Computers and Electronics in Agriculture 82 (2012) 128–133

Contents lists available at SciVerse ScienceDirect

Computers and Electronics in Agriculture

journal homepage: www.elsevier.com/locate/compag

Ripeness estimation of grape berries and seeds by image analysis

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ARTICLE INFO

Article history:
Received 23 July 2011
Received in revised form 19 November 2011
Accepted 6 January 2012

Keywords:
Colour
Grape seeds
Image analysis
Ripeness
Vitis vinifera

ABSTRACT

Digital imaging has become a powerful tool for the characterization and quality control of foodstuff. Because of the need to automate processes, faster tools are needed and Computer Vision is a good alternative to chemical analysis of many products in quality control. Appearance of grape seeds and grape berries change during the ripeness. These changes are closely related to the chemical composition, especially phenolics, which are very important compounds due to their implications on the intensity and stability of red wine colour. In this study, a complete characterization of grape seeds and grape berries by digital image analysis is described. The size of grapes and the veraison has been determined by image analysis and it has been also established an objective Browning Index of seeds. Morphological differences between varieties were studied by applying discriminant analysis models which allowed us to classify the grape seeds with high accuracy.

1. Introduction

1.1. Colorimetry and food stuff

Colour and appearance are closely related to sensory properties and chemical composition of food. Normally, colour is measured by tristimulus colorimetry. The colour stimulus is composed of three different sensations, giving to colour three-dimensional nature. These attributes are:

– Lightness: This feature makes a colour lighter or darker. It is a relative measure of the reflected light against the absorbed. Value 0 is assigned to black and value 100 is assigned to white.
– Chroma: It determines for each hue, the colour difference from the grey having the same lightness. It can take positive values from zero.
– Hue: It is the main attribute. It is a qualitative property which allows classifying colours as red, yellow, etc. It is related to differences in absorbance of radiant energy at different wavelengths. Hue is specified as an angle.

These attributes are often expressed as L^*, C^*_{ab} and h_{ab}, respectively, according to the CIELAB colour space.

It can be used different kinds of instruments, such as colorimeters, spectrophotometers and spectroradiometers. Nevertheless, these instruments require homogenizating the sample to achieve uniform colour, which becomes tedious and complicated task to measure colour of heterogeneous stuff or small objects, such as grape berries and grape seeds. In these cases, it is very advantageous the use of digital images for this purpose. Digital image analysis appears as successful complement since it can be determined not only colour but also other characteristics such as shape, texture and homogeneity (Savakar and Anami, 2009; Zheng and Sun, 2008b).

1.2. Imaging hardware and colour spaces

Computer Vision is a subfield of Artificial Intelligence. The aim of Computer Vision is 'teach' a computer for understanding a scene or the characteristics of an image. In this way, we can identify the seeds and grapes in these images, and then, morphological and colorimetric characteristics can be extracted from each one. Computer Vision is a powerful tool for testing quality in alimentary industry (Brosnan and Sun, 2004).

A Computer Vision system includes: an illumination system, a charge-coupled device (CCD), a frame grabber which converts the analogue image from the camera into a digitized one, and a computer with the suitable software for image processing and interpretation of results (Wang and Sun, 2002). Illumination system has high importance, not only because of the need to identify objects in the image, but because the colour calculation process needs a light standardization (CIE, 2007). Once the illumination is controlled, a digital camera receives images onto a CCD. It has

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doi:10.1016/j.compag.2012.01.004

capacitors which are stimulated by visible radiation and it is registered in gradations of three basic colours: red, green and blue (RGB). This is consistent with the theory that every colour can be reproduced by the combination of three primary colours.

The RGB colour space is an additive colour model that uses transmitted light to display colours. It is used for television and other devices screens, so this model is device-dependent (its appearance depends on the display) (Yam and Papadakis, 2004). The $L^*a^*b^*$ model is an international standard for colour measurement developed by the *Commission Internationale de l'Eclairage* (CIE) in 1976. Here, L^* has the same meaning that the L^* described previously. a^* (from green to red) and b^* (from blue to yellow) are Cartesian coordinates of Polar coordinates C^*_{ab} and h_{ab} previously described. This colour space is device-independent, providing consistent colour regardless of the input or output device such as digital camera, scanner, monitor and printer. CIELAB values are frequently used in food research (González-Miret et al., 2007).

Due to RGB colour space is not continuous each channel can take only integer values between 0 and 255. In order to calculate the colorimetric coordinated recommended by *Commission Internacionale de l'Eclairage* (CIE), it is necessary to transform from RGB to CIELAB colour spaces. This transformation requires calibration and it depends on illumination when images are taken (León et al., 2006).

One of the most important steps in image analysis is segmentation. Segmentation refers to the process of partitioning a digital image into multiple segments (sets of pixels). The goal of segmentation is to simplify and/or change the representation of an image into something more meaningful and easier to analyze (Zheng and Sun, 2008a).

1.3. Advantages of using image analysis for grapes and grape seeds characterization

The size of the grapes is measured in routine analysis during ripening. The diameter is normally measured with a calliper, which measures the size in only one direction and the mean of several grapes is given, making this a tedious task. In addition, the berry could be accidentally tightened during measurement and it induces error. For this purpose, the use of Feret diameter has been proposed (González Marcos et al., 2006). Feret diameter is the maximum length of chord for a counterclockwise angle with the x axis, defined between 0° and 180° (Fig. 1). Due to grape berries are not completely spherical, for each berry, the mean of all Feret diameters for all possible angles have been considered. This way, the size will be given as an average of all possible Feret diameters of all the grapes analyzed.

During the grapes ripening there is a loss of chlorophyll and the formation of the final dyes. This process starts at the veraison, the onset of ripening, when the colour of the grape berries change, being the transition from berry growth to berry ripening. In red grapes, the colour changes from green to purple, even almost black. The veraison is normally expressed as a percentage determined by visual inspection. It is possible to establish an objective veraison index by image analysis.

There are more appearance-related characteristics of the seeds and grapes associated to maturity, such as the browning of the seeds (Ristic and Iland, 2005). All these parameters can be estimated of an objective and automated manner by image analysis.

2. Material and methods

2.1. Sampling

The vineyards sampled are included under the "Condado de Huelva" Designation of Origin, in southwestern Spain, harvested in 2009 and 2010. Two red varieties (*Tempranillo* and *Syrah*) and one autochthonous white variety (*Zalema*) cultivated in two kinds of soil (*Sand* and *Clay*) were used. Samples were taken twice a week from early July until harvest (which occurred approximately at end of August depending on variety). Sampling was carried out taking a pair of berries from alternate grapevines and from both sides up to reach 2 kg of berries, ensuring this way the representativeness of the sample. Once in lab, one hundred berries were randomly taken and cleaned for acquiring the images. Seeds of these berries were removed and dried at room temperature for two hours before acquiring the image.

2.2. Apparatus

The DigiEye® imaging system based upon the calibrated digital camera was used (Luo et al., 2001). It includes an illumination box specially designed by VeriVide Ltd. (Leicester, UK) to illuminate the samples consistently and a digital camera connected to a computer (Fig. 2).

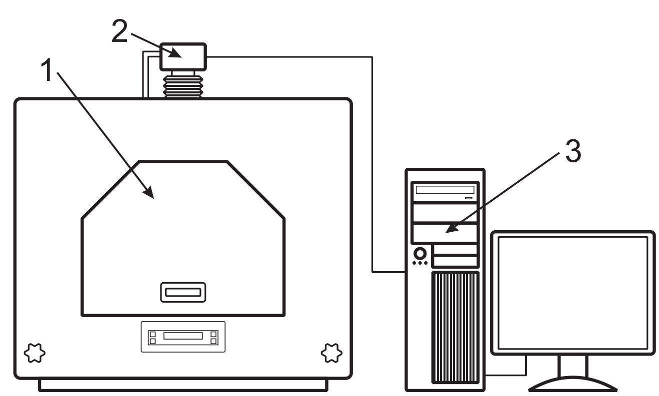

Fig. 2. The DigiEye® System: (1) illumination box; (2) digital camera; (3) computer.

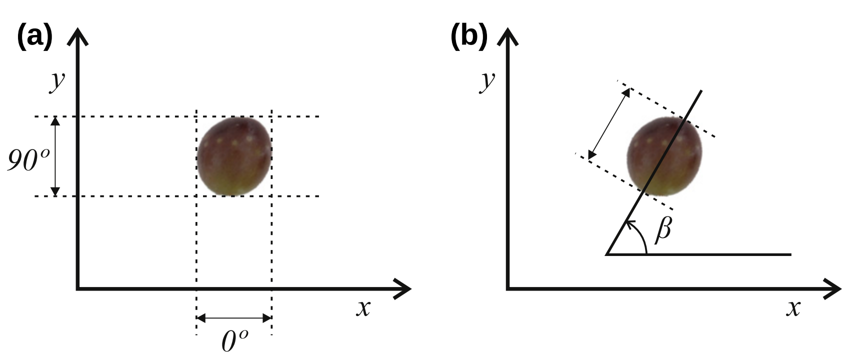

Fig. 1. (a) Feret diameters at 0° and 90°. (b) Feret diameter at an angle β.

The digital camera used for image acquisition was 10.2-megapixel Nikon® D80 with Nikkor® 35 mm f/2D objective. The camera was connected via USB to a computer with Pentium IV processor at 3.00 GHz. The cabinet is equipped with two fluorescent tubes that emulate the standard illuminant D65 and offer stable lighting conditions. Lamps were switched on at least ten minutes before being used, according to manufacturer indications, to stabilize them.

For obtaining morphological and appearance parameters, as well as CIELAB coordinates from RGB colour space, the software DigiFood® (Heredia et al., 2006) was used.

3. Results

3.1. Image acquisition

The camera parameters were set up for all images as shown in Table 1. To ensure that the camera always takes images the same way, calibration is required prior to use by a certified colour chart DigiTizer provided by VeriVide Ltd. (Leicester, UK) (Fig. 3), composed of a matrix of 12 × 20 squares of different colours. DigiEye have data of the $L^*a^*b^*$ coordinates of each square and adapt the RGB coordinates obtained by the camera for this calibration.

Several backgrounds were tested for image acquisition of grapes and seeds. Among them, it was found that the white surface increases the contrast between objects and background, and improves the segmentation process. Once the background was selected, both seeds and berries were put avoiding contact among them over a white thick sheet, considered as a good Lambertian surface (diffuse reflectance surface which does not vary depending on the viewing angle) (Jaglarlz et al., 2006).

3.2. Image processing

Although the analytical information was obtained from the CIELAB coordinates calculated, HSI (hue, saturation and intensity) colour space was used for segmentation process (Jack, 2008). HSI colour space is easier to understand than RGB, due to its likeness with the L^*, C^*_{ab} and h_{ab} stimuli.

The histogram thresholding segmentation technique was used (Cheng et al., 2001). It allows distinguish different objects in images that are composed of regions with different colour ranges. Histograms of HIS values were plotted, containing well separated peaks corresponding to the sample and the background. The lowest point between adjacent peaks was stated as threshold for segmentation process. After establishing the thresholds, the suitability of the criteria was confirmed by visual inspection.

For grape berries recognition, all regions having intensity (I) between 0 and 160 units were considered. Restrictions in hue (H) and saturation (S) values were not required for grapes berries. To prevent the recognition of small spots as grapes, the segmentation was restricted to objects having Feret diameter between 5 and 25 mm.

In the same way, for seeds recognition, sets of pixels with saturation (S) between 0 and 180 units and intensity (I) between 0 and 140 units were considered. All hues (H) were considered. Similarly, morphological restrictions were applied. All objects must have area between 6 and 35 mm^2 and length shorter than 15 mm for being considered (Fig. 4).

The following morphological and appearance parameters were measured from segmented images of seeds:

RGB colour was obtained from the raw images. CIELAB colour coordinates were obtained from the RGB values and through the camera calibration. All images were acquired under the same conditions. Thus, taking a picture of the calliper, it was possible to calculate the conversion between units of length and pixels. The size of seeds was measured in two directions: diameter along major axis of the seed (length) and diameter along the axis perpendicular to the major axis (width). In order to measure the elongation degree of each seed, aspect ratio was calculated, which is ratio is the ratio between major axis and minor axis of the ellipse equivalent to the seed. Roundness values were calculated using the following dimensionless equation (González Marcos et al., 2006):

$$\text{Roundness}: \frac{\text{perimeter}^2}{4 \times \pi \times \text{area}}.$$

In this sense, a perfect circle has a roundness value of 1 and it becomes higher while the shape elongates. Some authors use the inverse of this equation for the same purpose. The area of each pixel was calculated by the spatial calibration. Thus, the area of each seed was calculated by pixel count.

Measuring colour of each pixel, it is possible to have an estimation of the heterogeneity of the sample as the fraction of pixels that

Table 1
Camera setup.

Time exposure	1/15 s
Aperture	f/6.3
Sensitivity	200 ISO
Format	TIF
Image size	3872 × 2592
Resolution	96 pixels per inch (ppi)

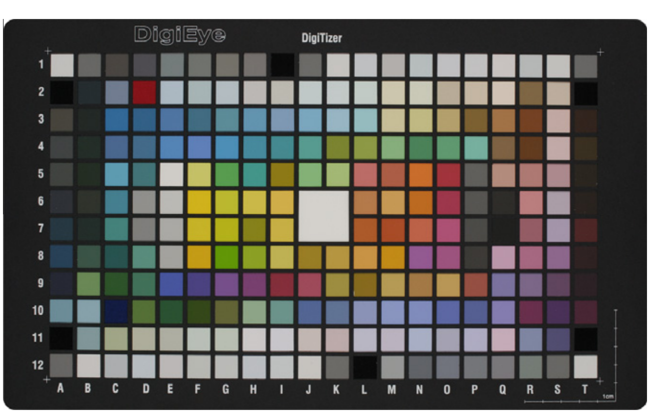

Fig. 3. Colour chart used for the calibration of the digital camera. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

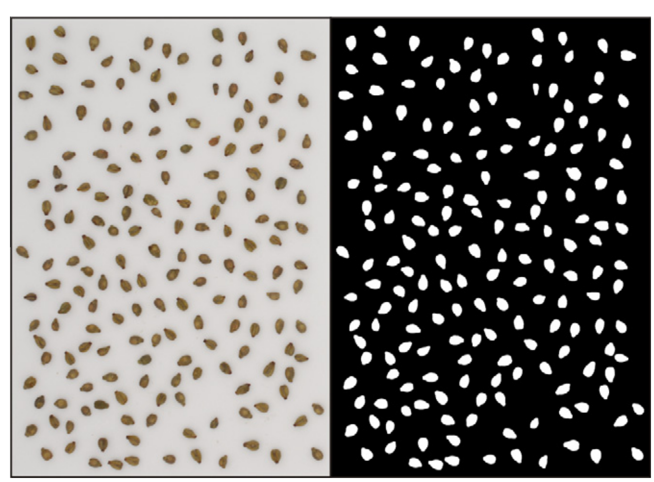

Fig. 4. Image of Syrah seeds and the resulting mask after segmentation.

Table 2
Some morphological parameters measured in seeds regarding the variety (mean ± Std. Dev.).

	Syrah	Tempranillo	Zalema (sand)	Zalema (clay)
Length (mm)	6.6 ± 0.2[a]	6.8 ± 0.2[a]	5.7 ± 0.1[b]	5.7 ± 0.1[b]
Width (mm)	3.8 ± 0.1[b]	4.0 ± 0.1[a]	3.6 ± 0.1[c]	3.6 ± 0.1[c]
Aspect ratio	1.80 ± 0.01[a]	1.72 ± 0.01[b]	1.64 ± 0.01[c]	1.64 ± 0.01[c]
Roundness	1.31 ± 0.02[a]	1.26 ± 0.03[b]	1.24 ± 0.03[b]	1.24 ± 0.02[b]
Perimeter (mm)	16.91 ± 0.08[b]	17.50 ± 0.09[a]	14.85 ± 0.07[c]	14.76 ± 0.07[c]
Area (mm^2)	17.3 ± 0.7[b]	19.1 ± 1.1[a]	14.1 ± 0.9[c]	13.9 ± 0.7[c]
Heterogeneity (%)	17.5 ± 0.6[a]	13.9 ± 0.7[b]	15.4 ± 0.5[ab]	14.7 ± 0.5[ab]

[a–c] Differents superscripts within rows for each parameter indicate statistically significant differences ($P < 0.05$).

deviate more than 10% from the average intensity. Other indicator of heterogeneity is obtained by using the Mean Colour Difference from the Mean (MCDM), proposed by Berns (2000):

$$\mathrm{MCDM} = \frac{\sum_{i=1}^{N}\left[\left(L_i^* - \overline{L^*}\right)^2 + \left(a_i^* - \overline{a^*}\right)^2 + \left(b_i^* - \overline{b^*}\right)^2\right]^{1/2}}{N}.$$

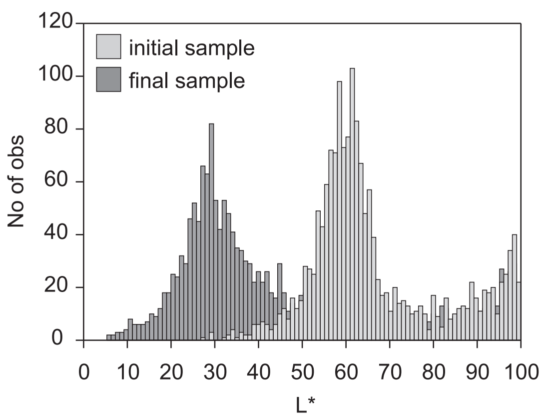

Fig. 5. Overlap of the histograms of L^* for the initial and final samples.

3.3. Study of appearance and colorimetric evolution of grapes and grape seeds

3.3.1. Varietal classification by morphology

From morphological data of all samples corresponding to the four grapes varieties considered in this study, analysis of variance ANOVA with Statistica® 8.0 (StatSoft Inc., 2007) was applied. The aim of considering all samples was to distinguish among varieties regardless the moment of sampling. Significant differences ($p < 0.05$) in the morphology of seeds from different grape varieties were found. No differences regarding the soil type for the white variety were found (Table 2).

Discriminant analysis was applied to classify the samples based on the features of appearance of the seed. Based on seeds from red grapes or white grapes, the classification was 100%. The classification between the two red varieties was 100% for Syrah and 87.5% for Tempranillo. When only the white variety was considered, it was not possible to discriminate correctly between soil types by morphological features. The classification for Zalema Sand was 71.43% while for Zalema Clay was only 57.14%. In general, the parameters aspect ratio, roundness and width gain more weight in the equations.

3.3.2. Evolution of the appearance of seeds during maturation

According to the variety, similar changes occur in grape seed during the maturation. Colorimetric parameters decrease quickly

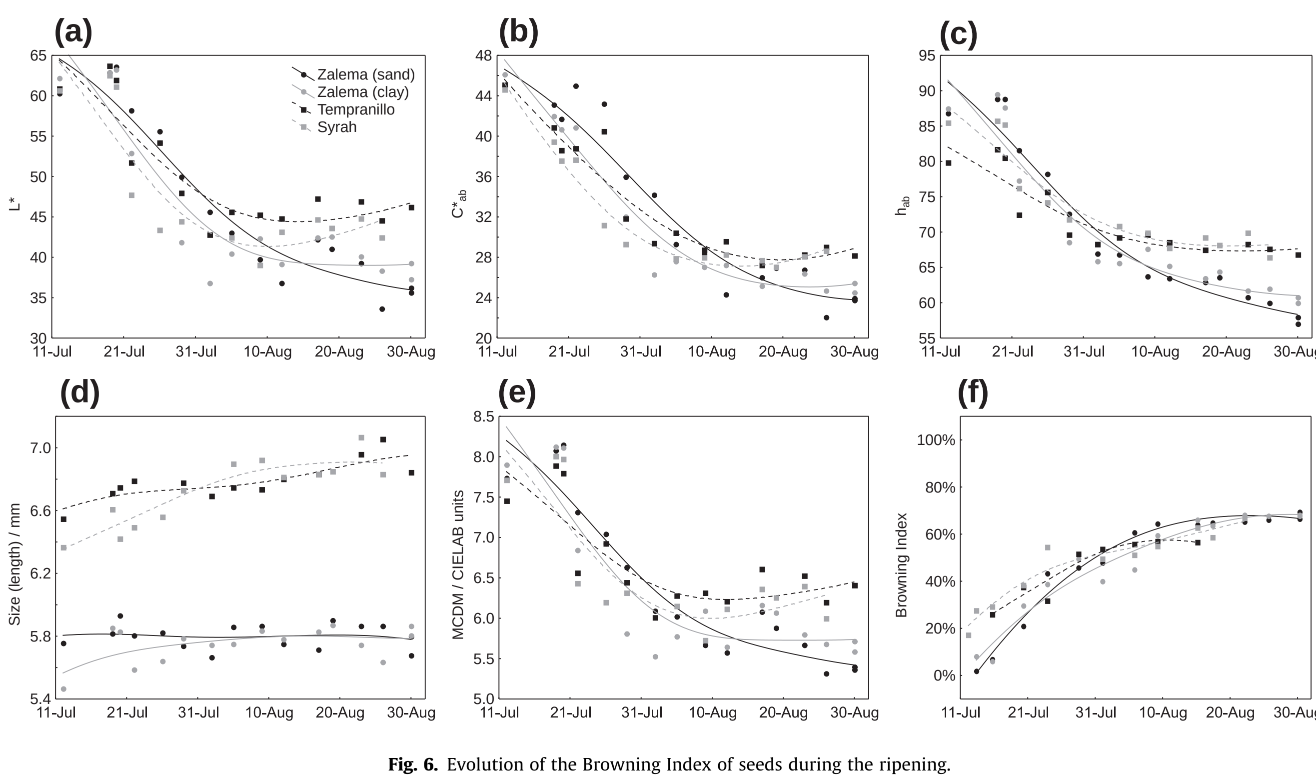

Fig. 6. Evolution of the Browning Index of seeds during the ripening.

in the first stages and stabilize themselves in the last ones (Fig. 6a–c). However, a related fit is visible in the figure depending on the type of grape (red or white). There was no noteworthy change in the shape of the seeds in this period. Nevertheless, the graph clearly shows the difference between the size of the seeds of red and white varieties (Fig. 6d). These differences were essential in the discriminant analysis described above. The Fig. 6f shows how natural darkening of seeds produces the decrease of the mean CIELAB colour difference from the mean MCDM, being this lastest colour more homogeneous.

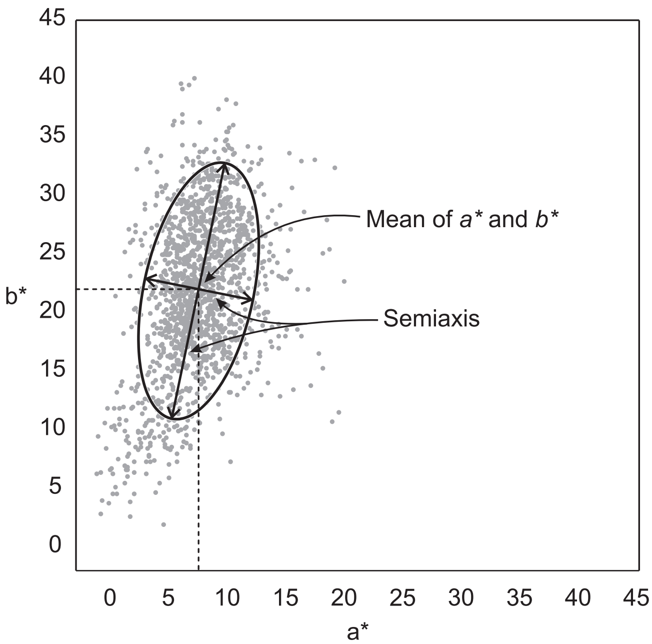

Fig. 7. Scatterplot of seed colour and ellipse fitting.

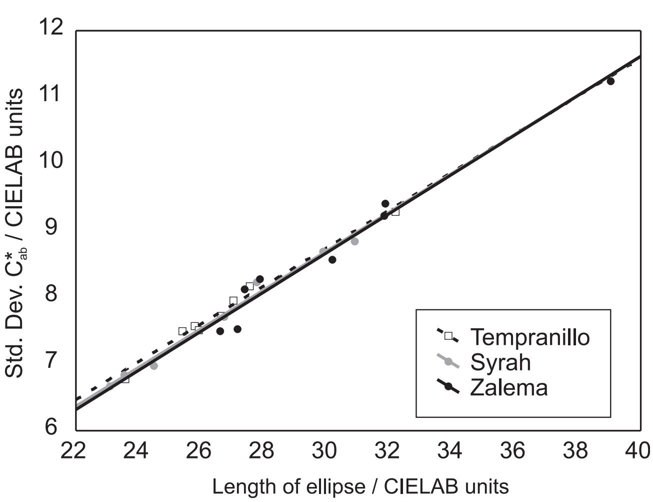

Fig. 8. Correlation between Std. Dev. of chroma (C^*_{ab}) and length of ellipse.

3.3.2.1. Browning Index. Colour heterogeneity increases in the early stages of maturation and decreases in the final stages, because browning process does not occur in a homogeneous manner. The seeds become dark brown bit by bit while the light green areas disappear in the same way. Histograms of L^* for the initial and final samples were overlaid in order to study the behaviour. Both histograms crossed in the value $L^* = 50$ (Fig. 5). Thus, "Browning Index" was defined the as fraction of pixel having L^* value lower than 50 CIELAB units.

$$\text{Browning Index} = \frac{\text{No. of pixels with } L^* < 50 \text{ units}}{\text{No. total pixels}}.$$

During maturation, the Browning Index increased rapidly at the early stages while it is stabilized at the final stages when the seed was almost completely dark brown (Fig. 6f).

3.3.2.2. Colour ellipses. By spectroradiometry, we obtain the average from the measured area. In colour analysis by digitalization, we obtain a measure from each pixel from the image.

After processing the images, all colour points corresponding to a sample were plotted in the a^*b^*-diagram. Regarding the evolution of these "clouds of points" it can be appreciated how the chromatic dispersion decreases over time. In the early stages, the seeds are pale green mixed with light brown, which evolve until the harvest, producing a clear convergence towards dark brown colours. These points give information about the chromatic heterogeneity of the sample, and they can be fitted to an ellipse for its quantification. The ellipse is oriented along the line of least squares linear fit of these points. The centre of the ellipse coincides with the mean of a^* and b^* values and its semiaxis are proportional to the dispersion of the points (Fig. 7). It was found high correlation ($r^2 = 0.98$) between the length of the ellipses containing the pixels of one sample and the standard deviation of chroma corresponding to these pixels (C^*_{ab}) (Fig. 8).

3.3.3. Evolution of the appearance of grapes during maturation

A group of berries were taken and their veraison were assigned by visual assessment. The unripe berries are clearly green. Then, its colour turns lighter and begins to appear pink zones. Finally, the skin colour moves forward purple almost black. For each berry, the veraison is considered complete when the colour reaches this deep purple.

A criterion based on the bright was enough to assess the veraison level. After identifying the grape berries within the images, the

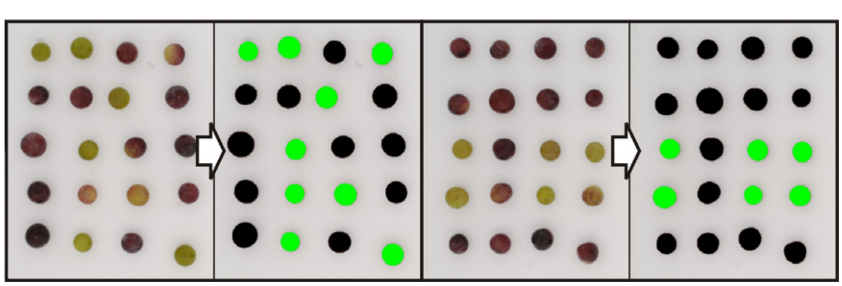

Fig. 9. Segmentation mask and veraison assessment by the selected criterion.

RGB values of grapes before and after veraison were studied. The following threshold was established:

$$\frac{R+G+B}{3} \geqslant 90 \rightarrow \text{pre-veraison},$$
$$\frac{R+G+B}{3} < 90 \rightarrow \text{post-veraison}.$$

As performed by visual assessment, the veraison was calculated for each sample (group of grapes) as the percentage of berries that meet the threshold set (Fig. 9).

4. Conclusions

The appearance of grape seeds has been assessed along two months corresponding to the final stages of ripening in two vintages. Within the same grape variety, the morphology of the seeds does not change during the period considered. However, there are significant differences ($p < 0.05$) among varieties, so we can use these parameters for varietal discrimination. Except for the Zalema cv., which two kinds of samples were considered regarding the type of soil, classification was successful, being the aspect ratio and the roundness the variables that induced the differences.

The threshold chosen from the overlap of initial and final histograms of lightness (L^*) was the same for the two vintages considered (2009 and 2010). So, the Browning Index proposed is a good criterion for estimating the maturity of grapes seeds.

The colour ellipses containing most of pixels from an image are measurable, clearly express the average colour and the chromatic heterogeneity of these samples, and there is a clear relationship between the size of the ellipse and the standard deviation of chroma (C^*_{ab}).

Veraison and the size of the grape berries are characteristics routinely assessed during maturation. It has been established an effective and objective criteria for the veraison assessment as well as a measure of size faster and more accurate than that obtained manually using a calliper based on digital image analysis.

In short, the image analysis is becoming a fast and successful tool for the evaluation of food products. It is possible to use these tools to characterize the colour, morphology and appearance of the grapes and their seeds in a comprehensive manner.

Acknowledgements

We thank the Ministry of Science and Innovation of Spain (Project AGL2008-05569-C02-02) and the concession of the fellowship (BES-2009-025429) for financial support.

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