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# Water content effect on the chromatic attributes of dehydrated strawberries during storage, as evaluated by image analysis☆

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ABSTRACT

The changes of chromatic attributes due to different hydration degree, anthocyanins degradation and browning development were studied in dehydrated strawberries. Strawberry slices were freeze-dried, equilibrated at different relative humidities and stored at 45 °C. The changes of several functions of the CIELAB color space were analyzed using a computer vision system, employing segmented image analysis. Darkening promoted by browning reactions could be evaluated through the decrease of luminosity and increase of hue angle and yellow coordinate in the whitish sections of the samples. During humidification the anthocyanin degradation increased as increasing water content. Of the several color functions, only the red coordinate values of the reddish sections of the samples correlated with anthocyanin degradation, but at relative humidities values higher than 43%. Light diffusion in the dried material caused a lighter appearance up to 75% relative humidity, while browning reaction and pigment degradation were accelerated at this relative humidity. These effects were related with the appearance of mobile water populations in the material, as detected by $^1$H NMR.



## 1. Introduction

Chromatic attributes are critical factors that determine food acceptance or rejection by the consumers. Thus, color measurement has gained much attention from food science and industry. Color meters typically measure the "average" color of the food material's surface exposed to the measuring window of the instrument (Balaban, 2008). However, many food products, such as strawberry slices, show heterogeneous color distribution. While averaging is acceptable for uniformly colored surfaces (Acevedo, Briones, Buera, & Aguilera, 2008; Acevedo, Schebor, & Buera, 2008; Briones & Aguilera, 2005; Mendoza, Dejmek, & Aguilera, 2006; Venir, Munari, Tonizzo, & Maltini, 2007), when the colors change with location or are non-homogeneous, averaging may result in inaccurate color determination (Balaban, 2008). Furthermore, even in homogeneously colored food products, discoloration can occur heterogeneously. For non-uniform colors, machine vision eliminates many restrictions of other instrumental methods. The contours method, or image segmentation, can play a useful role in the quantification of non-uniform colors of these fruits (Balaban, 2008). It also allows the quantification of areas with different chromatic characteristics.

Dehydrated strawberries are microbiologically highly stable and retain their structure; sublimation drying being more effective in preserving valuable food attributes than traditional methods of drying. However, freeze-dried fruits are prone to suffer discoloration during storage (Uddin, Hawlader, Ding, & Mujumdar, 2002). Anthocyanins are responsible for the red colors of strawberry. Interest in anthocyanin pigments has intensified in recent years because of their health benefits associated to their potent antioxidant properties (Benvenuti, Pellati, Melegari, & Bertelli, 2004; Hannum, 2004; Matchett, MacKinnon, Sweeney, Gottschall-Pass, & Hurta, 2005). Anthocyanins stability is affected by processing conditions, storage temperature, sugar, pH value, ascorbic acid, and water availability (Tsai, Hsieh, & Huang, 2004; Wrolstad, 2004). Besides anthocyanin losses, color changes can be caused by brown pigments that are formed due to the Maillard reaction (Acevedo, Briones, et al., 2008; Acevedo, Schebor, et al., 2008; Agudelo-Laverde, Acevedo, Schebor, & Buera, 2011). Critical appearance changes have also been reported, which could be related to water contents and water mobility. $^1$H NMR relaxation times have thus been and effective tool for interpreting the behavior of due to appearance changes in the RH scale (Agudelo-Laverde et al., 2011; Farroni & Buera, in press). The objective of this work was to evaluate

*Abbreviations:* RH, relative humidity; CVS, computer vision system; NMR, Nuclear magnetic resonance; CPMG, Carr–Purcell–Meiboom–Gill.

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the effect of hydration degree, anthocyanins degradation and browning development on the chromatic attributes of dehydrated strawberries by employing image segmentation analysis.

## 2. Materials and methods

### 2.1. Fruits

Fully ripe fresh strawberries were obtained from the local market and stored at 4 °C until the moment of the experiment. The fruits were washed and were cut transversally into slices (2.0 cm diameter and 0.5 cm thickness). The cut material was immediately frozen with liquid nitrogen and stored at −20 °C.

### 2.2. Materials preparation

Fruit slices were covered with liquid nitrogen before freeze-drying. A freeze drier (ALPHA 1-4 LD2 Martin Christ Gefriertrocknungsanlagen GMB, Germany) was used. The freeze drier was operated at −55 °C, at a chamber pressure of 4 Pa, and the process last 48 h. After freeze-drying, several pieces were distributed into vials for the determination of anthocyanins content. Samples used for all determinations were humidified in a range of 11–84% RH for 14 days at 20 °C (Greenspan, 1977). After humidification the strawberry slices used to determine color changes were placed inside rubber o-rings and sandwiched between two glass plates hermetically sealed to avoid water loss according to the method reported by Acevedo and coworkers (Acevedo, Briones, et al., 2008; Acevedo, Schebor, et al., 2008). Then they were placed in a forced air oven operated at 45 ± 1 °C during 120 h. Two glass plates containing six strawberry slices for each relative humidity were removed from the oven after adequate times to acquire images and measured in their glass-covered containers.

### 2.3. Molecular mobility

A pulsed nuclear magnetic resonance (NMR) instrument (Bruker mq 20 Minispec, with a 0.47 T magnetic field operating at resonance frequency of 20 MHz), was used for measurements. Humidified materials were removed from the desiccators, placed into10 mm diameter glass tubes and returned to the desiccators for additional 24 h prior to analysis.

Proton transverse relaxation times $T_2$ associated to slow relaxing protons (related to the populations of water molecules displaying less interaction with solids) were measured using the Carr–Purcell–Meiboom–Gill pulse sequence (CPMG) $90°x - (\tau - 180°y\ \tau\ \text{echo})_n$.

All determinations were performed in duplicate and the average and standard deviations are provided.

### 2.4. Anthocyanins extraction

Anthocyanins were extracted following the method described by Giusti and Wrolstad (2001). 1 g of fresh strawberries or 0.3 g of dehydrated fruits were blended with 25 mL 95% ethanol–1.5 N HCl (85:15) in order to release the red pigment. The mixture was kept overnight at 3–5 °C and then filtered. The solid residue was exhaustively washed with ethanol until final volume was 50 mL.

### 2.5. Anthocyanins determination

Total monomeric anthocyanins were determined by the pH-differential method described by Giusti and Wrolstad (2001) in fresh strawberries, after humidification at 20 °C and after heat treatment at 45 °C. Absorbance was read at 516 and 700 nm, using a spectrophotometer UV–visible Jasco V630 (Jasco Corporation, Japan). The major phenolic compounds identified in strawberry extracts are pelargonidin-3-glucoside and, in lesser quantities, cyanidin-3-glucoside (Proteggente et al., 2002). Thus, the anthocyanins content was calculated according to equation (1) and expressed as pelargonidin-3-glucoside per 100 g. An average value of three replicates was reported along with the standard deviation.

$$\text{Total Anthocyanins (mg/L)} = \frac{A \times \text{MW} \times \text{DF} \times 10^3}{\varepsilon \times l} \quad (1)$$

where: $A$ is the absorbance $(A_{\max} - A_{700\text{nm}})\text{pH}_{1.0} - (A_{\max} - A_{700\text{nm}})\text{pH}_{4.5}$, MW is the molecular weight of pelargonidin-3-glucoside (433.2 g/mol), DF is the dilution factor, $\varepsilon$ (31,600 L/mol × cm) is the molar extinction coefficient of pelargonidin (Wrolstad, 1976), $l$ is the path length (1 cm).

### 2.6. Computer vision system (CVS)

Strawberry color changes were determined by image analysis. The CVS consisted of three elements: a lighting system, a digital camera and a personal computer. The lighting system included a D65 lamp (this illuminant corresponds to solar irradiation with a color temperature of 6500 K (Lozano, 1978)) inside a grey chamber (N7 in the Munsell color space). The time of warm up of the lamps was of 15 min before to take pictures. The angle between the camera axis and the sample plane was 90° and the angle between the light source and the sample plane was 45°, in order to capture the diffuse reflection responsible for color, which is produced at this angle (Yam & Papadakis, 2004). A high-resolution (10.1 mega-pixel) digital camera, an EOS 40D (Canon Inc., Japan) was used, with an EF-S 60mm f2.8 macro lens (Canon Inc., Japan). Samples were transferred from the forced air oven to the grey box and images acquired at different times during the whole storage period. The digital camera was operated in manual mode, with the lens aperture at $f = 6.3$ and speed 1/8 s (no zoom, no flash) to achieve high uniformity and repeatability. The calibration of the camera and the parameters used for image capture are described in Briones and Aguilera (2005). Images have a resolution of 3888 × 2592 pixels and were stored in JPEG format using Canon's Remote Capture program (EOS Utility, Canon Inc., USA). The images were taken using white backgrounds.

### 2.7. Image segmentation

CVS permitted acquiring information for the whole pieces directly inside the glass plates. Color images were obtained in Lab values using Adobe Photoshop CS4 software (Adobe Systems Inc., San Jose, CA) and then were converted to the standard CIELAB space using mathematical formulas described by Papadakis and coworkers (Papadakis, Abdul-Malek, Kamdem, & Yam, 2000). From the CIELAB coordinates ($L^*$, $a^*$ and $b^*$), the hue angle and chroma variables have been calculated according to the following equations:

$$h'_{ab} = \arctan\left(\frac{b^*}{a^*}\right) \quad (2)$$

$$C^*_{ab} = \left[a^{*2} + b^{*2}\right] \quad (3)$$

The hue angle, $h'_{ab}$, has the advantage of providing information of the color quadrant in which a given analyzed sample is located, while the chroma, $C^*_{ab}$, represents a function of its color saturation (for example, from red to grey at a given hue angle).

It is possible for the software to identify every pixel in an image that has color attributes ($L$, $a$, $b$) lower than, or higher than a given threshold, or attributes between 2 threshold values. Once these pixels are identified and counted, then their percentage based on the total view area of an object can be calculated. In this way, the images of the strawberry slices were segmented in 2 different zones, according to their $a^*$ values. The selected intervals of $a^*$ values were: from −0.5 to 17 (representing the lighter sections); and from 17 to 28 (representing the reddish sections). The number of pixels of each zone of the strawberry slices was quantified and their corresponding percentage was calculated. The color functions selected to follow browning changes in fruits were the $L^*$, $a^*$, $b^*$, $h^*_{ab}$ and $C^*_{ab}$.

### 2.8. Statistical analysis

One way analysis of variance (ANOVA) was applied on the results of the chromatic coordinates, using the statistical program Statgraphics Centurion v15.0 (StatPoint®, Inc., USA).

## 3. Results and discussion

Strawberries slices presented heterogeneous color distribution. They showed a white to greenish center, surrounded by a bright red zone. Furthermore, on each slice different degrees of redness and some green or white sections were visually detected. These characteristics suggested that color should not be analyzed as it is usual for homogeneous materials. Instead, the different sections were studied separately. Thus, the images of strawberry slices were segmented in 2 different zones, according to their $a^*$ values (Fig. 1). The pixels proportion in each zone was calculated, as described in the Materials and Methods section.

Fig. 2 shows the CIELAB coordinates location of the two sample sections of the segmented images of fresh, freeze-dried and re-humidified strawberry slices in the 3D color space. The lighter sections of the segmented images ("whitish" zone) are located at higher luminosity ($L^*$) values and lower red ($a^*$) and yellow ($b^*$) values than the "reddish" zones of the images. The freeze-drying process promoted an increase of $L^*$ and a decrease of the yellow coordinate, which caused a decrease of hue angle ($h^*_{ab}$) values in both analyzed zones, with minimum change of the red coordinate and chroma ($C^*_{ab}$) values. The effect of the freeze-drying and humidification processes on the individual $L^*$, $b^*$ and $h^*_{ab}$ values of the different sections of the segmented images are shown in Fig. 3.

As shown in Figs. 2 and 3, the fresh sample presented low $L^*$ values and an intense red coloration, while the freeze-dried samples showed a much lighter appearance. This effect has been previously observed for strawberries (Agudelo-Laverde, Schebor, & Buera, 2012) and other food matrices, such as tomato and kiwi which became of dull appearance after drying (Lana, Hogenkamp, & Koehorst, 2006; Talens, Martínez-Navarrete, Fito, & Chiralt, 2001). In the present work, besides the higher $L^*$ values of the freeze-dried samples, a sharp decrease of the $b^*$ and $h^*_{ab}$ values was observed, as a consequence of drying, while $a^*$ and $C^*_{ab}$ changed to a much lesser extent. It is interesting to note that the fresh strawberries contained a mass fraction of water higher than 85%, and this amount decreased to less than 2% due to the drying process, causing a concentration of all the food components in the samples. Since anthocyanins are highly concentrated in the dried strawberries, it would be expected that the dried material would have a darker appearance than the fresh strawberries. During freeze-drying the water present in the inter and intra-cellular spaces of the fresh material is replaced by air, with a minimum shrinkage. Anthocyanins are then concentrated below the sample surface which became opaque due to the presence of the air cavities created the dried material. The reflectance measurements and visual appearance do not reflect the real pigment concentration. This phenomenon has been widely studied in the paper industry and is attributed to the diffusion of light inside a material at interphases of different refraction indexes (Saarela, Heikkinen, Fabritius, Haapala, & Myllylä, 2008).

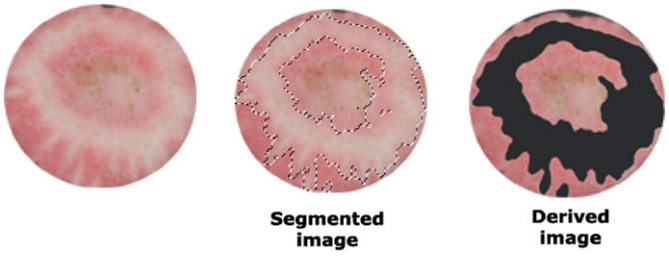


**Fig. 1.** Examples of color image segmentation in freeze-dried strawberry slices. The threshold values were imposed to the $a^*$ values: −0.5 to 17 for the lighter zones and from 18 to 28 for the reddish zones. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

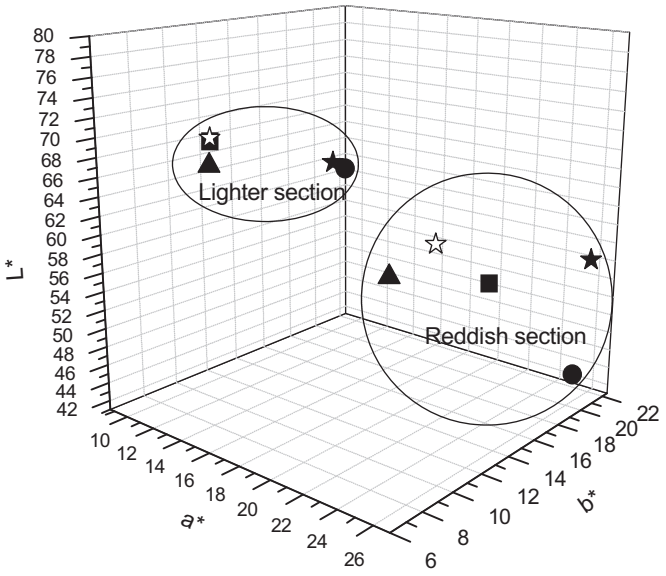


**Fig. 2.** Spatial location of light-whitish and reddish sections of the strawberry slices in the CIELAB color space: fresh (★), freeze dried (☆), and after humidification at 11% (▲), 43% (■) and 75% (●) RH.

The most important changes of the color functions due to freeze-drying and re-humidification ($L^*$, $b^*$ and $h^*_{ab}$ functions), are represented in Fig. 3. The $L^*$ values of samples at 11 and 43% RH were not significantly different to those of the freeze-dried samples, in any of the sections. The $L^*$ values of the re-humidified samples at 75% RH were lower than those of the fresh samples (Fig. 3), in the "reddish" sections. In the samples re-humidified at 75% RH the low $L^*$ values reflected some color intensification during the re-humidification stage at this RH at 20 °C. The $b^*$ and $h^*_{ab}$ values were significantly different in both sections. The decrease of $L^*$ and the increase of $b^*$ and $h^*_{ab}$ as increasing RH reflects the development of browning reactions (Agudelo-Laverde et al., 2011).

The main changes which occurred during storage at 45 °C are shown in Figs. 4–6. The color coordinates of the samples humidified at 11 and 43% RH did not change during storage at 45 °C, reflecting the high stability of these systems. However, for the samples re-humidified at 75% RH relevant changes were observed

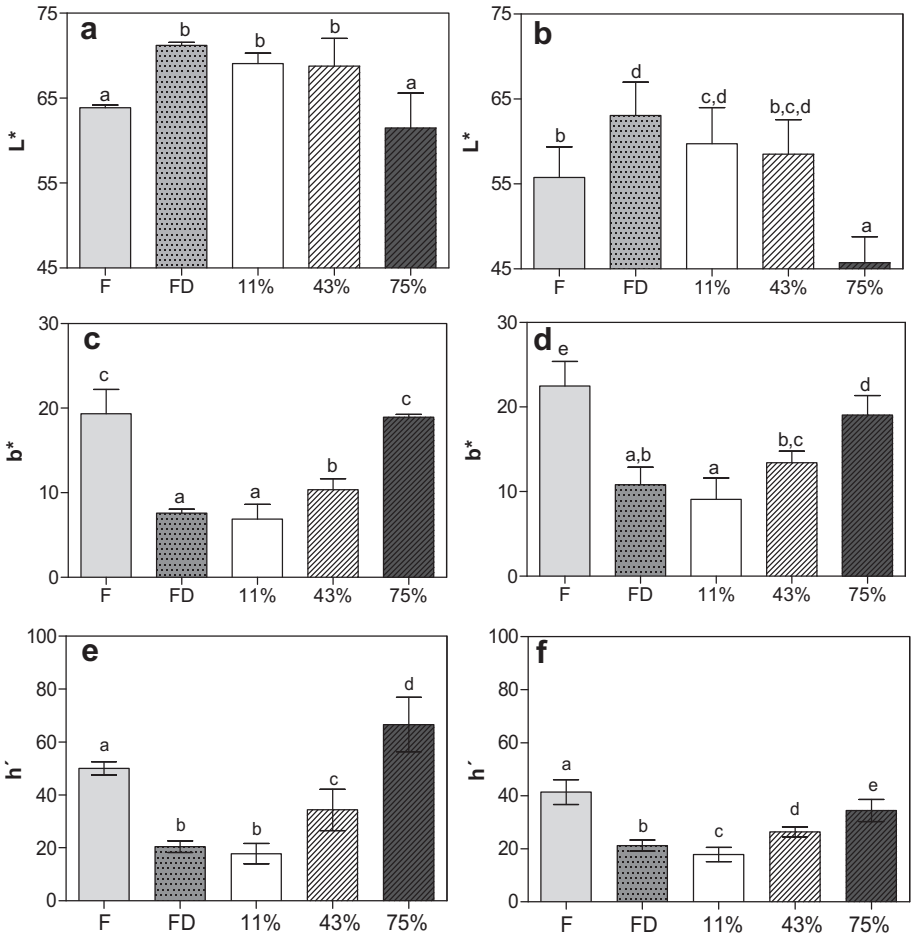


**Fig. 3.** $L^*$, $b^*$ and $h'$ values for the light-whitish sections (a,c,e) and reddish sections (b,d,f) of fresh (F), freeze-dried (FD) and re-humidified at 11% 43% and 75% RH strawberry slices. The averages with different letters show significant differences.

in the two sections of each sample. At that RH, $L^*$ and $h^*_{ab}$ values decreased in the lighter sections during storage, which is related to the visually perceptible browning experimented by the samples (Fig. 5). It has to be noted that the luminosity coordinate, $L^*$, decreased at all the browning stages, while the yellow coordinate $b^*$ and hue angle, increased at early stages, when the samples acquire a yellow coloration, as happened with the samples at 43% RH. However, $b^*$ and $h^*_{ab}$ decreased at advanced browning stages, when the samples acquire a neat brown coloration, as occurred with the samples at 75% RH. The darkening promoted by browning reactions could be evaluated through the decrease of $L^*$ value and the increase of $b^*$ and $h^*_{ab}$ in the lighter sections of the strawberry slices.

The decrease of $L^*$, $b^*$ and $h^*_{ab}$ during storage at 45 °C (Fig. 5), was enhanced at 75% RH, which corresponds to the higher browning rate in several fruit products (Agudelo-Laverde et al., 2011). In the case of the red zone of the segmented images of samples stored at 45 °C the main changes occurred in the $a^*$ chromatic coordinate, particularly at 75% RH (Fig. 6). $C^*_{ab}$ presented a similar trend, although with less relative changes.

The segmented image analysis was appropriate to evaluate some relevant characteristics of the visual appearance changes occurring in dehydrated strawberries, related to the humidification level and storage time. In contrast to the averaging method for measuring color changes, the full potential of image analysis can be exploited to evaluate several reactions occurring in food materials.

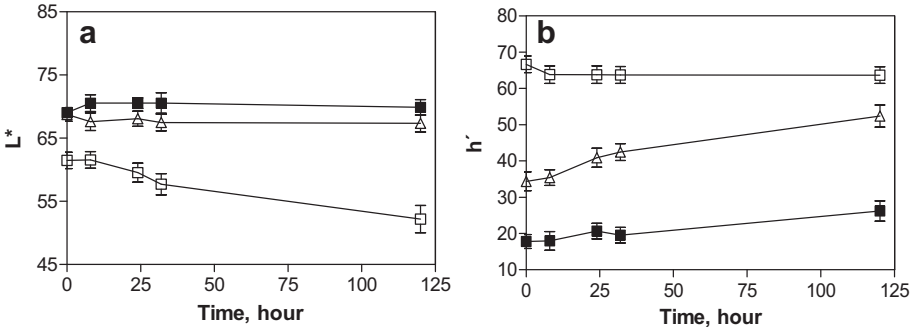


**Fig. 4.** Evolution of the variables $L^*$ and $h'$ for the lighter sections of strawberry slices during storage at 45 °C in samples re-humidified at 11% (■), 43% (△) and 75% (□) RH.

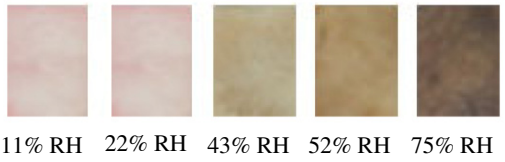


**Fig. 5.** Image gallery showing browning degree in the lighter section of the segmented images of strawberry slices re-humidified at different relative humidities at the final point of storage at 45 °C (120 h). The control samples were those at the end on freeze drying.

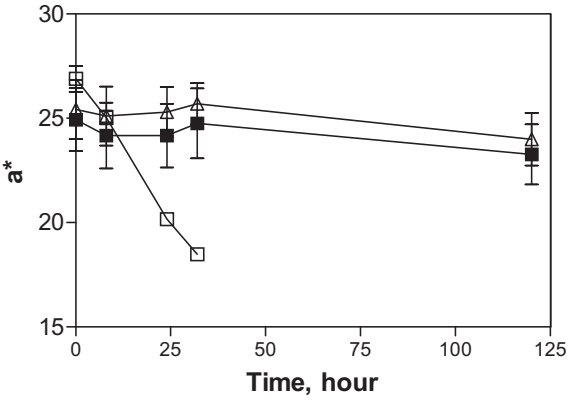


**Fig. 6.** Evolution of the $a^*$ chromatic coordinate for the reddish sections of the strawberry slices segmented images during storage at 45 °C in samples re-humidified at 11% (■), 43% (△) and 75% (□) RH.

In order to analyze if the observed decrease of $a^*$ values shown in Fig. 6 were related to pigment degradation, the total content of monomeric anthocyanins was determined at all stages: in fresh, freeze dried, and after humidification (Fig. 7a), and during storage at 45 °C (Fig. 7b), in a range of RH between 11 and 75%. It has been reported that several factors affect anthocyanins stability, some of them are storage time, sugar content, pH, ascorbic acid and water activity (Tsai et al., 2004). After freeze-drying, about 25% anthocyanin content was lost. The anthocyanin degradation during humidification increased as increasing RH, and was particularly important above 43% RH. At 75% RH almost 60% of the total anthocyanin content was lost (Fig. 7a). During storage at 45 °C further decrease of anthocyanin content occurred at 75% RH, at which total loss of anthocyanin content was verified (Fig. 7b).

Two reactions take place simultaneously in the stored strawberry samples: the destruction of anthocyanins and the formation of brown pigments. In order to evaluate the contribution of anthocyanin degradation to the color changes during storage at 45 °C, the $a^*$ value (red chromatic coordinate) of the reddish sections of the segmented images was related to the retention of anthocyanins (Fig. 8). At low RH values (11–33%), the anthocyanin content remained constant during storage at 45 °C, and the $a^*$ values slightly changed. At higher RH values (43, 52 and 75%) the decrease of anthocyanin content correlated with the decrease of the $a^*$ value of the reddish sections of the samples segmented images. These results indicate that the effect of water content on the chromatic appearance of the dried strawberries excludes the use of reflectance colorimetry as a method to analyze anthocyanins retention during storage. However, if the RH of the samples is adequately adjusted to a given value higher than 43%, $a^*$ values of the reddish sections could be used as an index of the anthocyanin degradation. The $a^*$ values were less affected by the light diffusion than $L^*$, which allowed to correlate $a^*$ values with the anthocyanin content.

The behavior of $L^*$, $b^*$ and $h^*_{ab}$ in the lighter section and of $a^*$ in the reddish section of the segmented images observed in Figs. 3–8, could be related to the water sorption stage in the samples. According to the sorption isotherm data by Moraga and coworkers (Moraga, Martinez-Navarrete, & Chiralt, 2004) at 75% RH, at which the decrease of $L^*$ and the increase of $b^*$ and $h^*_{ab}$ take place at 20 °C, correspond to the ascending part of the water sorption isotherm, above the so called "multilayer" zone.

The $^1$H NMR signal decay of the transversal relaxation times, $T_2$, were obtained by the CPMG pulse sequence in order to relate the observed behavior of the change of the chromatic attributes with the molecular mobility in the samples.

The decay envelopes of the $^1$H NMR signals were fitted to mono- or bi-exponential behavior with the following equation:

$$I = A_1\exp(-t/T_{2\text{-}1\text{-}CPMG}) + A_2\exp(-t/T_{2\text{-}2\text{-}CPMG}) \quad (4)$$

where $I$ represents the NMR signal intensity at time $t$. The relaxation time $T_{2\text{-}1\text{-}CPMG}$ corresponds to the protons in the less mobile water fraction. $A_1$ is proportional to the number of protons in the $T_{2\text{-}1}$ state. The relaxation time $T_{2\text{-}2\text{-}CPMG}$ corresponds to the more mobile water fraction. $A_2$ is proportional to the number of protons in the $T_{2\text{-}2}$ state. For the mono-exponential fit $A_2 = 0$.

At the RH value of 52% only one CPMG signal was detected with the selected sequence employed, while two protons populations were detected at RH values at and above 75%.

The relaxation times of each relative humidity at 20 and 45 °C are summarized in Table 1. These values correspond to quite mobile water molecules (Acevedo, Schebor, et al., 2008; Agudelo-Laverde et al., 2011). Thus, several aspects analyzed in present paper are related to the appearance of mobile water populations in the material. The effect of light diffusion in the dried tissues decreased at 75% RH, as discussed in relation to Fig. 2. On the other side, the

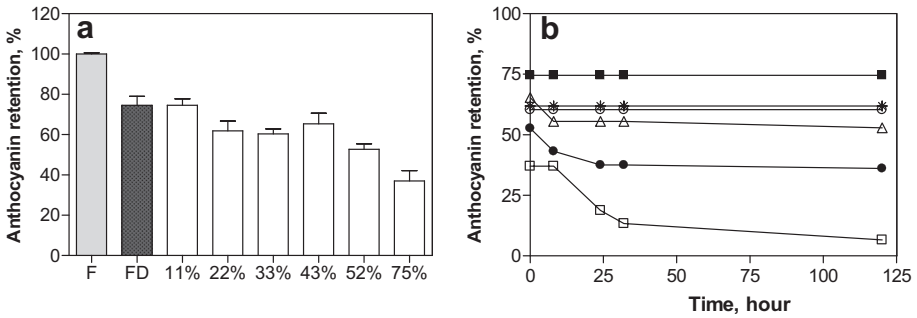


**Fig. 7.** Anthocyanins retention percentage for strawberry slices. Fresh (F), freeze-dried (FD) and after humidification at different RHs (a). Anthocyanins retention percentage during storage at 45 °C at 11% (■), 22% (✳), 33% (○), 43% (△), 52% (●) and 75% (□) RH as a function time (b).

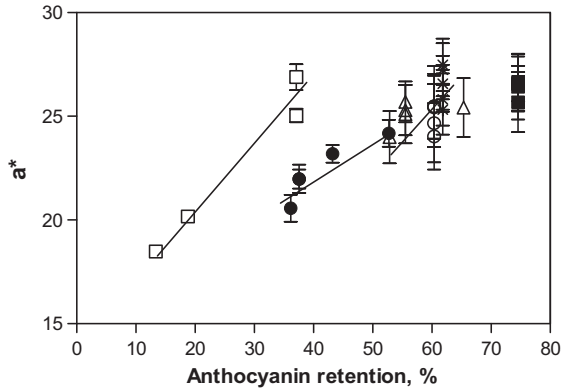


**Fig. 8.** Relationship between $a^*$ chromatic coordinate in the reddish section of the freeze-dried strawberries, during storage at 45 °C, for samples re-humidified at 11% (■), 22% (∗), 33% (○), 43% (△), 52% (●), and 75% (□) RH.

**Table 1**
$^1$H NMR relaxation times ($T_2$) of the protons populations obtained by CPMG pulse sequence, for freeze-dried strawberry humidified at 52%, 75% and 84% at 20 °C and at 45 °C.

| Relative humidity (%) | Temperature (°C) | $T_{2\text{-}1}$(ms) | $T_{2\text{-}2}$(ms) |
|---|---|---|---|
| 52 | 20 | 0.25 ± 0.004 | – |
| 52 | 45 | 1.08 ± 0.15 | – |
| 75 | 20 | 1.15 ± 0.05 | 3.78 ± 0.25 |
| 75 | 45 | 2.41 ± 0.69 | 8.05 ± 0.64 |
| 84 | 20 | 1.77 ± 0.17 | 5.25 ± 0.33 |
| 84 | 45 | 2.58 ± 0.3 | 9.72 ± 1.01 |

browning reaction as so as pigment degradation were accelerated at that RH, in correspondence to a significant increase of water mobility in the matrix, as determined by the sorption properties and by the $^1$H NMR relaxation times.

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