UNIVERSITAT POLITÈCNICA DE VALÈNCIA

INSTITUTO DE INGENIERÍA DE ALIMENTOS PARA EL DESARROLLO

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EFFECT OF DRYING CONDITIONS ON THE CAROTENOIDS CONTENT, ANTIOXIDANT ACTIVITY, STRUCTURE AND PHOTOLUMINESCENCE OF PERSIMMON (DIOSPYROS KAKI) IN THREE RIPENING STAGES

TRABAJO FIN DE MÁSTER UNIVERSITARIO EN CIENCIA Y TECNOLOGÍA DE LOS ALIMENTOS

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EFFECT OF DRYING CONDITIONS ON THE CAROTENOIDS CONTENT, ANTIOXIDANT ACTIVITY, STRUCTURE AND PHOTOLUMINESCENCE OF PERSIMMON (*DIOSPYROS KAKI*) IN THREE RIPENING STAGES

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RESUMEN

El incremento en el cultivo de persimmon en la Comunidad Valenciana junto con las estrictas exigencias de calidad, marcadas por la denominación "kaki Ribera del Xúquer", han provocado un aumento de excedentes de persimmon. Estas frutas desechadas, aunque presentan excelentes propiedades, no tienen valor comercial. El persimmon es un fruto estacional, rico en carotenoides y compuestos fenólicos. Sería interesante estudiar métodos para aprovechar estos excedentes de frutas y ofrecer nuevos productos al consumidor que puedan estar disponibles durante todo el año. El objetivo de este trabajo es estudiar el efecto del secado por aire caliente, en dos condiciones diferentes, 40ºC 24 h y 60ºC 9h, sobre el contenido, la actividad antioxidante, la estructura y las propiedades ópticas de los carotenoides del persimmon en tres estados de maduración. Los resultados muestran que el contenido en carotenoides aumenta con el avance de la maduración. El secado no afecta al contenido en carotenoides, pero sí disminuye la actividad antioxidante de los mismos. A medida que progresa la maduración y, después del secado, los tejidos pierden integridad, facilitando la difusión de los carotenoides por todo el tejido de persimmon. Las medidas de fotoluminiscencia muestran dos especies emisoras. La emisión a 380 nm se atribuye a los carotenoides en su forma cis y a 500 nm a los carotenoides en su forma trans. Después del secado, se observa un desplazamiento del espectro hacia el infrarrojo en la emisión a 500 nm, relacionado con la formación de productos de degradación térmica, y la formación de xantofilas con el avance de la maduración.

PALABRAS CLAVE: secado por aire caliente, "Rojo Brillante", valorización, FRAP, DPPH, microscopía, fluorescencia, β – caroteno, β – criptoxantina.

RESUM

L'increment en el cultiu de persimmon en la Comunitat Valenciana juntament amb les estrictes exigències de qualitat, marcades per la

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denominació “kaki Ribera del Xúquer”, han provocat un augment d’excedents de persimmon. El persimmon és un fruit estacional, ric en carotenoides i compostos fenòlics. Seria interessant estudiar mètodes per a aprofitar aquests excedents de fruita i oferir nous productes al consumidor que puguen estar disponibles durant tot l'any. L'objectiu d'aquest treball és estudiar l'efecte de l'assecat per aire calent, en dues condicions diferents, 40 °C 24 h i 60 °C 9h, sobre el contingut, l'activitat antioxidant, l'estructura i les propietats òptiques dels carotenoides del persimmon en tres estats de maduració. Els resultats mostren que el contingut de carotenoides augmenta amb l'avanç de la maduració. L’assecat no afecta el contingut en carotenoides, però sí disminueix l'activitat antioxidant d'aquests. A mesura que progressa la maduració i, després de l'assecat, els teixits perden integritat, facilitant la difussió dels carotenoides per tot el teixit del persimmon. Les mesures de fotoluminescència mostren dos especies emissores. L’emisió a 380 nm és atribuïble als carotenoides en la seua forma cis i a 500 nm als carotenoides en la seua forma trans. Després de l'assecat, s'observa un desplaçament de l'espectre cap a l'infraroig en l'emissió a 500 nm, que es relaciona amb la formació de productes de degradació tèrmica i la formació de xantófiles amb l’avanç de la maduració.

PARAULES CLAU: assecat per aire calent, “Rojo Brillante”, valorització, FRAP, DPPH, microscopía, fluorescència, β – carotè, β – criptoxantina.

ABSTRACT

The increase in the crop of persimmon in the Valencian Community together with the strict quality requirements set by the appellation “kaki Ribera del Xúquer” have caused an increase in persimmon’s surplus. Persimmon is a seasonal fruit, rich in carotenoids and phenolic compounds. It would be interesting to study methods to exploit these surpluses and offer new products to the consumer, making them available throughout the year. The objective of this work is to study the effect of hot air drying, in two different conditions, 40ºC 24 h and 60ºC 9h, on the content, antioxidant activity, structure and optical properties of persimmon carotenoids in three ripening stages. The results show that the content of carotenoids increases with the advance of maturation. Drying treatments do not affect the carotenoid content but they decrease its antioxidant activity. As maturation progresses and, after drying, the tissues lose integrity, easing the diffusion of carotenoids throughout the persimmon tissue. Photoluminescence measurements show two emitting species. Emission at 380 nm is attributable to cis forms of carotenoids and the emission at 500 nm corresponds to their trans forms. After drying, a shift of the spectrum to the infrared is observed in the emission at 500 nm,related to the formation of thermal degradation products and xanthophylls as the ripening progresses.

KEYWORDS: hot - air drying, “Rojo Brillante”, valorisation, FRAP, DPPH, light microscopy, fluorescence, β – carotene, β – cryptoxanthin

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1. INTRODUCTION

Persimmon (*Diospyros kaki L.)* is an original crop from Asia which has suffered a big growth along the Mediterranean area in the last decades because of its sensorial and nutritive properties. This fruit is a good source of bioactive compounds like polyphenols, ascorbic acid and carotenoids that have a favourable effect on human health (Giordani *et al.*, 2011). Thereby, it has been used in the treatment of mild pathologies as well as it's been proved its potential against cardiovascular diseases and some types of cancer (Slavin and Lloyd Beate, 2012).

"Rojo Brillante" persimmon (appellation of origin *Kaki Ribera del Xúquer,* Valencia, Spain) is a much-appreciated variety due to its size and texture and its crop has grown in Valencia region thanks to its high productivity (E. Giordani, 2002). However, due to quality control compulsory by the appellation of origin there is a loss of fruit fresh, which reaches up to 30%, generating an economic and environmental problem. Therefore, different techniques of valorisation have been studied to avoid and reduce persimmon excess and to offer new products to the consumer all year long (Cárcel *et al.*, 2007).

Drying techniques have been used since many years as a valorisation procedure for obtaining high quality and shelf-stable fruits (Hasan *et al.*, 2019). Hot-air drying (HAD) is a low-cost drying method and is one of the oldest and the most important food preservation methods practiced by humans. This process improves the food stability, as it, reduces the water and microbiological activity of the material while minimizing physical and chemical changes during its storage (Doymaz, 2012).

The effect of HAD on carotenoids is a complex matter. Several pieces of research have studied how HAD affects carotenoids in different plant - based foods. On one hand, Piyarach *et al.* (2020) found no differences in total carotenoids content when drying at 65ºC different vegetables, on the other hand, Zhang *et al.* (2018) evidenced the complex relation between HAD and carotenoids due to differences in the food matrix.

Carotenoids are a group of fat-soluble compounds synthetized by plants and some microorganisms (Britton, 1995). The basic structure is a tetraterpenoid chain and may exist rings on the extremes. In persimmon, most common carotenoids are β-cryptoxanthin, zeaxanthin and β-carotene. However, the amount of each one is variable depending on the ripening stage. β-carotene appears in the first ripening stages remaining constant throughout all the shell life of the fruit while β-cryptoxanthin appears at the later ripening stages (Bordiga *et al.*, 2019).

The most influential characteristic into the physical, chemical and biochemical properties of these molecules is the system of trans conjugated double bounds (although a small proportion of cis – isomers are encountered, having lower provitamin A activity than their trans counterparts), being the responsible of quite vital importance physiological processes such as

photosynthesis, structural stabilization of protein-pigment photosynthetic complexes and autooxidation to avoid cell damages (Nisar *et al.*, 2015).

The system of conjugated double bonds allows carotenoids to absorb UV-Vis light within the range of 400 – 500 nm. This feature allows carotenoids in solution to obey Lambert-Beer Law enabling its quantification from UV-Vis spectroscopic measures as many authors have reported before (Hernández-Carrión *et al.*, 2014; García-Cayuela *et al.*, 2018). Nevertheless, despite its simplicity, this system is not completely accurate to solve the complex nature of the excited states of carotenoids due to the low intensity of symmetry – forbidden π –> π* transitions (Tan and Soderstrom, 1989). For this reason, the high sensitive of the luminescence spectroscopy may become an alternative in the analysis of carotenoids.

Luminescence is the light emission from atoms and/or molecules due to the energetic fall from excited states to the ground state. Luminescence is formally divided into two categories - fluorescence and phosphorescence - depending on the nature of the excited state (Lakowicz, 2006). In the case of fluorescence, this deactivation occurs among same multiplicity states (singlet-singlet, triplet-triplet), what differs from the phosphorescence. Nevertheless, the light emission by electronic deactivation compete against other types of deactivation processes like the heat emission when vibrational relaxation occurs, overlapping between same multiplicity states and intercrossing if the process involves spin changes (Gehlen, 2020).

The aim of this work was to study the effect of two different hot-air drying conditions (40ºC, 24 h and 60ºC, 9 h) on content, antioxidant capacity, microstructure and optical emission spectroscopy by UV - Vis light absorption of persimmon's carotenoids in three different ripening stages.

2. MATERIALS AND METHODS

2.1. Fruit samples

Persimmon fruit cv. "Rojo Brillante" in three ripening stages (M1, M2 and M3) treated by a de-astringency treatment (CO_2 atmosphere 95% during 24 h at 20°C), were supplied by "Instituto Valenciano de Investigaciones Agrarias" (IVIA, Valencia, Spain). The choice of these three ripening stages was within the six different stages of characteristic commercial maturity (Besada, Arnal and Salvador, 2008). These three commercial ripening stages were from fruits harvested in mid-November and early December, which correspond to the ripening IV (M1), V (M2) and VI (M3) according to Salvador *et al*. (2007). The fruits were washed and cut into slices of 5 mm in thickness with a mandolin (OXO good grips mandolin slicer 2.0, USA). Hot-air drying was carried out in a cabinet dryer (Binder model FED 260 standard, Germany) at 40°C and 60°C for 24 and 9 h respectively, up to reach 15 ± 3 g water/ 100 g product, using an air velocity of 2 m/s. Thus, nine different samples were obtained and analysed in this work (Figure 1).

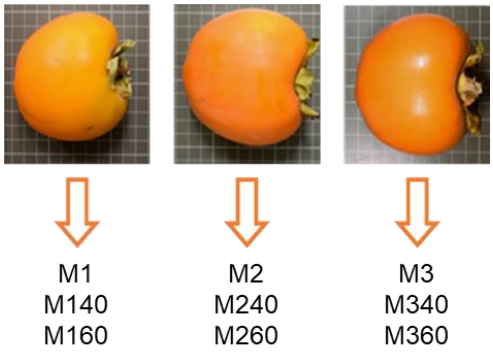

Figure 1 - Schematic representation of persimmon samples analysed. M1, M2 and M3 are referred to the three ripening stages. Numbers 40 and 60 are related with the temperature of the drying treatment.

2.2. Chemicals

Acetone (99.5%), Diethyl Ether (99.7% stabilized with ~ 6 ppm BHT), Iron (III) Chloride 6-hydrate pure, Sodium Sulphate Anhydrous (E-514i, F.C.C.) Food Grade, Sodium Acetate Anhydrous (Reag. Ph. Eur.), Sodium Chloride and Hydrochloric Acid 37% (Reag. USP) were obtained from PanReac AppliChem (Castellar del Valles, Barcelona, Spain). 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-Tris (2-pyridyn)-s-triazine (TPTZ) and β-carotene (provitamin A, ≥93% (UV), powder) were purchased from Sigma Aldrich (St. Louis, MO, USA). Trolox was obtained from Santa Cruz Biotechnology (Dallas, TX, USA).

2.3. Total carotenoids content

Total carotenoids content of fresh and dried samples were extracted according to Hernández-Carrión et al. (2014). Persimmon puree (5 g or the equivalent dried mass) was extracted six times with 25 mL cool acetone using an Ultraturrax (IKA Ultraturrax T25 Basic) and vacuum filtered, until no more colour was extracted. The extract was added gradually to 50 mL ethyl ether in a decanting funnel. With each addition of extract, enough NaCl solution (100 $g \cdot L^{-1}$) was added to separate the phases and to transfer the pigments to the ether, while the aqueous phase was removed. The process was carried out in several steps to ensure the greatest elimination of aqueous phase. The organic phase was treated several times with anhydrous Na_2SO_4 (20 $g \cdot L^{-1}$) to remove residual water. Then, it was evaporated until dry in a rotary evaporator (model RII; Buchi Labortechnik, Flawil, Switzerland) at a temperature ≥ 35 °C. Finally, the pigments were collected with acetone to a volume of 30 mL and the absorbance was measured at 450 nm using a spectrophotometer (CE 1021 1000 series, CECIL INSTRUMENTS Cambridge). The calibration curve was performed with different concentrations of β−carotene in acetone. Results were expressed as mg β−carotene/100 g of dry matter. Carotenoid extractions were made in triplicate.

2.4. Antioxidant activity

2.4.1. Determination of antioxidant activity by FRAP

The FRAP (ferric reducing antioxidant power) was performed according to method of Benzie and Strain, (1996) with some modifications. Three hundred mM sodium acetate buffer (pH 3.6), 20 mM ferric chloride solution and 10 mM TPTZ (2,4,6-Tris (2-pyridyl)-s-triazine) in 40 mM HCl solution were prepared. FRAP reagent was made by mixing 2.5 mL of sodium acetate buffer, 2.5 mL of TPTZ solution and 2.5 mL of ferric chloride solution. Then, it was warmed to 37 °C and kept in darkness for 30 min as minimum.

75 µL of distilled water, 75 µL of sample extract and 2.7 mL of FRAP reagent were mixed and warmed to 37 °C for 30 min away from any source of light. After, the absorbance was measured at 595 nm using spectrophotometer (CE 1021 1000 series, CECIL INSTRUMENTS Cambridge).

Standard curve was elaborated using Trolox standard and results were expressed as µmol Trolox eq·g^{-1}.

2.4.2. Determination of antioxidant activity by DPPH.

DPPH (2,2-diphenyl-1-picrylhydrazyl) antioxidant activity of carotenoids was measured according to the method described by Matsumura et al., (2016) with some modifications. Stable DPPH radical was reduced due to the carotenoids resulting in a reduction of the absorbance. 40 µg·mL-1 DPPH solution in acetone was prepared. 1 mL of sample extract was mixed with 4 mL of DPPH solution and stirred 30 s in a Vortex. After 30 min of incubation in dark at 37 °C, absorbance was measured at 517 nm using acetone as blank.

Results were expressed as a DPPH inhibition percent with equation 1:

$$\%\ \text{of DPPH inhibition} = \frac{\text{Abs control} - \text{Abs sample (extract)} - \text{Abs blank}}{\text{Abs control}} \cdot 100 \quad [1]$$

2.5. Microstructure

Microscopic analysis was performed with the aid of a Nikon Eclipse 80i® light microscope (Nikon Co. Ltd., Tokyo, Japan) which has incorporated a camera (ExwaveHAD, nº DXC-19, Sony Electronics Inc., Park Ridge, New Jersey, USA). 20 µm cryostat sections were obtained from persimmon slices and were transferred to a glass slide. Sections were displayed by bright-field (BF), with and without 1% toluidine blue as staining agent, and by fluorescence, using a mercury arc lamp with a FITC filter as excitation source

(482/35 nm and 536/40 nm, excitation and emission wavelengths, respectively). The images were captured and stored at 1280 x 1024 pixels using the microscope software (NIS-Element M, version 4.0., Nikon, Tokyo, Japan).

2.6. Carotenoid determination by fluorescence

Fluorescence measurements for persimmon extracts were recorded on Jasco FP-8500 Spectrofluorometer (Jasco Inc., Easton, MD). The emission spectra were measured in the range of 360 to 650 nm and using an excitation wavelength of 340 nm. The excitation spectra were recorded at 420 nm emission wavelength in the range of 300 to 380 nm. Data interval was 0.5 nm, scan speed was 500 $nm \cdot min^{-1}$ and the emission and excitation band with were 2.5 nm.

Absorbance of the extracts was adjusted and the measurements were performed at weather conditions.

All the data were analysed using OriginPro 9.0.0. software package (OriginLab Co., Northampton, MA)

2.7. Statistical analysis

A categorical multifactorial experimental design with two factors – ripening stage and drying treatment – was used to characterize all the samples. The honest significant difference (Tukey's HSD test) with a 95% confidence was used to compare the mean values obtained ($p < 0.05$).

All the data were analysed using XLSTAT statistical software (2019.4.2 (Addinsoft, Barcelona, Spain)).

3. RESULTS AND DISCUSSION

3.1. Total carotenoids content

The effect of both, ripening stage and drying treatment, on total carotenoids content (TCC) is presented in Figure 2A and 2B. There were no significant interactions ($p < 0.05$) between the ripening stage and the drying treatment factors. The only factor with significant effect ($p < 0.05$) on the TCC was the ripening stage.

Regarding to Figure 2A, it was observed an increase in the TCC as the ripening stage progressed, presenting M3 persimmon the highest TCC values. However, M2 persimmon showed TCC values with no significant differences ($P > 0.05$) neither with M1 nor M3. Figure 2B shows the effect of the drying treatment on the TCC. There were no significant differences ($P > 0.05$) among the untreated samples and the dehydrated samples, so the total carotenoids content remained after both dehydration treatments applied in the samples.

3.2. Antioxidant activity

As regards to the antioxidant activity (AA), it was measured the capacity to reduce the ferric - tripyridyltriazine (Fe^{III}-TPTZ) complex to the ferrous form (Fe^{II}) and the ability to inhibit DPPH free radical of carotenoids present in the persimmon samples.

FRAP method did not show significant ($p > 0.05$) interactions between factors, but both the ripening stage and drying treatment factors had a significant ($p < 0.05$) effect on the antioxidant capacity by FRAP method.

Figure 2C shows the effect of the ripening stage factor in the FRAP method. As the ripening stage progressed, there was an increase in the AA. As occurred with TCC, only there were significant ($p < 0.05$) differences between M1 and M3. No differences were found between M2 and M3 neither between M2 and M1. As to the influence of the ripening state, FRAP method followed the same tendency as TCC, meaning that the higher content of bioactive could be related with a higher AA. As was also concluded by Yoo and Moon, (2016) in three citrus varieties. Figure 2D shows the effect of the drying treatment in the FRAP method. This factor showed higher significant ($p < 0.05$) effect than the ripening stage factor. It was observed a significant ($p < 0.05$) decrease in the antioxidant properties after the drying treatment at 40 and 60ºC. This effect has been reported previously by Martínez-Las Heras *et al.* (2017). This reduction in the AA could be related with the formation of cis-isomers when the samples were dehydrated (Marx *et al.*, 2003). This might change the conformation of carotenoids within the lipid bilayer membrane due to changes in polarization affecting its protective properties (Grudzinski *et al.*, 2017).

Regarding to the DPPH method, there was no significant interactions ($p > 0.05$) between the ripening stage and the drying treatment factors, but both factors had a significant effect ($p < 0.05$) on the AA by DPPH method.

Figure 2E shows the effect of the ripening stage in the DPPH. It was obtained similar tendency to the FRAP method, increasing the AA when the ripening stage progressed. The samples M2 and M3 presented the highest ($p < 0.05$) DPPH values without significant differences between them. Figure 2F shows the effect of the drying treatment factor in the DPPH. As it can be seen in the FRAP method, there was a significant ($p < 0.05$) decrease of the AA after both drying treatments, without significant differences between them.

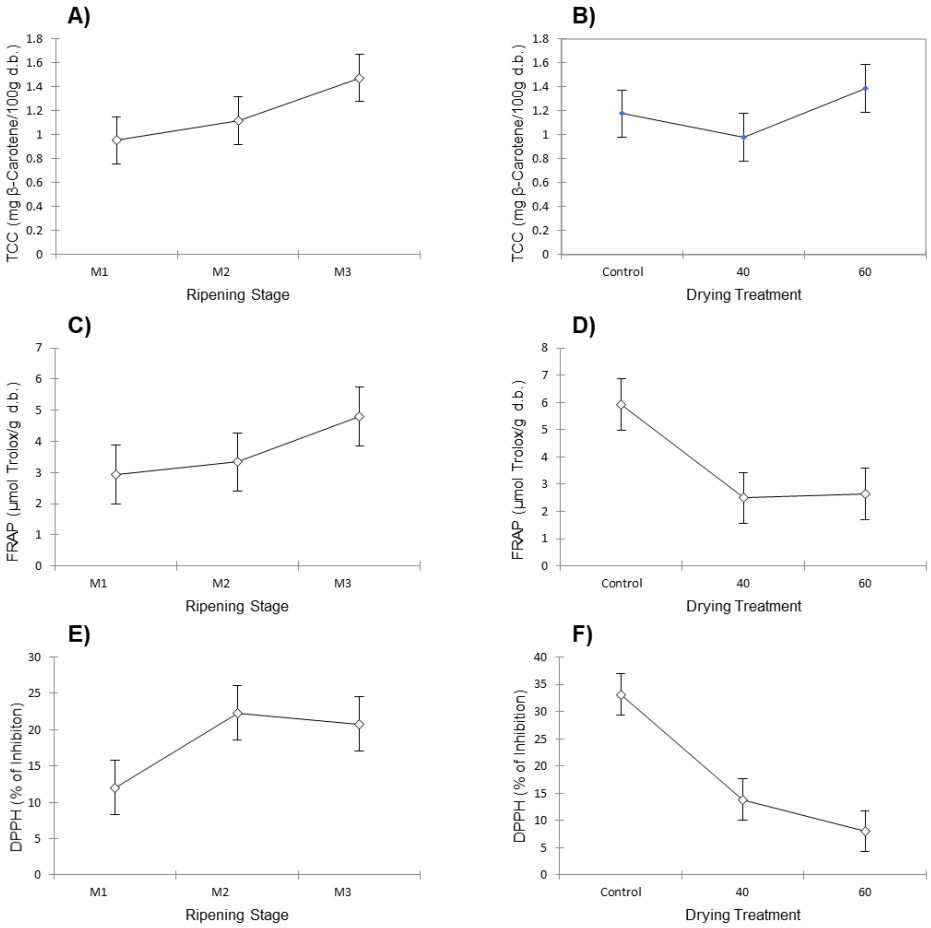

Figure 2 - Mean plots with Tukey HSD intervals. A, B: mean values for the TCC according to ripening stage and drying treatment, respectively. C, D: mean values for the FRAP according to ripening stage and drying treatment, respectively. E, F: mean values for the DPPH according to ripening stage and drying treatment, respectively.

3.3. Microstructure

Figure 3 shows images of persimmon in the three ripening stages. It was seen how the cell walls appeared turgid, compact and with high physical integrity in the three stages of ripening (Figure 3A, D and G). In sections of unstained persimmon (Figure 3B, E and H), the carotenoids could be seen, with their characteristic yellow-orange color, distributed homogeneously throughout the cellular tissue. Carotenoid substances were observed grouped in two different ways. On the one hand, they were inside the chromoplasts, close to the cellular wall, adopting a globular structure or spherical bodies. On the other hand, carotenoids appeared forming crystalline clusters both, inside the cell and in the cell wall. Comparing the three ripening stages, there was an increase on the carotenoid's accumulation in M3 samples (Figure 3H and I). These results were in accordance with the TCC, as M3 samples had more carotenoids than M1 samples. During ripening took place the transformation of chloroplasts into chromoplasts, which is the most frequent modification of plastids taking place in ripening fruits (Egea *et al.*, 2010; Quiles and Hernando, 2011; Schweiggert *et al.*, 2011). Autofluorescent emission of persimmon

samples was attributed to carotenoids since An *et al.*, (2000) concluded that by excitation with a 488 nm argon ion laser, the autofluorescence emitted at lengths greater than 515 nm was mainly due to carotenoids. Autofluorescence images (Figure 3C, F and I) showed that a large proportion of carotenoids were in the cell walls, in the three ripening stages.

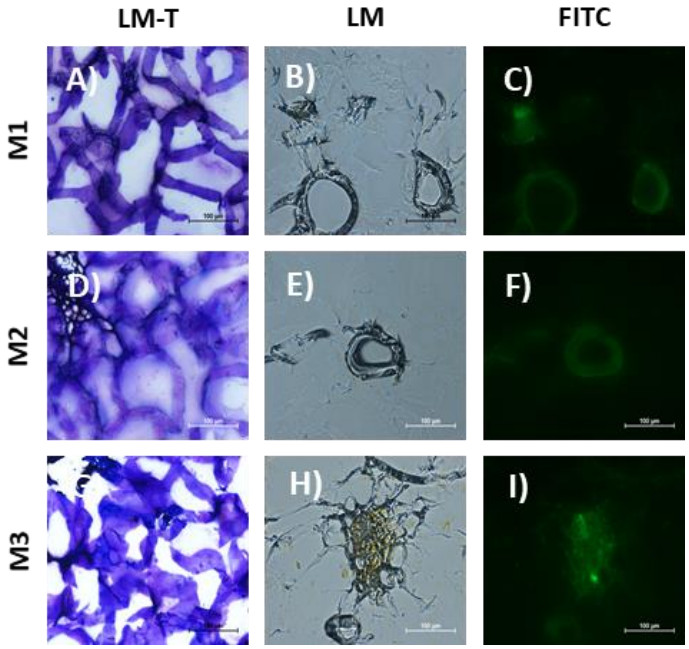

Figure 3 - Microstructure of persimmon samples at three ripening stages (M1, M2, M3). A, D, G: Blue toluidine images. B, E, H: unstained samples. C, F, I: fluorescence images. Magnification 20x.

Figure 4 shows images of dehydrated persimmon at the three ripening stages. Toluidine blue staining (Figure 4A, D, G, J, M and P) allowed to observe differences and changes in the cell wall structure of the samples submitted to different drying treatments. In the unstained (Figure 4B, E, H, K, N and Q) and fluorescence (Figure 4C, F, I, L, O and R) persimmon images it could be observed changes in the structure and location of the carotenoids.

When comparing persimmon samples, in the same ripening state, it was observed, in all stages of ripening, that both drying treatments produced degradation of walls and membrane cells and loss of cellular structural integrity (Figure 4A, D, G, J, M and P). This degradation of the tissue could facilitate the diffusion of carotenoids. In all dried persimmon samples, independently of the ripening stage, carotenoids lost a part of their crystalline appearance and they were observed spread throughout the tissue (Figure 4B, E, H, K, N and Q). Thus, the drying of the samples in the three stages of ripening enhanced the circulation and diffusion of carotenoids throughout the tissue, as can be seen in the autofluorescence images (Figure 4C, F, I, L, O and R). The carotenoids diffusion could be related to a high extractability of the carotenoids after the drying treatment. Hence, it could improve the bioaccesibility of carotenoids since they would be more dispersed. However, this release from the cells might put carotenoids in contact with environmental conditions affecting its antioxidant properties.

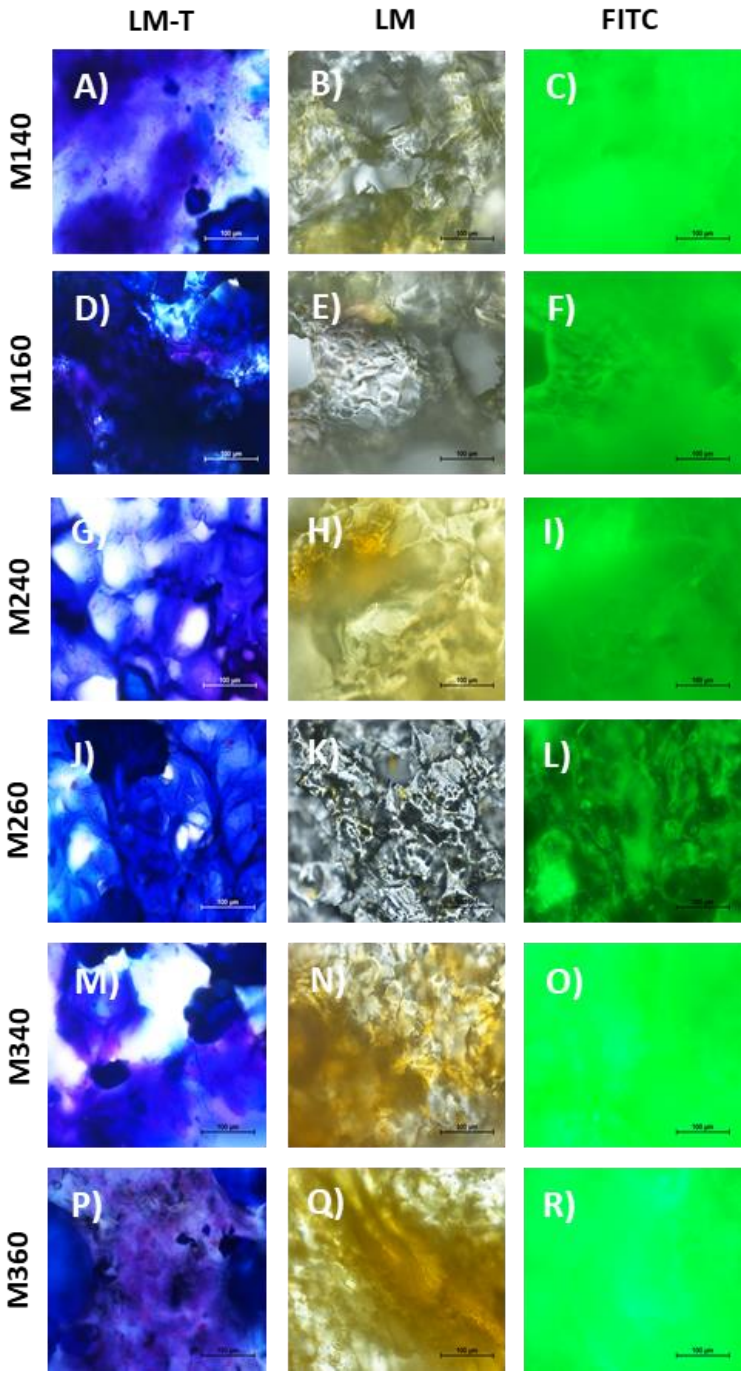

Figure 4 - Microstructure of persimmon samples submitted to different drying treatments at three ripening stages. A, D, G, J, M, P: Blue toluidine images.B, E, H, K, N, Q: unstained samples. C, F, I, L, O, R: fluorescence images. Magnification 20x

3.4. Fluorescence of carotenoids

Different excitation wavelengths were explored in order to determine the energy at which maximum emission occurs. In Figure 5A is shown the normalized emission and excitation spectra of M1 sample which allowed to select the 340 nm wavelength used in the fluorescence analysis. Besides,

emission spectra of pure β – carotene was carried out to compare with our extracted compound, observing similar features, although with more definition as it can be seen in Figure 5B. The emission spectra of β – carotene is characterised by a band from 400 to 500 nm with three characteristics peaks.

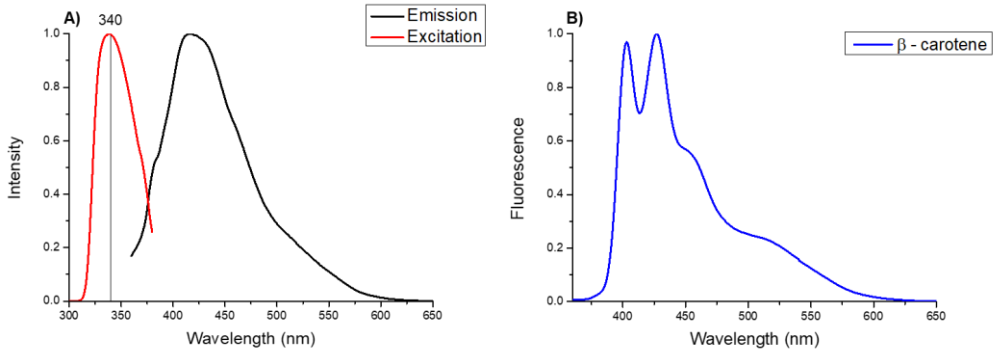

Figure 5 - A: Normalized emission and excitation spectra of M1 sample. B: Normalized emission spectra of β-carotene at 340 nm.

Figure 6 shows the normalized fluorescence spectra for the different samples exciting at 340 nm and recorded between 360 to 650 nm. In Figure 6A, it is observed the effect of the ripening stage while ripening progresses and Figure 6B, 6C and 6D presents the effect of the drying treatments in the earlier, intermediate and later ripening stages, respectively.

All the samples were found to be strong emitters when excited at 340 nm. A broad emitting band ranging from 375 nm to 500 nm was observed which corresponds to a hidden band in the UV/Vis spectra and is associated to a symmetry-forbidden π –> π* transition (Jørgensen, Stapelfeldt and Skibsted, 1992). This fact is evidenced in Figure 6 when observed all the normalized spectra of the different samples.

Green circles in Figure 6 were indicated in order to emphasize the appearance of a emitting peak at 380 nm. In Figure 6A it was possible to notice a small difference on the intensity among M1, M2 and M3. This peak might be associated with different isomers of β – carotene as reported previously by Zaghdoudi *et al.*, (2017). Although carotenoids are present in an all - trans conformation in the nature in predominant proportion, cis – isomers represent a minor fraction (Aman, Schieber and Carle, 2005) in fresh fruits. In Figure 6B, 6C and 6D, this peak was remarkable. Dehydrated samples showed a higher intensity at 381 nm if compared with the control samples. When heated, some all – trans - β – carotene were partially converted into its cis – isomers. The temperature increased the amount of cis – isomers as it was possible to notice when comparing the treated samples in Figure 6C and 6D.

Vertical green arrow in Figure 6A was drawn to point out the appearing of a red – shift in the emission spectra evidenced by the new band at 550 nm. This could be related with the formation of new compounds such as β – cryptoxanthin, which is in agreement with the study of Bordiga *et al.*,(2019). Furthermore, the emission at longer wavelengths caused by β – cryptoxanthin is strongly supported by Jørgensen, Stapelfeldt and Skibsted, (1992), due to the nonradiative deactivation produced by the presence of n – states in

xanthophylls that reduces the energy gap between higher singlet state and the emissive state.

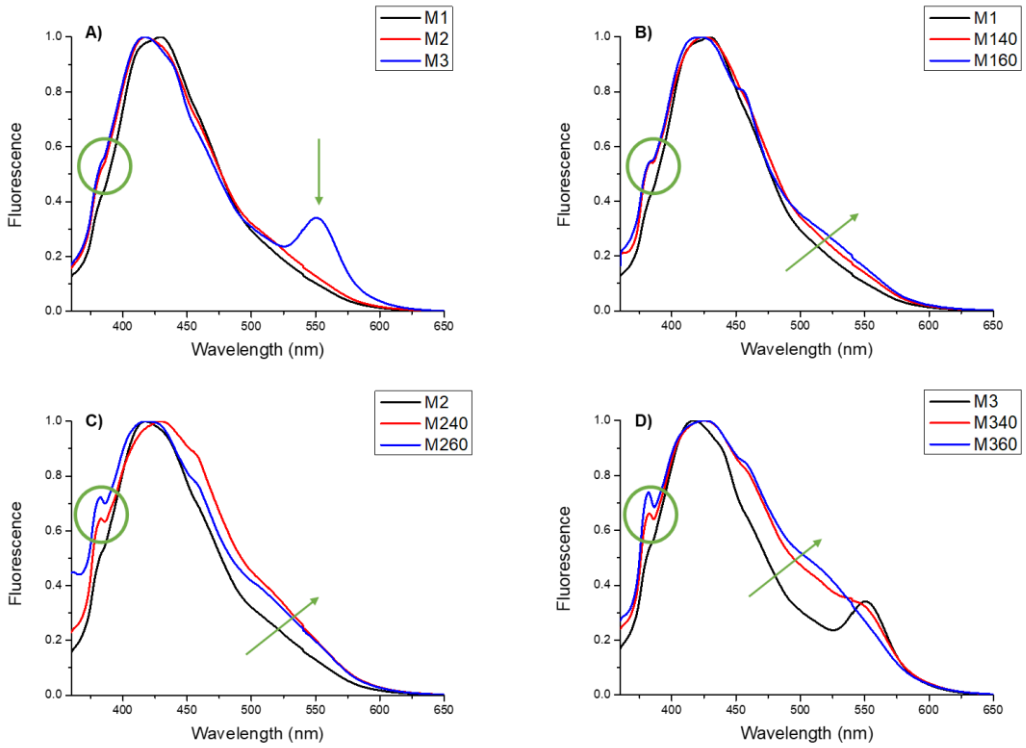

Figure 6 - Normalized emission spectra of the different samples at $\lambda_{excitation}$ = 340 nm. A: effect of the ripening stage in the fluorescence. B, C and D: effect of the drying treatment in M1, M2 and M3 fluorescence, respectively.

Concerning the horizontal arrow marked in Figure 6B, 6C and 6D, several authors have studied the thermal degradation of carotenoids and proved that isomerisation and oxidation were the main degradative reactions of carotenoids (Kim *et al.*, 2006; Colle *et al.*, 2016). Énicaud *et al*, (2011) supposed that isomerisation could be the first step of oxidation, so that could lead to the formation of apocarotenones and apocarotenals from the epoxides. Like that, the evolution of the fluorescence spectra of the dehydrated samples towards the near infrared might be yielded by the thermal degradation of carotenoids as described previously by Ehlers *et al*, (2007).

4. CONCLUSIONS

As ripening stage progresses, persimmon presents higher total carotenoids content and higher antioxidant activity values. Microstructural analysis allows to confirm that the higher accumulation of carotenoids, both in the cell walls and inside the cell, are present in the later ripening stage. Hot – air drying treatments (40ºC 24h and 60ºC 9h) don't affect the total carotenoids content but decrease the carotenoids antioxidant activity values. Structurally, in the three ripening stages, both treatments produce a loss of the persimmon cellular integrity and favour the movement and diffusion of the carotenoids through the vegetal tissue. This could improve the bioaccesibility of

carotenoids but might put carotenoids in contact with conditions which may affect its antioxidant properties.

Samples photoluminescence study allows us to prove that there are two emission bands corresponding to carotenoids, one at 380 nm corresponding to cis – isomers and other at 500 nm corresponding to trans – isomers. The appareance of a new peak at longer wavelengths, in latter ripening stages, implies the formation of new carotenoids. In the dehydrated samples, of a definite peak at shorter wavelengths shows that drying induces the isomerization of trans - carotenes into cis – forms. Besides, thermal degradation transforms carotenoids in epoxides producing a red – shift in the spectra.

The combination of analytical, structural and photoluminescence techniques allows to quantify, detect and study the presence and evolution of carotenoids in persimmon tissue.

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