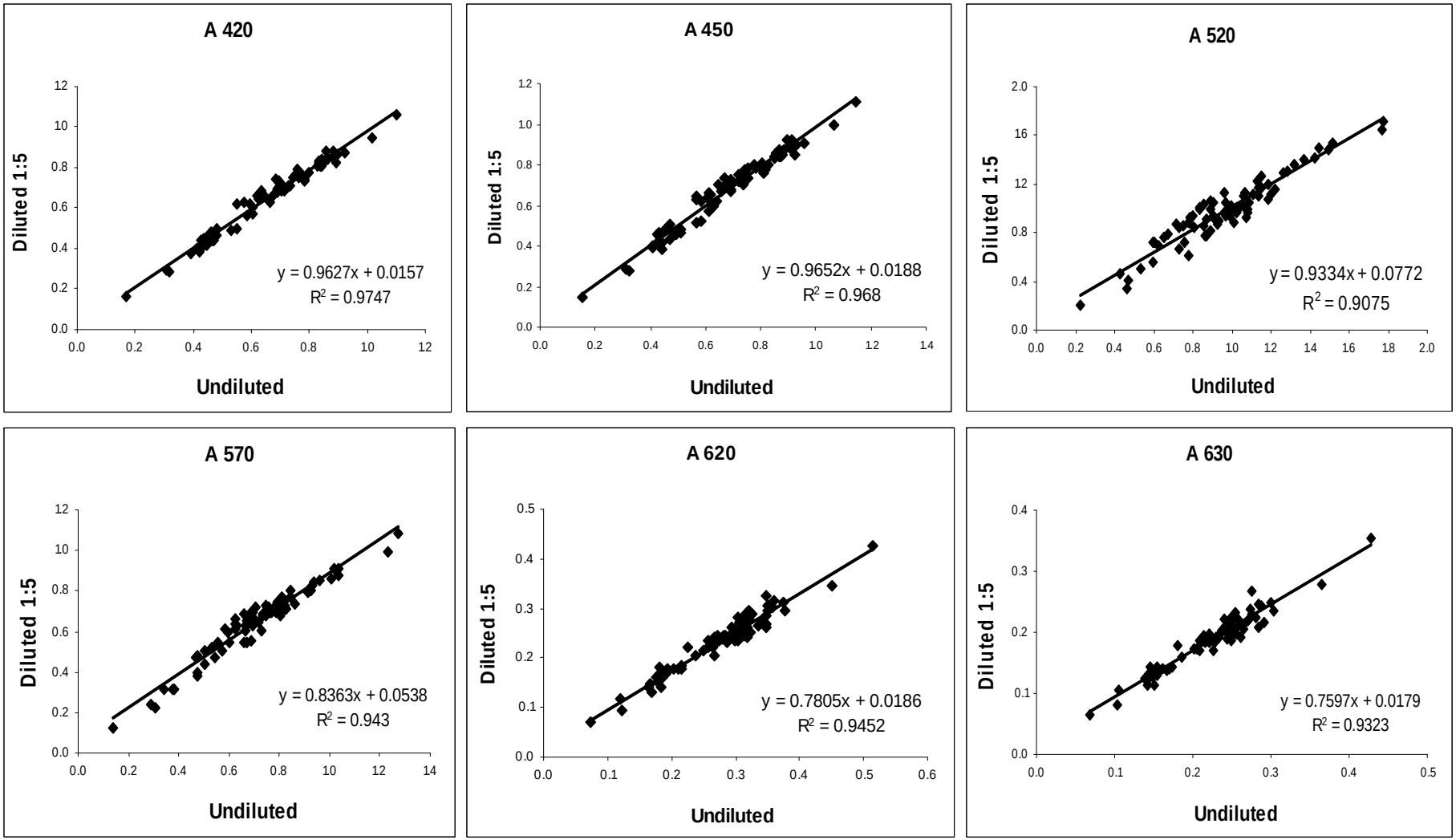


Fig. 4. Absorbances in arbitrary units of diluted (1:5) vs non-diluted samples at different wavelengths (nm), for all studied samples