Carotenoids in dehydrated persimmon: Antioxidant activity, structure, and photoluminescence

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ABSTRACT

In this study, the effect of two hot air drying conditions (40 °C for 23 h and 60 °C for 9 h) on the content, antioxidant activity, microstructure, and luminescence properties of persimmon carotenoids in three ripening stages was studied. Based on the results from total carotenoids content and HPLC analysis, the carotenoid's content increased with the advance of ripening, highlighting the β-cryptoxanthin fraction. In addition, drying treatments did not affect the carotenoid content and profile but decreased the antioxidant activity. Microstructural studies showed that the ripening progress and/or drying treatments, led persimmon tissues to lose integrity, allowing the diffusion of carotenoids and their degradation. Photoluminescence measurements evidenced the synthesis of β-cryptoxanthin during the fruit ripening. After drying, a new emitting specie at 340 nm was attributed to the carotenoid's isomerisation while the emission at 500 nm experienced a shift that was related to the formation of thermal degradation products. Both facts could explain the loss of antioxidant activity in persimmon submitted to drying treatments. In this sense, photoluminescence, in combination with spectrophotometric, chromatographic and structural techniques, helps to understand the phenomena caused by both, ripening and drying treatments, in the persimmon's carotenoids fraction.

1. Introduction

Persimmon (*Diospyros kaki L.*) is a fruit crop originating from Asia, which has experienced a large growth along the Mediterranean over 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 beneficial effect on human health (Giordani, Doumett, Nin, & Del Bubba, 2011). Thus, it has been used to treat mild pathologies, plus its potential against cardiovascular diseases and some cancer types has been proven (George & Redpath, 2008). Rojo Brillante persimmon is a much-appreciated variety because of its size and texture, and its crop has grown over years thanks to its high productivity (Cárcel, García-Pérez, Sanjuán, & Mulet, 2010). However, because of the compulsory quality control by the origin appellation there is a loss of ≤30% fruit, generating an economic and environmental problems. Therefore, valorisation techniques have been studied to avoid and reduce persimmon excess and to offer new products to the consumer throughout the year (Cárcel, García-Pérez, Riera, & Mulet, 2007).

Drying techniques have been used for many years as a valorisation procedure for obtaining high quality and shelf-stable fruits (Hasan et al., 2019). Hot air drying (HAD) is one of the oldest and the most important food preservation methods practised; this improves the food stability and reduces the water and microbiological activity of the material, minimising physical and chemical changes during its storage (Doymaz, 2012). The effect of HAD on carotenoids is complex; several studies have shown how HAD affects carotenoids in different plant-based foods. Piyarach, Nipawan, Chadapon, Daluwan, and Kunjana (2020) found no differences in total carotenoid content in various vegetables when drying at 65 °C; whereas Zhang et al. (2018) showed the complex relationship between HAD and carotenoids because of differences in the food matrix.

Carotenoids are a group of hydrophobic molecules synthesised by plants and some microorganisms; the basic structure comprises a tetraterpenoid chain with possible terminal rings (Britton, 1995). 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 early ripening stages remaining constant throughout the shelf-life of the fruit whereas β-cryptoxanthin appears at advanced ripening stages (Bordiga et al., 2019).

The typical structure of trans-conjugated double bonds influences chemical and biochemical properties of carotenoids. They are responsible for vital physiological processes such as photosynthesis, structural stabilisation of protein-pigment photosynthetic complexes, provitamin A activity, and autoxidation to avoid cell damage. A small proportion of cis-isomers have been observed having lower provitamin A activity than their trans-equivalents (Nisar, Li, Lu, Khin, & Pogson, 2015). The system of conjugated double bonds allows carotenoids to absorb UV–Vis light between 400 and 500 nm. This feature allows carotenoids in solution to obey the Lambert-Beer Law, thus its quantification from UV–Vis spectroscopic measurements can be obtained; therefore, several authors have reported the application of UV–Vis spectroscopy to determine carotenoid content (García-Cayuela, Quiles, Hernando, Welti-Chanes, & Cano, 2018; Hernández-Carrión, Tárrega, Hernando, Fiszman, & Quiles, 2014). However, despite its simplicity, this system is not accurate enough to solve the complex nature of the excited states of carotenoids because of the low intensity of symmetry-forbidden, $\pi \rightarrow \pi^*$ transitions (Jørgensen, Stapelfeldt, & Skibsted, 1992). Therefore, the molecular and chemical changes that occur in the entire absorption spectrum cannot be detected. Although, luminescence spectroscopy may become an alternative in the analysis of carotenoids.

Luminescence is the light emission from atoms and/or molecules because of the energetic fall from excited states to the ground state. Luminescence is formally divided into two categories, fluorescence and phosphorescence, depending on the excited state. In fluorescence, deactivation occurs among the same multiplicity states (singlet-singlet and triplet-triplet) (Lakowicz, 2006). Fluorescence is the most common and useful type of luminescence in analytical chemistry. Excitation-emission spectrofluorimetry is an analytical technique of moderate selectivity and high sensitivity, which can be applied to the detection of a very wide range of analytes in environmental and biological samples. The capacity of detection is approximately one order of magnitude greater than molecular absorption spectroscopy, and its selectivity is greater than other spectroscopic methods (Andrade-Eiroa, de-Armas, Estela, & Cerdà, 2010). Fluorescence experiments are relatively easy to perform and can provide important information concerning molecular structure and chemical interactions (Strasburg & Ludescher, 1995). Many compounds in foods have been analysed by fluorometry, such as proteins, peptides, amino acids, vitamins, carbohydrates, enzymes, steroids, some inorganic compounds, and toxins (Jihad-René, 2000). To the best of our knowledge, this technique has not been used to study changes of carotenoids in foods.

Therefore, this study aimed to observe the changes in carotenoid fractions in persimmon because of air drying. Thus, their content, antioxidant activity, and microstructure were studied. Furthermore, the photoluminescence was investigated as a potential tool to easily show changes in carotenoid fractions.

2. Materials and methods

2.1. Sample preparation

Persimmon (*Diospyros kaki* Thunb cv Rojo Brillante) unseeded fruits in three ripening stages (M1, M2, and M3), treated using a de-astringency treatment (95% CO_2 atmosphere over 24 h at 20 °C), were provided by "Instituto Valenciano de Investigaciones Agrarias" (IVIA, Valencia, Spain). These three commercial ripening stages were from fruits harvested from a local grove in L'Alcudia (Valencia, Spain) between mid-November and early December 2018. The criterion for harvesting each ripening stage was the visual evolution of skin colour

corresponding to ripening IV (yellow orange), V (orange), and VI (intense orange) (Tessmer et al., 2016). The average water content of persimmon fruits was 80%. The fruits were washed and cut into slices (5 mm thick) with a mandolin (mandolin slicer 2.0, OXO good grips, Sheffield, UK). HAD was conducted in a cabinet dryer (FED 260 standard model, Binder, Tuttlingen, Germany) at 40 and 60 °C for 23 and 9 h respectively, using an air velocity of 2 m/s and 30% relative humidity to achieve ≈ 15 g water/100 g product (wet basis). The final point were set based on the literature and previous experimental trials (Doymaz, 2012; Megías-Pérez, Gamboa-Santos, Soria, Villamiel, & Montilla, 2014). These groups of fruits are named as shown in Fig. 1. The analytical determinations were made in the pulp of the analysed samples.

2.2. Chemicals and standars

Acetone (99.5%): Panreac Quimica S.A. (Spain), diethyl ether (99.7% stabilised with ≈7 ppm BHT): Scharlab S. L. (Spain), food grade sodium sulphate anhydrous (E-514i, F.C.C.): Panreac Quimica S.A. (Spain), sodium acetate anhydrous (Reag. Ph. Eur.) Panreac Quimica S. A. (Spain), anhydrous sodium sulphate: Scharlab S. L. (Spain), basic magnesium carbonate (CO_3Mg) ≥ 40%: Riedel-de Haën (Germany), sodium chloride (NaCl) 99,0–100,5%: Panreac Quimica, S. A. (Spain), hydrochloric acid 37% (Reag. USP): Panreac Quimica S.A. (Spain), potassium hydroxide (KOH) ≥ 86%, Sigma-Aldrich (France), Triethylamine 99.5%: Panreac Quimica, S. A. (Spain), Ethyl acetate, acetonitrile, methanol and water were of LC-MS grade, purchased from Scharlab S. L. (Spain). β-carotene ≥ 97% (UV): Fluka Biochemika, Sigma-Aldrich (USA), β-cryptoxanthin 97% (UV): Extrasynthese (France), β-apo-8′-carotenal (trans) ≥ 96% (UV): Fluka Biochemika, Sigma-Aldrich (Switzerland), Violaxanthin 0.897 mg/L solution in ethanol: Carbosynth Ltd (UK), Lutein 88%: Carbosynth (UK), Zeaxanthin: Carbosynth (UK). 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). 2,6-Di-tert-butyl-4-methyl phenol (BHT) 99% was obtained from Panreac Quimica, S. A. (Spain).

2.3. Total carotenoid content

Total carotenoid content of fresh and dehydrated samples were extracted according to Hernández-Carrión et al. (2014) with some modifications. Homogenized persimmon pulp samples (5 g of fresh samples and the equivalent dried mass from dehydrated samples) were extracted six times with 25 mL cool acetone using an Ultraturrax (T25 Basic, IKA, Staufen, Germany) 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/L) was added to separate the phases and to transfer the pigments to the ether, while the aqueous phase was removed. The process was conducted in several steps to ensure the greatest elimination of the aqueous phase. The organic phase was treated several times with anhydrous Na_2SO_4 (20 g/L) to remove residual water. Then, the sample was evaporated until dry in a rotary evaporator (model RII; Buchi Labortechnik, Flawil, Switzerland) at a temperature ≤ 45 °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, UK). The calibration curve was performed with different concentrations of β-carotene in acetone ($R^2 = 0.9984$). Results were expressed as mg β-carotene/100 g of dry matter. Carotenoid extractions were made in triplicate.

2.4. Individual carotenoids by HPLC-DAD analysis

Five grams of fresh tissue was homogenized (Polytrom PT3100 homogenizer (Kinematica AG, Switzerland) with 0.2 g of basic magnesium
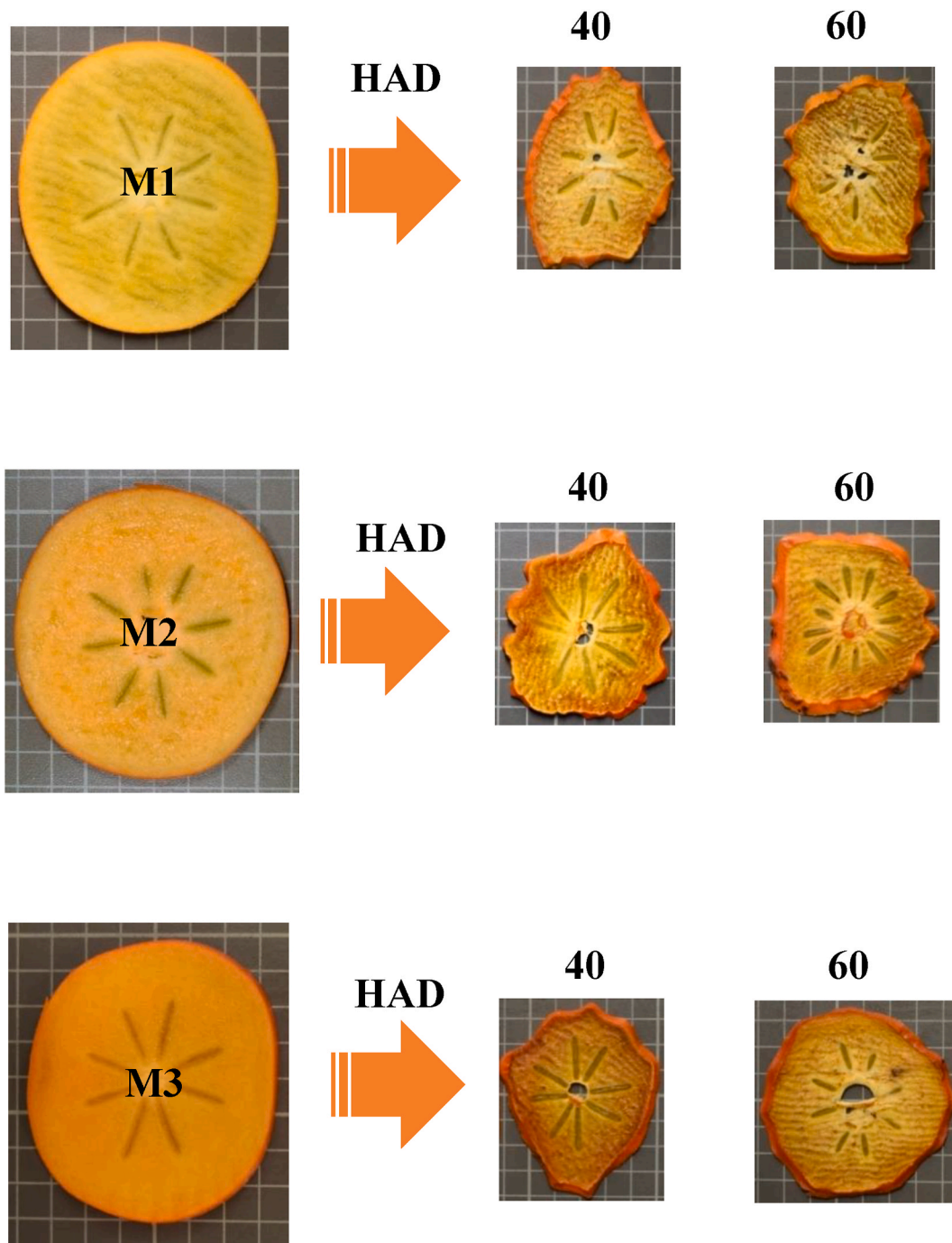
Fig. 1. Images of persimmon samples analysed. M1, M2, and M3 are related with the three ripening stages. Numbers 40 and 60 correspond to the hot air drying treatments (HAD) applied at 40 and 60 °C, respectively.

carbonate and 15 mL of ethyl acetate (0.05% BHT). A volume of 100 mL of β-apo-8′-carotenal internal standard was added to sample. The homogenate was centrifuged (Centrifuge Eppendorf 5810R, Eppendorf Iberica, Madrid, Spain) at 12000 rpm at 4 °C for 30 min, the supernatant was collected in a 100 mL decantation funnel and the pellet was re-extracted with 5 mL of ethyl acetate (0.05% BHT) and the supernatant transferred to the decantation funnel. The organic phase was washed three times with water and NaCl-saturated water, and then collected and dried on a bed of anhydrous sodium sulphate. Ethyl acetate was removed in a rotary vacuum evaporator at 40 °C. The residue was dissolved in 5 mL of diethyl ether and saponified with 2.5 mL 20% KOH in methanol, and kept overnight in darkness. The saponified extract was

transferred to a 100 mL decantation funnel, mixed with 2 mL of diethyl ether and washed three times with water and NaCl-saturated water. Diethyl ether was collected, then dried with anhydrous sodium sulphate, and evaporated. Dried samples were dissolved in 0.4 mL ethyl acetate and analysed by HPLC-DAD Alliance liquid chromatographic system (Waters, Barcelona, Spain) equipped with a 2695 separations module coupled to a 2996 photodiode array detector, using a reverse-phase column ZORBAX Eclipse XDB-C_{18} (150 mm × 4.6 mm), 5 μm (Agilent). The flow rate was 1 mL/min and the injection volume was 5 μL. The mobile phase was of water (A): acetonitrile-water-triethylamine (900:99:1) and (B) ethyl acetate. The gradient elution was: 0–5 min, 100%–75% A; 5–10 min, 75%–30% A; 10–13 min, 75%–0% A; 13–14

min, 0%–100% A; and 14–15 min, 100% A, with a total run time of 15 min. Compounds were identified on the basis of comparison of their retention times and absorption spectrum characteristics. Quantification of carotenoids was achieved using calibration curves with commercially available authentic standards: violaxanthin, lutein, zeaxanthin, β-cryptoxanthin and β-carotene. Their quantity was corrected for extraction efficiency based on the β-apo-8′-carotenal internal recovery standard. Empower 2 software was used for data acquisition. Results were expressed as percentage (mg individual carotenoid/total carotenoids) in dry basis.

2.5. Antioxidant activity

2.5.1. Determination of antioxidant activity using the FRAP method

The FRAP (ferric reducing antioxidant power) was performed in the carotenoid extracts obtained from fresh and dehydrated samples according to the method of Benzie and Strain (1996) with some modifications. Sodium acetate buffer (300 mM; 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. The 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. Furthermore, 75 μL of distilled water, 75 μL of the sample extract, and 2.25 mL of the FRAP reagent were mixed and incubated at 37 °C for 30 min in darkness. After, the absorbance was measured at 595 nm using a spectrophotometer (CE 1021 1000 series, CECIL INSTRUMENTS Cambridge, UK). A standard curve was performed using Trolox as a standard and results were expressed as μmol Trolox eq/g.

2.5.2. Determination of antioxidant activity using the DPPH method

The DPPH (2,2-diphenyl-1-picrylhydrazyl) method was performed on the carotenoid extracts obtained from fresh and dehydrated samples according to the method described by Matsumura et al. (2016) with some modifications. A stable DPPH radical was reduced because of the carotenoids resulting in a reduction of the absorbance; 40 μg/mL 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 at 37 °C in darkness, absorbance was measured at 517 nm using acetone as a blank. Results were expressed as a DPPH inhibition percent (Eq. (1)):

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

(1)

2.6. Microstructure

Microscopic analysis was performed in fresh and dehydrated samples with the aid of a Nikon Eclipse 80i® light microscope (Nikon Co. Ltd., Tokyo, Japan) which has incorporated a camera (Exwave HAD, nº DXC-19, Sony Electronics Inc., Park Ridge, New Jersey, USA). Twenty micrometre cryostat sections were obtained from persimmon slices and were transferred to a glass slide. Sections were displayed using brightfield, with (LM-T) and without (LM), 1% toluidine blue as staining agent, and by fluorescence, using a mercury arc lamp with an 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).

2.7. Carotenoid determination using fluorescence

Fluorescence measurements for persimmon extracts from fresh and dehydrated samples were recorded using a Jasco FP-8500 Spectrofluorometer (Jasco Inc., Easton, MD). The emission spectra were measured between 360 and 650 nm using an excitation wavelength of 340 nm. The

excitation spectra were recorded at 420 nm emission wavelength between 300 and 380 nm. The data interval was 0.5 nm, scan speed 500 nm/min, and the emission and excitation band were 2.5 nm. Absorbance of the extracts was adjusted, and the measurements were performed at ambient conditions. All the data were analysed using the OriginPro 9.0.0 software package (OriginLab Co., Northampton, MA, USA).

2.8. Statistical analysis

A categorical multifactorial experimental design with two factors, ripening stage and drying treatment, was used to characterise the carotenoid content and the antioxidant activity of the samples. The honest significant difference (Tukey's HSD test) with a 95% confidence compared the mean values obtained ($p < 0.05$). All the data were analysed using the XLSTAT statistical software 2019 4.2 (Addinsoft, Barcelona, Spain).

3. Results and discussion

3.1. Total carotenoid content (TCC)

The effect of both factors, ripening stage, and drying treatment conditions on the TCC of samples is presented in Fig. 2A and B. There were no significant interactions ($p < 0.05$) between the ripening stage and the drying treatment conditions. The only factor with a significant effect ($p < 0.05$) on the TCC was the ripening stage. There was an increase in the TCC as the ripening stage progressed (Fig. 2A), where M3 persimmon showed the highest TCC values; however, M1, M2, and M3 persimmon showed no significant differences ($p > 0.05$) TCC values. Fig. 2B shows the effect of the drying treatment conditions on the TCC of samples. There were no significant differences ($p > 0.05$) among fresh and dehydrated samples; therefore, the TCC remained after both drying treatments.

3.2. Individual carotenoids by HPLC-DAD analysis

The different carotenoids detected by HPLC-DAD are shown in Fig. 3 β-cryptoxanthin and β-carotene were the main carotenoids in both fresh and dehydrated samples. A carotenoid increase was observed with the fruit ripening highlighting β-cryptoxanthin (Fig. 3A); the content of violaxanthin, lutein and zeaxanthin was detected in a lower percentage. Therefore, the increase observed in the TCC (Fig. 2A) could be related with the increase in β-cryptoxanthin. In agreement with De Ancos, Gonzalez, and Cano (2000), the same carotenoids were also detected in the 'Rojo Brillante' persimmon. In Fig. 3B, C, and 3D no differences were found in the percentage of individual carotenoids between fresh and dehydrated samples. This behaviour correlates with TCC determination (Fig. 2B).

3.3. Antioxidant activity (AA)

The capacity to reduce the ferric-tripyridyl triazine (Fe^{III}-TPTZ) complex to the ferrous form (Fe^{II}) and the ability to inhibit DPPH free radical content of carotenoids in the fresh and dehydrated persimmon samples shows the AA. Results of the FRAP method showed there were no significant ($p > 0.05$) interactions between the factors; however, the ripening stage and drying treatment conditions had a significant effect ($p < 0.05$). While the ripening stage progressed (Fig. 2C), there was an increase in the AA. Significant differences ($p < 0.05$) were found between M1 and M3 as seen with the TCC. The FRAP method followed the same tendency as TCC along with the increase of β-cryptoxanthin fraction detected in the HPLC-DAD analysis, thus the higher content of carotenoids could be related with a higher AA; also determined by Yoo and Moon (2016) in three citrus varieties. Fig. 2D shows the effect of the drying treatment conditions on the AA, determined by the FRAP method with a significant decrease ($p < 0.05$) in the AA after the drying
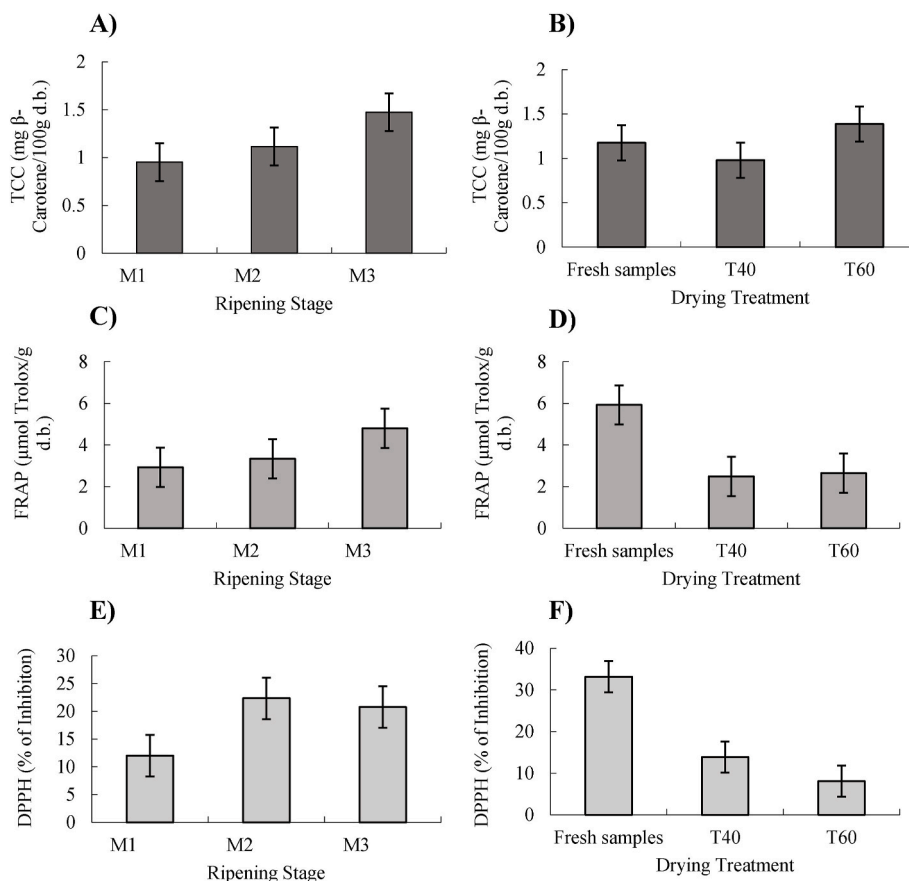
Fig. 2. Mean plots with Tukey HSD intervals. A, B: mean values for the TCC according to the ripening stage and drying treatment, respectively for fresh and dehydrated samples. C, D: mean values for the FRAP according to the ripening stage and drying treatment, respectively for fresh and dehydrated samples. E, F: mean values for the DPPH (% inhibition) according to the ripening stage and drying treatment, respectively for fresh and dehydrated samples.

treatments at 40 and 60 °C was observed. This effect has also been reported previously by Martínez-Las Heras, Landines, Heredia, Castelló, and Andrés (2017). This reduction in the AA could be related with the formation of cis-isomers when the samples were dehydrated (Marx, Stuparic, Schieber, & Carle, 2003). The conformation of carotenoids within the lipid membrane bilayer might have changed because of polarisation changes affecting its antioxidant properties (Grudzinski et al., 2017).

AA values obtained using the DPPH method show no significant interactions ($p > 0.05$) between the factors, but both factors had a significant effect ($p < 0.05$). Fig. 2E shows the effect of the ripening stage factor in the DPPH values. A similar tendency to the FRAP method was obtained, where an increasing AA was seen when the ripening stage progressed. The samples M2 and M3 presented the highest ($p < 0.05$) DPPH values without significant differences. Considering drying treatment (Fig. 2F), there was a significant decrease ($p < 0.05$) in the AA after both drying treatments without significant differences between them; also observed in the FRAP method.

3.4. Microstructure

Fig. 4 shows images of the fresh persimmon samples in the three ripening stages. In sections stained with toluidine blue, the cell walls appeared turgid, compact and with high physical integrity in the three ripening stages (Fig. 4A, D, and 4G). In unstained sections of persimmon (Fig. 4B, E, and 4H), the carotenoids could be seen with their characteristic yellow orange colour, distributed homogeneously throughout the cellular tissue. Carotenoid substances were observed grouped in two separate ways. They were inside the chromoplasts, close to the cellular

wall, adopting a globular structure or spherical bodies. In addition, carotenoids appeared formed as crystalline clusters inside the cell and cell wall. There was an increase in the carotenoid's accumulation in M3 samples (Fig. 4H and I) compared to M1 and M2. These results agreed with the TCC, as M3 samples had more carotenoids than M1 samples.

During ripening the transformation of chloroplasts into chromoplasts occurred, which is the most frequent modification of plastids in the ripening of fruits (Egea et al., 2010; Schweiggert, Steingass, Heller, Esquivel, & Carle, 2011; Vázquez-Gutiérrez, Quiles, Hernando, & Pérez-Munuera, 2011). Autofluorescent emission was attributed to carotenoids because An, Suh, Kwon, Kim, & Johnson, 2000 concluded that, using excitation with a 488 nm argon ion laser, the autofluorescence emitted at lengths greater than 515 nm was because of carotenoids. Autofluorescence images (Fig. 4C, F, and 4I) showed that a substantial proportion of carotenoids were in the cell walls, in the three ripening stages.

Fig. 5 shows images of dehydrated persimmon samples at the three ripening stages. The sections stained with toluidine blue (Fig. 5A, D, 5G, 5J, 5M, and 5P) showed differences and changes in the cell wall structure of the samples after drying at 40 and 60 °C. In the unstained (Fig. 5B, E, 5H, 5K, 5N, and 5Q) and autofluorescence (Fig. 5C, F, 5I, 5L, 5O, and 5R) persimmon images the changes in the structure and location of the carotenoids can be observed.

When comparing the fresh and dehydrated persimmon samples (Figs. 4 and 5; blue toluidine stained images), both drying treatments produced the degradation of walls and membrane cells, thus loss of cellular structural integrity (Fig. 5A, D, 5G, 5J, 5M, and 5P) in the three ripening stages. This degradation of the tissue could facilitate the diffusion of carotenoids. In all dehydrated persimmon samples,
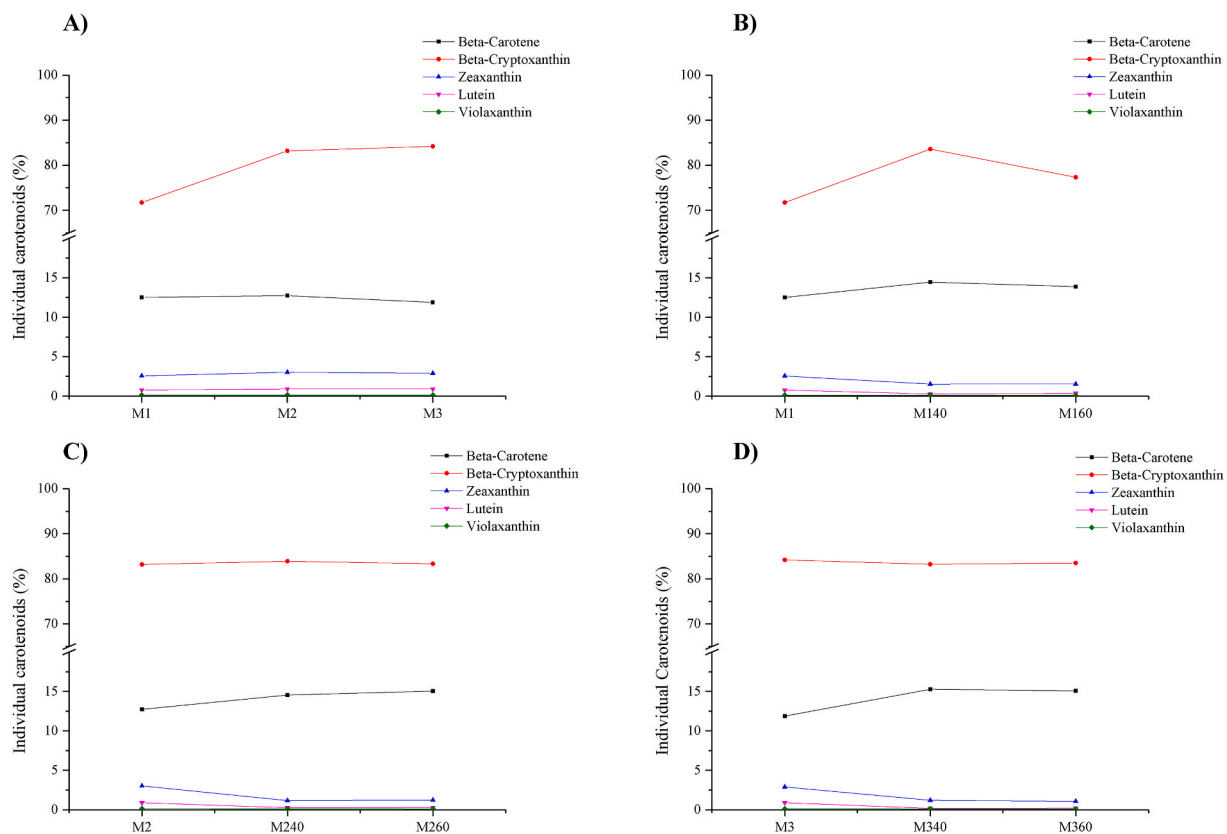
Fig. 3. A, B, C, D: Individual carotenoids percentage detected by HPLC-DAD. A: effect of ripening stage (M1, M2, M3) in fresh samples. B, C and D: effect of dehydration at 40 and 60 °C in M1 (B), M2 (C), and M3 (D), respectively.
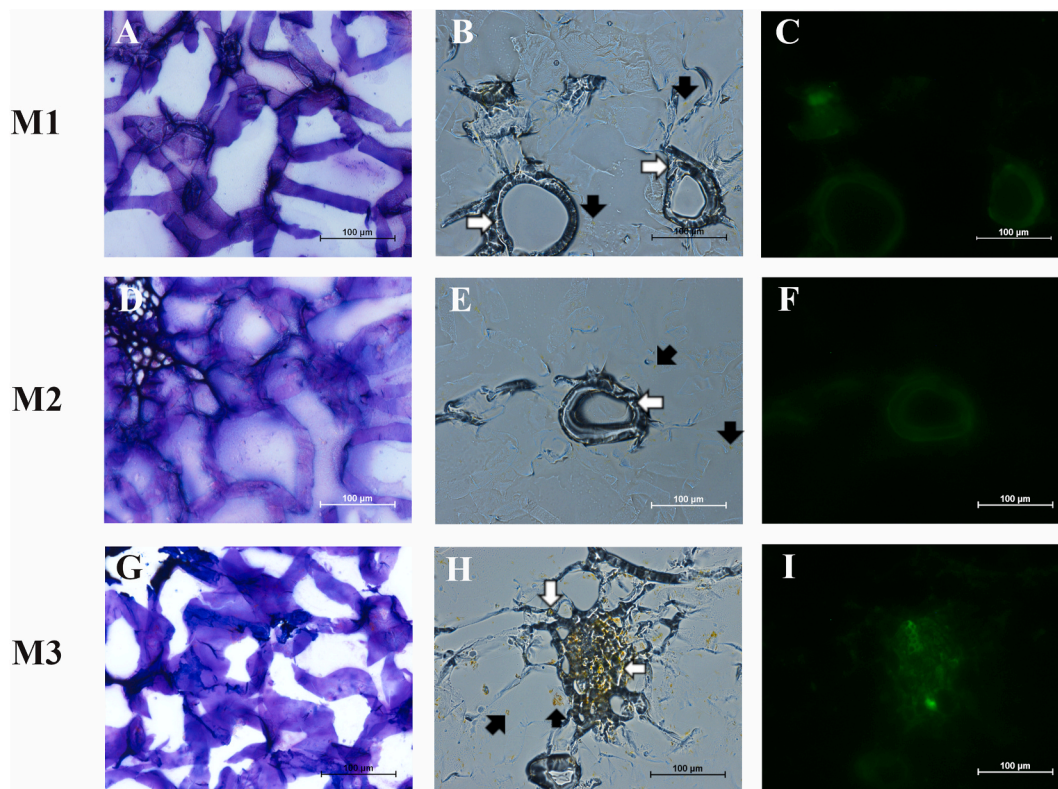

Fig. 4. Microstructure of the fresh persimmon samples at the three ripening stages (M1, M2, M3). A, D, G: Blue toluidine stained images. B, E, H: unstained samples (White arrows: crystalline clusters of carotenoids; Black arrows: spherical bodies of carotenoids). C, F, I: autofluorescence images. Magnification 20x. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Fig. 5. Microstructure of dehydrated persimmon samples dried at 40 and 60 °C at the three ripening stages (M1, M2, M3). A, D, G, J, M, P: Blue toluidine stained images. B, E, H, K, N, Q: unstained samples. C, F, I, L, O, R: autofluorescence images. Magnification 20x. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
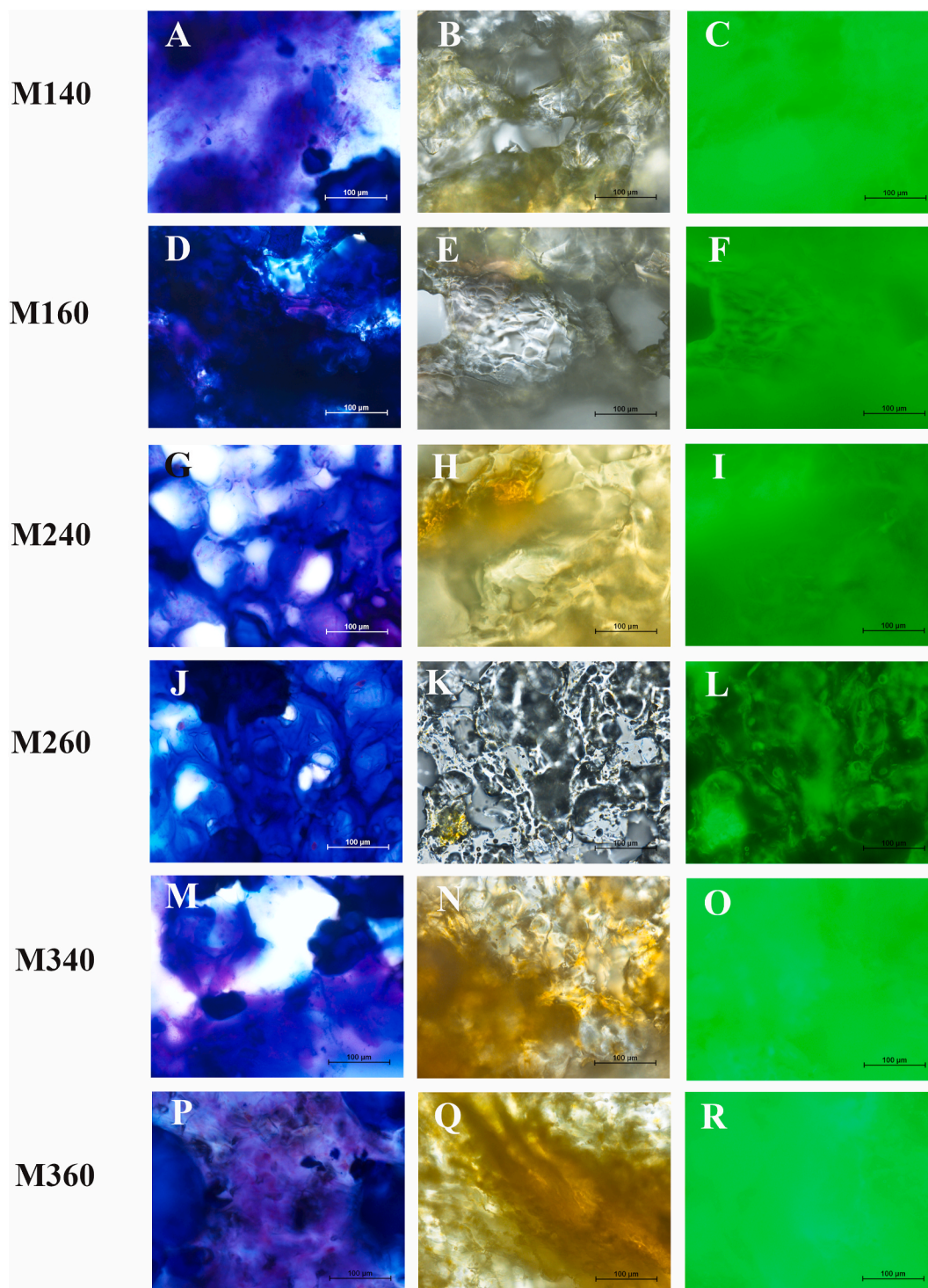
independent of the ripening stage, carotenoids lost a part of their crystalline appearance and were spread throughout the tissue (Fig. 5B, E, 5H, 5K, 5N, and 5Q). In addition, the drying treatments enhanced the circulation and diffusion of carotenoids throughout the tissue, as seen in the autofluorescence images (Fig. 5C, F, 5I, 5L, 5O, and 5R). The carotenoids diffusion could be related to the extractability of the carotenoids after the drying treatment. Therefore, it could improve the bioaccessibility of carotenoids because they would be more dispersed.

However, this release from the cells could leave the carotenoids more exposed to environmental conditions affecting its antioxidant properties.

3.5. Fluorescence of carotenoids

Different excitation wavelengths were studied to determine the energy at which maximum emission occurs. Fig. 6A shows the normalised
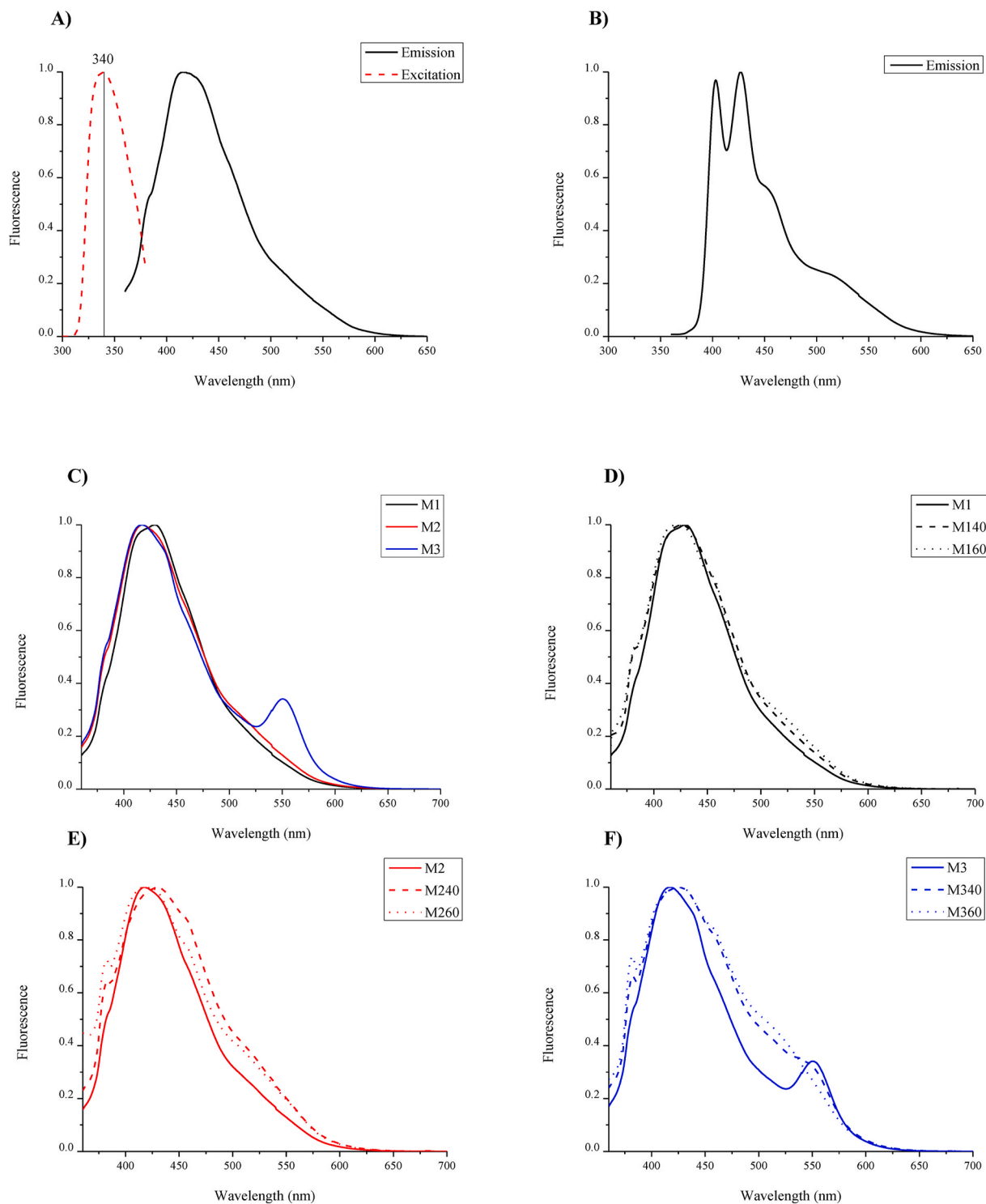
Fig. 6. A: Normalised excitation ($\lambda_{excitation} = 340$ nm; – – –) and emission (—) spectra of the carotenoids extract from fresh M1 sample. B: Normalised emission (—) spectra of the β-carotene pure extract excited at 340 nm. C, D, E, F: Normalised emission spectra of the carotenoids extract from fresh and dehydrated persimmon samples at λ excitation = 340 nm. C: the effect of the fresh samples in M1, M2, M3 ripening stages in the fluorescence. D, E, and F: the effect of the fresh and dehydrated samples dried at 40 and 60 °C in M1, M2, and M3 ripening stages on the fluorescence, respectively.

excitation and emission spectra of the carotenoids extracted from the M1 fresh sample, which allowed selection of the 340 nm wavelength, used in the fluorescence analysis. Besides, emission spectra of pure β-carotene was conducted to compare with the extracted carotenoids, observing similar features, although with more definition, seen in Fig. 6B. The emission spectra of β-carotene is characterised by a band from 400 to 500 nm with three characteristics peaks.

Fig. 6 also shows the normalised fluorescence spectra for the fresh and dehydrated samples excited at 340 nm and recorded between 360 and 650 nm. Fig. 6C shows the effect of the ripening stage, while ripening progresses on the spectra; Fig. 6D, E, and 6F present the effect of the drying treatments in the earlier (M1), intermediate (M2), and advanced (M3) ripening stages, respectively.

All the samples were 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, associated to a symmetry-forbidden $\pi \rightarrow \pi^*$ transition (Jørgensen et al., 1992). This was demonstrated when the normalised spectra of the different samples were observed.

In Fig. 6C a small difference of intensity among M1, M2, and M3 was noticed. An emitting peak at 380 nm can be observed in the M2 and M3 persimmon samples. This peak might be associated with different cis-isomers of β-carotene, as reported previously by Zaghdoudi et al. (2017). Although carotenoids are seen in an all samples, trans-conformation are the predominant proportion in nature and cis-isomers represent a minor fraction in fresh fruits (Aman, Schieber, & Carle, 2005). In Fig. 6D, E, and 6F, this peak was remarkable with dehydrated samples, which showed a higher intensity at 380 nm if compared with the fresh persimmon samples, especially in the M2 and M3 ripening stages. Previous authors found some all-trans-β-carotene could be partially converted into cis-isomers by the heat action during the drying treatments (Schieber & Carle, 2005). Therefore, the formation of these cis-isomers could be related with the reduction of the AA after the drying treatments (Fig. 2D and F) because the cis-isomers have lower AA (Pénicaud, Achir, Dhuique-Mayer, Dornier, & Bohuon, 2011).

In Fig. 6C a shift in the emission spectra, evidenced by a new band at 550 nm, was observed; especially in the most advanced ripening stage (M3). This could be related with the formation of new compounds such as β-cryptoxanthin. As seen in Fig. 3A, β-cryptoxanthin was one of the main carotenoids that increased in advanced ripening stages. This also agrees with the study of Bordiga et al. (2019) where β-cryptoxanthin was the most abundant carotenoid as ripening progressed. Furthermore, the emission at longer wavelengths caused by β-cryptoxanthin is strongly supported by Jørgensen et al. (1992), because of the nonradiative deactivation produced by n-states in xanthophylls, which reduces the energy gap between higher singlet state and the emissive state.

In Fig. 6D, E, and 6F, there was a shift in the emission spectra of the dehydrated samples towards broader wavelengths. The evolution of the fluorescence spectra of the dehydrated samples towards longer wavelengths could be explained by the thermal degradation of carotenoids as described previously by Ehlers, Wild, Lenzer, and Oum (2007). In addition, several authors have studied the thermal degradation of carotenoids and proved that isomerisation and oxidation were the main degradative reactions of carotenoids (Colle, Lemmens, Knockaert, Van Loey, & Hendrickx, 2016; Kim et al., 2006). Pénicaud et al. (2011) investigated that isomerisation could be the first step of oxidation, leading to the formation of apocarotenones and apocarotenals from the epoxides.

4. Conclusion

As the ripening stage progresses, persimmon samples present higher TCC and AA values. The appearance of an emission peak at longer wavelengths, in the advanced ripening stage implies changes in the carotenoids fraction as the synthesis of β-cryptoxanthin confirmed by HPLC.

Hot air drying treatments do not affect the TCC or the carotenoids fraction but decrease the carotenoids AA values. At the microstructural level, both treatments lead to persimmon cellular integrity loss and favour the diffusion of the carotenoids throughout the vegetal tissue. This could expose them to external conditions favouring their degradation, which may explain the decrease in the persimmon antioxidant activity. The appearance of a new emission peak, at shorter wavelengths, and a shift in the spectra, both induced by drying treatments, could be related to isomerisation reactions and thermal degradation, respectively. Therefore, the photoluminescence help to understand the phenomena caused by both, ripening stage and drying treatments, in the persimmon's carotenoid fraction.

The combination of spectrophotometric, chromatographic, structural, and photoluminescence techniques allows quantifying, detecting, and studying the changes in carotenoids of persimmon tissue produced by drying treatments.

CRediT authorship contribution statement

Cristina M. González: Investigation, Validation, Methodology, Formal analysis, Writing - original draft, preparation. **Adrián López García:** Investigation, Validation, Methodology, Formal analysis. **Empar Llorca:** Investigation, Validation, Methodology, Formal analysis. **Isabel Hernando:** Supervision, Resources. **Pedro Atienzar:** Supervision, Conceptualization, Writing-Review. **Almudena Bermejo:** HPLC methodology, Analysis. **Gemma Moraga:** Supervision, Resources, Conceptualization, Funding acquisition, Writing - review & editing. **Amparo Quiles:** Investigation, Supervision, Resources, Conceptualization, Funding acquisition, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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