Carotenoid composition of strawberry tree (*Arbutus unedo* L.) fruits

Raúl Delgado-Pelayo, Lourdes Gallardo-Guerrero, Dámaso Hornero-Méndez

PII: S0308-8146(15)30273-9
DOI: http://dx.doi.org/10.1016/j.foodchem.2015.11.135
Reference: FOCH 18476

To appear in: *Food Chemistry*

Received Date: 25 May 2015
Revised Date: 21 November 2015
Accepted Date: 28 November 2015

Please cite this article as: Delgado-Pelayo, R., Gallardo-Guerrero, L., Hornero-Méndez, D., Carotenoid composition of strawberry tree (*Arbutus unedo* L.) fruits, *Food Chemistry* (2015), doi: http://dx.doi.org/10.1016/j.foodchem.2015.11.135

Carotenoid composition of strawberry tree (*Arbutus unedo* L.) fruits.

Raúl Delgado-Pelayo, Lourdes Gallardo-Guerrero, Dámaso Hornero-Méndez*

Group of Chemistry and Biochemistry of Pigments. Food Phytochemistry Department. Instituto de la Grasa (CSIC). Campus Universidad Pablo de Olavide, Ctra. de Utrera km. 1. 41013 - Sevilla (Spain).

* Corresponding author.

Telephone: +34 954611550; Fax: +34 954616790; e-mail: hornero@ig.csic.es

Abstract

The carotenoid composition of strawberry tree (*A. unedo*) fruits has been characterised in detail and quantified for the first time. According to the total carotenoid content (over 340 μg/g dw), mature strawberry tree berries can be classified as fruits with very high carotenoid content (> 20 μg/g dw). (all-*E*)-Violaxanthin and 9*Z*-violaxanthin were found to be the major carotenoid pigments, accounting for more than 60%, responsible for the bright colour of the flesh of ripe fruits. In addition other 5,6-epoxide carotenoids, such as (all-*E*)-neoxanthin, (9'*Z*)-neoxanthinn (all-*E*)-antheraxanthin and lutein 5,6-epoxide, together with (all-*E*)-lutein, (all-*E*)-zeaxanthin and (all-*E*)-β-carotene were found at high levels (> 5-20 μg/g dw). The LC-MS (APCI+) analysis of the xanthophyll fraction in their native state (direct extract) revealed that most of them (> 90%) were totally esterified with saturated fatty acids (capric, lauric, myristic, palmitic and stearic). Monoesters, homodiesters and heterodiesters of (all-*E*)-Violaxanthin and 9*Z*-violaxanthin were the major pigments.

Keywords: *Arbutus unedo* L.; HPLC-DAD; LC-MS(APcI+); GC; carotenoids; xanthophyll esters; fatty acids

Chemical compounds studied in this article:

(all-E)-β-Carotene (PubChem CID: 5280489); (all-E)-Zeaxanthin (PubChem CID: 5280899); (all-E)-Lutein (PubChem CID: 5368396); (all-E)-Violaxanthin (PubChem CID: 448438); (9Z)-Violaxanthin (PubChem CID: 5282218); (9'Z)-Neoxanthin (PubChem CID: 5282217); (all-E)-Neoxanthin (PubChem CID: 5281247); (all-E)-Antheraxanthin (PubChem CID: 5281223); Lutein 5,6-epoxide (PubChem CID: 5281244)

1. Introduction

The strawberry tree (*Arbutus unedo* L.) is an evergreen tree, usually smaller than 5 m, belonging to the *Ericaceae* family, and typical of the Mediterranean basin and climate, although it can be also found in the Near East and Transcaucasian and Macaronesian areas, and in some Atlantic regions such as France and Ireland (Torres, Valle, Pinto, García-Fuentes, Salazar, & Cano, 2002). Other three *Arbutus* species can be found in the Mediterranean region: *A. andrachne* L., *A. pavarii* Pampanini and *A. canariensis* Veill., the last two endemic to Libya and Canary Islands, respectively, and the first one found at the eastern Mediterranean regions (mainly in Greece and Turkey). The fruits are spherical berries, about 1.5-2 cm in diameter, dark red and tasty when fully ripen. It matures during autumn and winter with a colour ranging from red to deep crimson when ripe. The tree bears mature fruits and flowers at the same time, since the fruits take the whole year to ripen (Maleš, Plazibat, Vundać, & Žuntar, 2006). The berries can be eaten as fresh fruits, preferred when they reach an overripe stage, but they are frequently used to prepare preserves, jams, jellies and marmalades, as well as for pastry and pie fillings. The name 'unedo' is explained by Pliny the Elder as being derived from *unum edo* ("I eat one"), which may be related to the alcoholic content of overripe fruits. In some countries the fruits are used to produce alcoholic beverages such as wines, liquors or distillates, e.g. "aguardente de medronho" in Portugal, "Koumaro" in Greece and "licor de madroño" in Spain (Soufleros, Mygdalia, & Natskoulis, 2005).

A. unedo is well-known in folk medicine for the antiseptic, diuretic, laxative, urinary antiseptic, antidiarrheic, astringent, depurative and antihypertensive properties of the infusions and decoctions prepared from roots, barks, leaves and fruits (Fortalezas et al., 2010; reviewed by Oliveira, Baptista, Bento, & Pereira, 2011; Miguel, Faleiro, Guerreiro, & Antunes, 2014). Some studies have proved vasorelaxant and antiaggregant

effects of *A. unedo* leaves extracts in human platelets (El Haouari, López, Mekhfi, Rosado, & Salido, 2007; Legssyer et al., 2004), and antihemolytic and radical scavenging activities have been characterised in leaf and fruit extracts, suggesting that *A. unedo* leaves are a promising source of natural antioxidants (Mendes, de Freitas, Baptista, & Carvalho, 2011; Oliveira, Coelho, Baltasar, Pereira, & Baptista, 2009). These health promoting properties have been related with the antioxidant activity of the diverse phytochemical profile of the different parts of this plant, which has prompted a renewed interest in this underutilized wild fruit-tree species (Molina, Pardo-de-Santayana, Aceituno, Morales, & Tardío, 2011; Ruiz-Rodríguez et al., 2011), including some efforts for the selection and breeding of genotypes for fruit production and extensive cultivation (Celikel, Demirsoy, & Demirsoy, 2008; Sulusoglu, Cavusoglu, & Erkal, 2011; Molina et al., 2011).

Bioactive compounds characterized in *A. unedo* fruits include terpenoids, organic acids, vitamin C, tocopherols, carotenoids and phenolic compounds such as flavonoids (anthocyanins, proanthocyanidins and flavonols) and phenolic acids (Ayaz, Kucukislamoglu, & Reunanen, 2000; Barros, Carvahlo, Morais, & Ferreira, 2010; Guimarães et al., 2013; Maleš et al., 2006; Pallauf, Rivas-Gonzalo, del Castillo, Cano, & de Pascual-Teresa, 2008; Pawlowska, de Leo, & Braca, 2006). Most of the phytochemical studies have focused on the characterization of the phenolic composition, and despite the striking and attractive colour of the fruits the identification of the responsible pigments has been partially addressed. Whereas the external red colour of the fully mature strawberry tree fruits has been associated to the presence of anthocyanins (Guimarães et al., 2013; Pawlowska et al., 2006), there are few reports about the carotenoid pigments that might be involved in providing the yellow colour of unripe fruits and the intense orange colour of ripe fruits. Prospective analysis carried out

in our laboratory suggested that the carotenoid composition of strawberry tree fruits is not as simple as shown in previous studies. Thus, in the first known work about the carotenoid composition of *A. unedo,* Schön (1935) described the presence of three major carotenoids (violaxanthin, cryptoxanthin and β-carotene) and traces of lycopene and α-carotene, whereas the incomplete characterization performed in other recent studies concluded that β-carotene is major and single carotenoid found in these fruits (Alarcão-E-Silva, Leitão, Azinheira, & Leitão, 2001) or sometimes accompanied by small amounts of lycopene (Barros et al., 2010; Ruiz-Rodríguez et al., 2011) as well as lutein and zeaxanthin (Pallauf et al., 2008). Most of these studies lack of an adequate identification of the reported carotenes and xanthophylls, which underline the necessity of a detailed characterization of the carotenoid profile of strawberry tree fruits.

Plant carotenoids (carotenes and xanthophylls) are C_{40} isoprenoids having a polyene skeleton consisted of a long conjugated double bound system, which constitutes the chromophore responsible for the attracting colour that these pigments conferred to most fruits and vegetables. About 750 carotenoids have been described in nature (Britton, Liaaen-Jensen, & Pfander, 2004). Carotenoids are essential components of the photosynthetic apparatus and are involved in the light harvesting process, as well as in the photo-protection mechanisms of plants. Animals are unable to synthesize *de novo* carotenoids, and therefore they need to be incorporated from the diet, and important biological activities are derived from the ingested carotenoids: antioxidant, provitamin A activity (β-carotene, α-carotene, β-cryptoxanthin, etc), inhibition of carcinogenesis, enhancement of the immune response and cell defence against reactive oxygen species and free radicals, and the reduction on the risk of developing cardiovascular and other degenerative diseases. Epidemiological studies have shown a direct correlation between the progression of age-related macular degeneration (AMD) and cataracts and the high

intake of lutein and zeaxanthin rich-vegetables (Britton, Liaaen-Jensen, & Pfander, 2009)

The growing interest in the role of dietary phytonutrients, such as carotenoids, in human health has increased the searching for new natural sources, including many underutilized wild fruits and vegetables which may serve as biological sinks of phytochemicals with nutritional properties and health benefits (Cortés Sánchez-Mata, Cabrera Loera, Morales, Fernández-Ruiz, Cámara, Díez-Marqués, Pardo-de-Santayana, & Tardío, 2012; Ruiz-Rodríguez, Sánchez-Moreno, de Ancos, Cortés Sánchez-Mata, Fernández-Ruiz, Cámara, & Tardío, 2014; Morales, Ferreira, Carvalho, Fernández-Ruiz, Cortés Sánchez-Mata, Cámara, Morales, & Tardío, 2013). In the case of strawberry tree fruits, very few studies have been performed with the aim of providing information about quality traits (agronomical, morphological, chemical and genetics) to be used in the selection of varieties for extensive cultivation and breeding programs, as well as to improve their commercial value (Sulusoglu, Cavusoglu, & Erkal, 2011; Celikel et al., 2008; Mulas et al., 1998; Takrouni & Boussaid, 2010; Molina et al., 2011). In order to contribute to the phytochemical characterization of strawberry tree fruits, the aim of the present study was to carry out the detailed identification and quantification of the carotenoid pigments responsible for the characteristic yellow-orange colour of the flesh of *A. unedo* fruits. In addition, the effect of the fruit maturation state on the carotenoid composition of fully developed fruits has also been investigated.

2. Materials and methods

2.1. Plant material and sample preparation

Fully mature strawberry tree fruits were collected during the autumn and winter from trees at the Sierra Norte de Sevilla Natural Park (Sevilla, Spain; 37º37'44.3424'': -6º24'22.4316''). In addition fruits at four different stages of ripening (based on the external colour: green, yellow, orange and red) were also harvested (**Table 1**). Samples were taken to the laboratory in the same day of harvesting, frozen in liquid nitrogen and subsequently freeze-dried (lyophilized). The dry samples were ground in a mortar and kept in sealed plastic bags at -80 ºC until the extraction and analysis of pigments. The relative moisture content (%) of the fresh samples was measured in triplicate by using an Ohaus moisture balance model MB35 (Ohaus, Switzerland).

2.2. Chemicals and reagents

HPLC grade acetone, n-heptane, methanol and toluene were supplied by BDH Prolabo (VWR International Eurolab, SL, Barcelona; Spain). HPLC grade deionized water was produced with a Milli-Q 50 system (Millipore Iberica SA, Madrid, Spain). Diethyl ether (containing 7 ppm BHT) and *N,N*-dimethylformamide were purchased from Scharlau (Scharlab, SL, Barcelona, Spain). Heptadecanoic acid, butylated hydroxytoluene (BHT), 2,2-dimethoxypropane, and fatty acid methyl ester (FAME) standard mixtures were purchased from Sigma-Aldrich Quimica, SA (Madrid, Spain). The rest of the reagents were all of analytical grade. Plates of silica gel 60 GF_{254} (20 x 20 cm plates, 0.7 mm thickness; supplied by Merck, Darmstadt, Germany) were used for TLC analysis.

2.3. Extraction of carotenoids

Pigment extraction was performed in accordance with the method developed by Fernandez-Orozco, Gallardo-Guerrero, & Hornero-Méndez (2013). Briefly, 1.5 g of the lyophilized sample was placed in a round-capped polypropylene 50-mL centrifuge tube, and extracted with 20 mL of N,N-dimethylformamide (DMF) saturated with $MgCO_3$ by using an Ultra-Turrax T-25 homogenizer at maximum speed (24,000 rpm) for 1 min. Subsequently, the mixture was centrifuged at 4,500 $\times g$ for 5 min at 4 ºC, and the extracted pigments, contained in the DMF, were collected. The extraction operation was repeated trice more with the pellet, and the DMF fractions were pooled in a separation funnel. Pigments were transferred to diethyl ether (80 mL) by adding 90 mL of 10% (w/v) NaCl solution saturated with $MgCO_3$ at 4 ºC. The mixture was vigorously shaken and allowed to stand until complete separation of phases. The lower phase (aqueous DMF) was re-extracted with diethyl ether (50 mL), and the ether fractions were pooled and washed with 50 mL 2% (w/v) Na_2SO_4 solution. The extract was filtered through a solid bed of anhydrous Na_2SO_4, and the solvent was evaporated under vacuum in a rotary evaporator at 30 ºC. The pigments were dissolved in 1 mL of acetone (containing 0.1% BHT), and an aliquot of 200 µL (direct extract) was kept and stored at -30 ºC for the analysis of xanthophyll esters and chlorophylls. The rest (800 µL) of the sample extract was transferred to a round-capped polypropylene 15-mL centrifuge tube, evaporated under a stream of nitrogen and submitted to a micro-scale saponification procedure (Delgado-Pelayo & Hornero-Méndez, 2012) by the addition of 2 mL of diethyl ether and 1 mL of 10% (w/v) KOH-methanol, which were left to react for 40 min at room temperature under nitrogen atmosphere and with periodic shaking. The organic phase was washed several times with distilled water until the washings were neutral, washed with 2% (w/v) Na_2SO_4 solution and filtered through with an anhydrous Na_2SO_4 bed. The solvent was evaporated to dryness and the dry extract (saponified

extract) was dissolved with 1 mL of acetone for subsequent chromatographic analysis. Samples were centrifuged at 13,000 ×*g* for 5 min at 4 ºC prior to the chromatographic analysis, which it was carried out in the same day of the preparation of the extracts. All operations were made under dimmed light to prevent isomerization and photo-degradation of pigments. Samples were analysed in triplicate (n=3).

2.4. Pigment identification

Routine procedures were used for the identification of pigments (carotenoids and chlorophylls), which consisted of: separation and isolation of pigments by TLC and co-chromatography (TLC and HPLC) with pure standards, analysis of the UV-visible and mass spectra, and comparison with the literature values (Britton, 1991; Britton, 1995; Britton, Liaaen-Jensen, & Pfander, 2004; Mínguez-Mosquera, Gandul-Rojas, Gallardo-Guerrero, Roca, & Jarén-Galán, 2008) and micro-scale chemical test for 5,6-epoxide groups of xanthophylls by controlled treatment with diluted HCl in ethanol following the analysis of the changes in the UV-visible spectrum and the chromatographic mobility. Authentic pigment samples of most pigments used for identification were isolated, and purified by means of TLC, from natural sources: (9′*Z*)-neoxanthin, (all-*E*)-violaxanthin, (all-*E*)-lutein, (all-*E*)-β-carotene, chlorophyll *a* and chlorophyll *b* were obtained from spinach leaves (*Spinacea oleracea* L.), and (all-*E*)-β-cryptoxanthin and (all-*E*)-zeaxanthin were isolated from red pepper fruits (*Capsicum annuum* L.) (Mínguez-Mosquera & Hornero-Méndez, 1993). (all-*E*)-Antheraxanthin and lutein 5,6-epoxide were prepared by the epoxidation with 3-chloroperoxybenzoic acid of zeaxanthin and lutein. For identification purposes, carotenoids containing 5,8-epoxide groups (auroxanthin, luteoxanthin, neochrome and mutatoxanthin) were prepared from

the corresponding 5,6-epoxide parent pigments (violaxanthin, neoxanthin and antheraxanthin) (Britton, 1991).

The identification of *Z* isomers was based on the presence and relative intensity ($\%A_B/A_{II}$) of the "*cis* peak" at about 330-340 nm in UV-visible spectrum, a reduction in the fine structure and a small hypsochromic shift in λ_{max} with respect to the all-*E* counterpart, and the chromatographic behaviour in the C18 HPLC column (the *Z* isomers show slightly longer retention times than the all-*E* isomer) (Britton, 1995).

The fragmentation pattern obtained by means of mass spectrometry linked to liquid chromatography (LC-MS (APCI+)) was used for the structural assignment of the major carotenoid esters, including the identification of the fatty acid moiety. The APCI (positive mode) mass spectra of xanthophyll acyl esters are characterized for presenting high abundant fragments corresponding to the neutral loss of the fatty acid moiety allowing the identification of the esterification nature (Breithaupt and Schwack, 2000; Mellado-Ortega and Hornero-Méndez, 2012; Rivera, Christou, and Canela-Garayoa, 2013; Delgado-Pelayo, Gallardo-Guerrero and Hornero-Mendez, 2014; Delgado-Pelayo and Hornero-Mendez, 2012).

2.5. Quantitative HPLC analysis of pigments

The HPLC method developed by Mínguez-Mosquera and Hornero-Méndez (1993), with some minor modifications, was used for the quantitative analysis of chlorophylls and carotenoids (Delgado-Pelayo and Hornero-Méndez, 2012). The chromatographic system consisted of a Waters e2695 Alliance chromatograph linked to a Waters 2998 photodiode array detector (DAD), and controlled with Empower2 software (Waters Cromatografía, SA, Barcelona, Spain). An analytical reversed-phase C18 column (200 mm × 4.6 mm i.d., 3 μm, Mediterranea SEA18; Teknokroma,

Barcelona, Spain), fitted with a guard column of the same material (10 mm × 4.6 mm), was used. Separation was achieved by a binary-gradient elution using an initial composition of 75% acetone and 25% deionised water, which was increased linearly to 95% acetone in 10 min, then hold for 7 min and raised to 100% in 3 min, and maintained constant for 10 min. Initial conditions were reached in 5 min. Column and sample compartments were maintained at 25 ºC and 15 ºC, respectively. An injection volume of 10 μL and a flow rate of 1 mL/min were used. Detection was performed at 450 nm, and the online spectra were acquired in the 330-700 nm wavelength range with a resolution of 1.2 nm. Detection was performed simultaneously at 402, 430, 450, 645 and 666 nm, and the online spectra were acquired in the 330-700 nm wavelength range with a resolution of 1.2 nm. Chlorophylls and esterified xanthophylls were quantitated in the direct extracts, whereas individual carotenoids were quantitated in the saponified extract. Calibration curves (up to eight concentration levels) were prepared with standard stock solutions for each carotenoid and chlorophyll in the concentration range 5-100 μg/mL. Calibration curves were constructed by plotting the peak area (at 402 nm for auroxanthin; at 450 nm for lutein, zeaxanthin, β-cryptoxanthin, violaxanthin, neoxanthin, neochrome and β-carotene; at 645 nm for chlorophyll *b*; at 666 nm for chlorophyll *a*) versus the pigment concentration. Antheraxanthin and luteoxanthin were quantified by using the calibration curves of zeaxanthin and neochrome, respectively. The concentration of xanthophyll ester fractions (sum of partially and totally esterified) was estimated by using the calibration curve of free violaxanthin due to the main occurrence of this pigment within the esterified fraction as deducted from the online UV-visible spectra (the esterification of xanthophylls with fatty acids does not modify the chromophore properties; Britton, 1995). Analogously, the quantification of the *Z* isomers was performed by using the calibration curve of the all-*E* counterpart.

Concentration values were calculated as µg of pigment per gram of dry sample weight (µg/g dw).

2.6. Liquid Chromatography - Mass Spectrometry (LC-MS (APCI+))

LC-MS was performed with the same chromatographic system (HPLC-DAD) described above, by coupling a Micromass ZMD4000 mass spectrometer equipped with a single quadrupole analyzer (Micromass Ltd, Manchester, United Kingdom) and an APCI (Atmospheric Pressure Chemical Ionisation) probe to the outlet of the DAD detector. The system was controlled with MassLynx 3.2 software (Micromass Ltd, Manchester, United Kingdom). The mass spectrometer condition parameters were: positive ion mode (APCI+); source temperature, 150 ºC; probe temperature, 400 ºC; corona voltage, 3.7 kV; high voltage lens, 0.5 kV; and cone voltage, 30 V. Nitrogen was used as the desolvation and cone gas at 300 and 50 L/h, respectively. Mass spectra were acquired within the *m/z* 300-1200 uma range. The chromatographic conditions were the same as described for quantitative analysis of pigments.

2.7. Analysis of fatty acids by Gas Chromatography

Fatty acids methyl esters (FAMEs) were prepared for the analysis of fatty acid profile of strawberry tree fruits. Extraction and gas chromatographic analysis of FAMEs was carried out according to the method of Garcés & Mancha (1993). Briefly, 0.5 g of lyophilized sample was placed in a glass tube with Teflon caps together and 100 µL of heptadecanoic acid (C17:0) 20.0 mg/mL were added as internal standard. Subsequently, 1.65 mL of methanol/toluene/2,2-dimethoxypropane/H_2SO_4 (39:20:5:2, by vol.) and 0.85 mL of heptane (containing 0.05% BHT) were added and the mixture was flushed with nitrogen and heated at 80 ºC during 2 h. After cooling to room temperature, 0.5 mL

of the upper organic phase was transferred to a glass vial for GC analysis. FAMEs were separated on a Supelcowax 10 fused silica capillary column (30 m length; 0.32 mm i.d.; 0.25 μm film thickness) (Sigma-Aldrich Química, S.A., Madrid, Spain) by using an Agilent Technologies 7890A gas chromatograph (Agilent Technologies España, S.L., Madrid, Spain) fitted with a flame ionization detection (FID), a split/splitless injector and a 7683B Series automatic liquid sampler. Helium was used as carrier gas with a constant linear flow of 1.75 mL/min. The injector and detector temperature were 250 and 260 °C, respectively. The oven temperature program started at 145 °C, increasing with a ramp of 15°C/min to 230 °C with a 10 min hold. Injection volume was 1 μL at a split ratio of 1:50. Fatty acids were identified by comparison with known standards. Quantitative determination was performed using the area of the internal standard. Analyses were carried out in triplicate.

2.8. Statistical analysis

The compositional data were expressed as mean and standard deviation (SD). The existence of significant differences between means was determined by one-way ANOVA, followed by a *post-hoc* test of mean comparison with the Tukey's Honestly Significant Difference (HSD) test for a confidence level of 95% ($p<0.05$) by using the STATISTICA 6.0 software (StatSoft Inc.).

3. Results and discussion

3.1. Characterization of the carotenoid profile of *A. unedo* fruits

The carotenoid pigments responsible for the intense yellow-orange colour of the flesh of the strawberry tree fruits have been characterized and identified according to their chemical, chromatographic and spectroscopic properties (UV-visible and mass

spectroscopic). **Figure 1** shows the reversed phase C18 HPLC chromatograms corresponding to the direct (A) and the saponified (B) carotenoid extracts obtained from mature fruits. The disappearance upon saponification of the long retained peaks and the appearance of new major peaks, and the increase of the area of some others, at shorter retention times revealed the presence of xanthophyll acyl esters in the direct extract. Previous works on other fruit and vegetables have demonstrated that the chromatographic analysis, under reverse-phase conditions, of a direct carotenoid extract containing xanthophyll esters can be resolved in three major fractions with different esterification degree, namely free, partially and totally esterified xanthophylls, respectively (Fernandez-Orozco, Gallardo-Guerrero, & Hornero-Méndez, 2012; Delgado-Pelayo & Hornero-Méndez, 2012). The partially esterified xanthophylls (monoesters in the case of a dihydroxy-xanthophyll, i.e. lutein) acylated with common saturated fatty acids, such as lauric, myristic, palmitic and estearic acids, have shorter retention times than β-carotene, and the totally esterified forms (monoester for a monohydroxy-xanthophyll - i.e. β-cryptoxanthin - and diester for a dihydroxy-xanthophyll), are more retained than β-carotene due to their lower polarity and stronger interactions with the stationary phase. The identification of individual free carotenoids was carried out in a first instance in the saponified extracts, and in a second phase the esterified forms of the xanthophylls, and the presence of some chlorophylls, were investigated in the direct extracts (non-saponified) in order to determine and characterise for the first time the native carotenoid composition in fruits of *A. unedo*. **Table 2** summarises the identification for each major chromatographic peak (see **Figure S3** in the Supplementary material for the corresponding chemical structures). In total fifteen carotenoids, including some geometrical *cis-trans* (*Z*/*E*) isomers, were identified in the saponified extract.

Peaks **1** (Rt=5.81 min) and **2** (Rt=6.10 min) were identified as (all-*E*)-neoxanthin and (9'*Z*)-neoxantin, respectively. The UV-visible spectrum of peak **1**, with λ_{max} at 419, 444, 472 nm, and the characteristic pronounced fine structure (%III/II=99) was in agreement with a chromophore consisted of nine conjugated double bonds and at least one β-ring, and data reported in the literature (Britton, 1995). In contrast, peak **2** presented an UV-visible spectrum with maxima shifted to lower values (hypsochromic shift), a less marked fine structure and the appearance of a `*cis* peak´ at 328 nm (328, 415, 439, 468 nm; %III/II=91). Both peaks disappeared upon treatment of the saponified extract with diluted HCl (epoxide test), with the subsequent formation of a new peak, corresponding to neochrome (peak **3**; Rt=6.53 min), generated by the acid-catalyzed rearrangement of one 5,6-epoxy group. Neochrome was detected in the saponified extract at very low levels. The UV-visible spectrum of neochrome showed λ_{max} at 402, 424, 452 nm, corresponding to a hypsochromic shift of about 12-16 nm with respect to the maxima of neoxanthin, and consequently in agreement with the presence of only one 5,6-epoxy group in its structure. The LC/MS (APCI+) spectra of peaks **1** and **2** showed a protonated molecule $[M+H]^+$ at *m/z* 601, which was consistent with the molecular formula $C_{40}H_{56}O_4$ (Mw=601.4257). The presence of various less abundant fragments at *m/z* 583 $[M+H-18]^+$, 565 $[M+H-18-18]^+$ and 547 $[M+H-18-18-18]^+$, derived from the neutral losses of waters molecules, confirmed the presence of three hydroxyl groups. In addition, the fragment at *m/z* 521 $[M+H-80]^+$ was in agreement with the presence of one 5,6-epoxy group (Britton, 1991), which was confirmed with the positive result of the chemical test for 5,6-epoxy groups. The identification of peak **1** was supported by the co-chromatography with an extract prepared from potato tubers in which the presence of (all-*E*)-neoxanthin has been

previously demonstrated (Fernandez-Orozco, Gallardo-Guerrero, & Hornero-Méndez, 2013), whereas peak **2** co-eluted with (9'*Z*)-neoxantin isolated from spinach leaves.

Peaks **4** (Rt=7.28 min) and **5** (Rt=8.03 min) were assigned as (all-*E*)-violaxanthin and (9*Z*)-violaxantin, respectively. The identity of (all-*E*)-violaxanthin was confirmed by co-chromatography with the standard isolated from spinach leaves, as well as by the comparison and matching of their spectroscopic properties, whereas the identification of (9*Z*)-violaxantin was demonstrated, as in the case of (all-*E*)-neoxanthin, by co-chromatography with carotenoid extracts prepared from potato tubers and apple fruits (Fernandez-Orozco, Gallardo-Guerrero, & Hornero-Méndez, 2013; Delgado-Pelayo, Gallardo-Guerrero, & Hornero-Méndez, 2014). The UV-visible spectra for peaks **4** and **5** were in accordance with data reported in the literature (Britton, 1995; Meléndez-Martínez, Vicario, & Heredia, 2007). The LC/MS (APCI+) spectra of (all-*E*)-violaxanthin and (9*Z*)-violaxantin presented a protonated molecule ion $[M+H]^+$ at *m/z* 601 [$C_{40}H_{56}O_4$ (Mw=601.4257)] and fragments at *m/z* 583 $[M+H-18]^+$ and 565 $[M+H-18-18]^+$ produced from the neutral losses of one and two molecules of water, respectively, which confirmed the presence of two hydroxyl groups. In addition, as described before for neoxanthin, the occurrence of fragments at *m/z* 521 $[M+H-80]^+$ indicates the presence of at least one 5,6-epoxy group (Britton, 1991). The epoxide test for both chromatographic peaks derived in the formation of new ones generated by the acid-catalyzed rearrangement of the 5,6-epoxy groups, which showed the characteristic hypsochromic shift in the UV-visible spectra (Eugster, 1995). Taking into consideration the chromatographic and spectroscopic properties, peak **6** (Rt=8.17 min) was identified as luteoxanthin, whereas peak **8** (Rt=8.80 min) was assigned as auroxanthin. As in the case of neochrome, luteoxanthin and auroxanthin were detected at trace levels in the saponified extract.

Peak **7** (Rt=8.33 min) was identified as (all-*E*)-antheraxanthin. The UV-visible spectrum, with λ_{max} at 424, 448, 476 nm (%III/II=66), was in agreement with a chromophore consisted of nine conjugated double bonds and at least one β-ring. In addition, when the pigment extract was treated with diluted HCl (epoxide test) the resulting HPLC chromatogram (data not shown) revealed the disappearance of antheraxanthin and the formation of two peaks corresponding to C8-epimers (8*R* and 8*S*) of mutatoxanthin (5,8-epoxy-5,8-dihydro-β,β-carotene-3,3´-diol), which is the 5,8-epoxide derivative of antheraxanthin generated by the acid-catalyzed rearrangement of the 5,6-epoxy group. Mutatoxanthin-epimers showed UV-visible spectrum with λ_{max} at 408, 430, 456 nm, corresponding to a hypsochromic shift of about 20 nm, which indicated the presence of one 5,6-epoxy group (Eugster, 1995). Mutatoxanthin epimers were not found at detectable levels in the saponified extract. The MS(APCI+) spectrum for antheraxanthin was characterized by a major ion for the protonated molecule $[M+H]^+$ at *m/z* 585, which was consistent with the formula $C_{40}H_{56}O_3$ (Mw=584.4229). Other fragments at *m/z* 567 $[M+H-18]^+$ and 549 $[M+H-18-18]^+$ confirmed the presence of two hydroxyl groups and, as previously shown for neoxanthin and violaxanthin, the fragment at *m/z* 505 $[M+H-80]^+$ was in agreement with the presence of at least one 5,6-epoxy group (Britton, 1991). The co-elution of peak **7** with the authentic sample of antheraxanthin confirmed the identification.

Peak **9** was tentatively identified as lutein 5,6-epoxide by means of co-chromatography with the standard prepared in the laboratory and the comparison of the spectroscopic properties with the data reported in the literature.

The chemical, chromatographic and spectroscopic properties of peak **10** (Rt=9.35 min) were in agreement with those for (all-*E*)-zeaxanthin. The UV-visible spectrum, with λ_{max} at 428, 454, 481 nm, and a low spectroscopic fine structure

(%III/II=18), indicated a chromophore structure comprised by nine conjugated double bonds and two β-ring (Britton, 1995). The MS spectrum was dominated by an abundant ion corresponding to the protonated molecule $[M+H]^+$ at *m/z* 569, which is in agreement with the $C_{40}H_{56}O_2$ formula (Mw=568.4280). The presence of additional fragments at *m/z* 551 $[M+H-18]^+$ and 533 $[M+H-18-18]^+$ confirmed the existence of two hydroxyl groups in the pigment structure. The identity of this compound was confirmed by its co-elution with an authentic sample of zeaxanthin.

(all-*E*)-Lutein (peak **11,** Rt=9.56 min) was in first instance identified by co-chromatography with a standard lutein sample isolated from spinach leaves. The UV-visible spectrum showed λ_{max} at 423, 450, 476 nm and a marked spectroscopic fine structure (%III/II=70), in accordance with a chromophore with nine conjugated double bonds and presumably one β-ring and one ε-ring (Britton, 1995). As shown in previous works (Mellado-Ortega & Hornero-Méndez, 2012), the APCI(+) mass spectrum of lutein can be used for the unambiguous structural determination of this compound, being characterized by the presence of a protonated molecule ($[M+H]^+$) at *m/z* 569 with low intensity (10-20%), whereas the most abundant ion (100%) appeared at *m/z* 551, which is produced by the neutral loss of a water molecule ($[M+H-18]^+$) from the hydroxy group at position 3´ of the ε-ring. The subsequent additional loss of a second water molecule from the hydroxy group from the carbon 3 of the β-end ring derived in a fragment at *m/z* 533 ($[M+H-18-18]^+$) with very low intensity (1-3%). Peaks **14** and **15** (Rt=10.11 and 10.22 min) were tentatively assigned as geometric isomers of lutein ((9*Z*)- and (13*Z*)-lutein, respectively), as deducted from their UV-visible spectra, with lower %III/II and slightly shorter λ_{max} when compared with the all-*E* relative compounds, together with the occurrence of a "*cis* peak" at around 330 nm. The

characteristics of their mass spectra were in agreement with the fragmentation pattern described for (all-*E*)-lutein.

The preliminary structural assignment of (all-*E*)-β-cryptoxanthin for peak **16** (Rt=14.13 min) was based on the HPLC co-elution with an authentic standard isolated from red pepper. The on-line UV-visible spectrum was characterised by λ_{max} at 428, 454, 480 nm (%III/II=18), suggesting a chromophore consisted of nine conjugated double bonds and two β-rings. The mass spectrum was characterized by a protonated molecule $[M+H]^+$ at *m/z* 553 ($C_{40}H_{56}O$) and a fragment at *m/z* 535 $[M+H-18]^+$ which is in agreement with the presence of only one hydroxy group.

(all-*E*)-β-Carotene (peak **17**; Rt=20.68 min) was identified straightforward by comparing, both the chromatographic and the spectroscopic properties with the standard samples and the literature data (Britton, 1995; Britton, Liaaen-Jensen, & Pfander, 2004). The mass spectrum showed the expected protonated molecule $[M+H]^+$ at *m/z* 537, which is in agreement with the $C_{40}H_{56}$ formula, and the typical fragment corresponding to the in-chain loss of toluene ($[M+H-92]^+$) at *m/z* 445. β-Carotene presented an UV-visible spectrum with λ_{max} at 427, 454, 479 nm and a low fine structure (%III/II=16), which is accordance with a chromophore with nine conjugated double bonds and two β-rings.

The quantitative analysis of mature *A. unedo* fruits (**Table 2**) revealed a total carotenoid content of 343.4 ± 17.8 µg/g dw with (all-*E*)-violaxanthin and (9*Z*)-violaxanthin as the major carotenoids (123.5 ± 5.9 and 87.8 ± 5.7 µg/g dw, respectively), accounting for more than 60%, followed by other 5,6-epoxide-containing xanthophylls such as (all-*E*)-neoxanthin (26.4 ± 1.8 µg/g dw), (9'*Z*)-neoxantin (24.6 ± 1.9 µg/g dw), (all-*E*)-antheraxanthin (18.9 ±1.5 µg/g dw) and lutein 5,6-epoxide (20.5 ± 0.9 µg/g dw). Nutritionally important carotenoids such as lutein, zeaxanthin and β-

carotene were found also found at important concentration levels (5-20 μg/g dw). Xanthophylls with 5,8-epoxide (furanoid) groups, such as neochrome, luteoxanthin, auroxanthin and mutatoxanthin were observed at very low concentration levels or even not detected. Therefore, according to Britton and Khachik (2009) the mature fruits of *A. unedo* can be classified as a very high (> 2 mg/100g) or high (0.5-2 mg/100g) carotenoid-containing natural source, at both individual and total carotenoid contents. As stated in the introduction these data are much higher than those from previous works (Barros et al., 2010; Pallauf et al., 2008; Ruiz-Rodríguez et al., 2011) in which the total carotenoid concentration ranged from 0.5 to 11 μg/g fw (1.2-27.5 μg/g dw, assuming a moisture content of 60%). A partial extraction of carotenoid pigments together with an inadequate characterization of the individual profile seems to be the reason for these lower values.

3.2. Identification of esterified xanthophylls

As deducted from the chromatogram corresponding to the direct extract, the main native state of the xanthophylls in *A. unedo* fruits was esterified, especially as totally esterified. The structural assignment of the carotenoid esters, including the acyl moiety, was performed by liquid chromatography mass spectrometry (LC-DAD-MS(APCI+)) analysis. The acylation of a xanthophyll with fatty acids does not modify the chromophore and its light absorption properties, and therefore the UV-visible spectrum of the acylated carotenoid is identical to the spectrum of the free pigment (Britton, 1995). Thus the combined information provided by the on-line UV-visible spectrum, obtained with the DAD detector, and the MS(APCI+) fragmentation pattern allowed the identification of the major xanthophyll esters in the *A. unedo* fruits. As an example, **Figure 2** shows the MS(APCI+) spectra for (all-*E*)-violaxanthin

monomyristate and (all-*E*)-violaxanthin dimyristate. The mass spectra of these compounds have in common the presence a major ion corresponding to the protonated molecule $[M+H]^+$. In the case of the monoester, the molecular ion is accompanied by other abundant fragments derived the neutral loss of a fatty acid moiety $[M+H\text{-fatty acid}]^+$ (myristic acid in this example, Mw=228.21) from one end (position 3) of the xanthophyll molecule, and another from the neutral loss of water $[M+H-18]^+$ from hydroxy group at position 3´. In the case of the diester, the APCI+ mass spectrum comprised the molecular ion together with a prominent fragment corresponding to the loss of the one fatty acid moiety at either end of the xanthophyll ester molecule. A fragment at *m/z* 565 was observed for monoesters ($[M+H\text{-18-myristic}]^+$) and diesters ($[M+H-2\times\text{myristic}]^+$), corresponding to the backbone of the carotenoid after the neutral loss of the fatty acid or waters molecules from both ends of the molecule. Surprisingly, neutral loss of water was also observed in the diester APCI+ mass spectrum as accompanying fragments for the main ions ($[M+H-18]^+$; $[M+H\text{-myristic-18}]^+$; $[M+H-2\times\text{myristic-18}]^+$). The absence of free hydroxyl groups suggested that the fragmentation of the 5,6-epoxide group might be the origin of this additional ions which are difficult to be deducted from the MS spectrum of free violaxanthin.

As observed in **Figure 1**, (all-*E*)-violaxanthin and (9*Z*)-violaxanthin were the major esterified xanthophylls, whereas lutein, neoxanthin, zeaxanthin and β-cryptoxanthin esters were at lower relative concentrations. The fatty acid involved in the esterification of the xanthophylls were saturated ones, mostly capric (C10:0), lauric (C12:0), myristic (C14:0) and palmitic (C18:0) acids. In contrast, the GC analysis of the fatty acid profile of *A. unedo* fruits (see Supplementary material) revealed that the total lipid pool was mainly composed by unsaturated fatty acids (linolenic acid, 30.9%; oleic acid, 24.6%; linoleic acid, 24.4%) and, with the exception of palmitic (10.7%) and

stearic acids (5.3%), the saturated fatty acids were present at very low levels (capric acid, trace levels; lauric acid, 0.4%; myristic acid, 2.6%; eicosanoic acid, 0.7%; gadoleic acid, 0.4%). In agreement with previous works, the lack in correlation between the fatty acid profiles of the total lipid pool and the acyl moieties of the xanthophyll esters suggested the high selectivity of the enzymes involved in this process (xanthophyll acyl transferases; XAT) (Breithaupt & Schwack, 2000; Delgado-Pelayo & Hornero-Méndez, 2012; Mellado-Ortega & Hornero-Méndez, 2012; Delgado-Pelayo, Gallardo-Guerrero & Hornero-Méndez, 2014). Although the individual xanthophyll esters were not quantified, the relative abundance of the different peaks in the direct chromatogram allowed reporting that the main representatives of the partially esterified fraction were (all-*E*)-violaxanthin monolaurate, (9*Z*)-violaxanthin monolaurate, (all-*E*)-violaxanthin monomyristate, (9*Z*)-violaxanthin monomyristate, (all-*E*)-violaxanthin monopalmitate and (9*Z*)-violaxanthin monopalmitate. Analogously, (all-*E*)-violaxanthin dilaurate, (9*Z*)-violaxanthin dilaurate, (all-*E*)-violaxanthin laurate-myristate, (9*Z*)-violaxanthin laurate-myristate, (all-*E*)-violaxanthin dimyristate, (9*Z*)-violaxanthin dimyristate, (all-*E*)-violaxanthin myristate-palmitate, (9*Z*)-violaxanthin myristate-palmitate, (all-*E*)-violaxanthin dipalmitate, and (9*Z*)-violaxanthin dipalmitate were the major constituents of the totally esterified fraction.

3.3. Effect of ripening on the carotenoid profile

Given the fact that the *A. unedo* fruits take the whole year to ripen, the tree bears fruits at different growing and ripening stages at the same time. In order to investigate the effect of ripening on the carotenoid profile of fully developed fruits, berries at four maturation stage according to external colour (green, yellow, orange and red) were harvested and analysed. As summarised in **Table 4**, whereas the chlorophyll pigments

content markedly and progressively decreased from the green to red stage, the carotenoid concentration remained constant ($p<0.05$) for the four ripening stages. Xanthophylls were mostly in the esterified state, and the degree of esterification increased throughout the ripening process from 88 to 95 % for the green and red fruits, respectively. Analogously, the ratio between totally and partially esterified xanthophylls increased from 4.3 to 12.5, for the green and red fruits, respectively, indicating an active esterification process.

4. Conclusions

The carotenoid composition from strawberry tree (*A. unedo*) fruits has been identified in detail and quantified for the first time. The carotenoid profile was mainly composed by violaxanthin (all-*E* and 9*Z* isomers) and neoxanthin (all-*E* and 9'*Z* isomers), followed by lower amounts of antheraxanthin, lutein, zeaxanthin, β-cryptoxanthin and β-carotene. The analysis of the xanthophyll fractions in their native state (direct extract), by means of LC-MS (APCI+), revealed that most of them were totally esterified with saturated fatty acids (capric, lauric, myristic, palmitic and stearic).

Acknowledgements

This work was supported by funding from the Ministerio de Ciencia e Innovación (Spanish Government, Project AGL2010-14850/ALI) and the Consejería de Economía, Innovación, Ciencia y Empleo (Junta de Andalucía, Project P08-AGR-03477). Raúl Delgado Pelayo holds a predoctoral grant from Junta de Andalucía (Proyecto de Excelencia P08-AGR-3477). Authors are members of the IBERCAROT Network, funded by CYTED (ref. 112RT0445).

References

Alarcão-E-Silva, M. L. C. M. M., Leitão, A. E. B., Azinheira, H. G., & Leitão, M. C. A. (2001). The arbutus berry: Studies on its color and chemical characteristics at two mature stages. *Journal of Food Composition and Analysis*, *14*, 27–35.

Ayaz, F. A., Kucukislamoglu, M., & Reunanen, M. (2000). Sugar, non-volatile and phenolic acids composition of strawberry tree (*Arbutus unedo* L. var. *ellipsoidea*) fruits. *Journal of Food Composition and Analysis*, *13*, 171–177.

Barros, L., Carvahlo, A. M., Morais, J. S., & Ferreira, I. C. F. R. (2010). Strawberry-tree, blackthorn and rose fruits: Detailed characterisation in nutrients and phytochemicals with antioxidant properties. *Food Chemistry*, *120*, 247–254.

Breithaupt, D. E., & Schwack, W. (2000). Determination of free and bound carotenoids in paprika (*Capsicum annuum* L.) by LC/MS. *European Food Research and Technology, 211*, 52–55.

Britton, G. (1991). Carotenoids. In P. M. Dey, & J. B. Harborne (Eds.), *Methods in Plant Biochemistry*. (pp. 473-518). London: Academic Press.

Britton, G. (1995). UV/visible spectroscopy. In G. Britton, S., Liaaen-Jensen, & H. Pfander (Eds.), *Carotenoids. Volume 1B: Spectroscopy* (pp. 13–62). Basel: Birkhäuser Verlag.

Britton, G., & Khachik, F. (2009). Carotenoids in Food. In G. Britton, S. Liaaen-Jensen, & H. Pfander, *Carotenoids. Volume 5: Nutrition and Health* (pp. 45-66). Basel: Birkhäuser Verlag.

Britton, G., Liaaen-Jensen, S., & Pfander, H. (2004). *Carotenoid Handbook*. Basel: Birkhäuser Verlag.

Britton, G., Liaaen-Jensen, S., & Pfander, H. (2009). *Carotenoids*. *Volume 5*: *Nutrition and Health*. Basel: Birkhäuser Verlag.

Celikel, G., Demirsoy, L., & Demirsoy, H. (2008). The strawberry tree (*Arbutus unedo* L.) selection in Turkey. *Scientia Horticulturae*, *118*, 115–119.

Delgado-Pelayo, R., Gallardo-Guerrero, L., & Hornero-Méndez, D. (2014). Chlorophyll and carotenoid pigments in the peel and flesh of commercial apple fruits varieties. *Food Research International*, *65*, 272–281.

Delgado-Pelayo, R., & Hornero-Méndez, D. (2012). Identification and quantitative analysis of carotenoids and their esters from sarsaparrilla (*Smilax aspera* L.) berries. *Journal of Agricultural and Food Chemistry*, *60*, 8225–8232.

El Haouari, M., López, J. J., Mekhfi, H., Rosado, J. A., & Salido, G. M. (2007). Antiaggregant effects of *Arbutus unedo* extracts in human platelets. *Journal of Ethnopharmacology*, *113*, 325–331.

Fernandez-Orozco, R., Gallardo-Guerrero, L., & Hornero-Méndez, D. (2013). Carotenoid profiling in tubers of different potato (*Solanum* sp) cultivars: Accumulation of carotenoids mediated by xanthophyll esterification. *Food Chemistry*, *141*, 2864–2872.

Fortalezas, S., Tavares, L., Pimpão, R., Tyagi, M., Pontes, V., Alves, P., McDougall, G., Stewart, D., Ferreira, R., & Santos, C. (2010). Antioxidant properties and neuroprotective capacity of strawberry tree fruit (*Arbutus unedo*). *Nutrients*, *2*, 14–229.

Garcés, R., & Mancha, M. (1993). One-step lipid extraction and fatty acid methyl esters preparation from fresh plant tissues. *Analytical Biochemistry, 211,* 139-143.

Guimarães, R., Barros, L., Dueñas, M., Carvalho, A. M., Queiroz, M. J., Santos-Buelga, C., & Ferreira, I. C. (2013). Characterisation of phenolic compounds in wild fruits from Northeastern Portugal. *Food Chemistry*, *141*, 3721–3730.

Legssyer, A., Ziyyat, A., Mekhfi, H., Bnouham, M., Herrenknecht, C., Roumy, V., Fourneau, C., Laurens, A., Hoerter, J., & Fischmeister, R. (2004). Tannins and catechin gallate mediate the vasorelaxant effect of *Arbutus unedo* on the rat isolated aorta. *Phytotherapy Research*, *18*, 889–894.

Maleš, Z., Plazibat, M., Vundać, V. B., & Žuntar, I. (2006). Qualitative and quantitative analysis of flavonoids of the strawberry tree – *Arbutus unedo* L. (Ericaceae). *Acta Pharmaceutica*, *56*, 245–250.

Meléndez-Martínez, A. J., Vicario, I. M., & Heredia, F. J. (2007). Geometrical isomers of violaxanthin in orange juice. *Food Chemistry*, *104*, 169–175.

Mendes, L., de Freitas, V., Baptista, P., & Carvalho, M. (2011). Comparative antihemolytic and radical scavenging activities of strawberry tree (*Arbutus unedo* L.) leaf and fruit. *Food and Chemical Toxicology*, *49*, 2285–2291.

Mellado-Ortega, E., & Hornero-Méndez, D. (2012). Isolation and identification of lutein esters, including their regioisomers, in tritordeum (×*Tritordeum* ascherson et graebner) grains. Evidences for a preferential xanthophyll acyltransferase activity. *Food Chemistry, 135*, 1344–1352.

Miguel, M., Faleiro, M., Guerreiro, A., & Antunes, M. (2014). *Arbutus unedo* L.: Chemical and biological properties. *Molecules*, *19*, 15799–15823.

Mínguez-Mosquera, M. I., Gandul-Rojas, B., Gallardo-Guerrero, L., Roca, M., & Jarén-Galán, M. (2008). Chlorophylls. In W. J. Hurst (Ed.), *Methods of analysis for functional foods and neutraceuticals. 2nd Ed.* (pp. 237-400). Boca Raton, FL, USA: CRC Press LLC.

Mínguez-Mosquera, M. I., & Hornero-Méndez, D. (1993). Separation and quantification of the carotenoid pigments in red peppers (*Capsicum annuum* L.),

paprika, and oleoresin by reversed-phase HPLC. *Journal of Agricultural and Food Chemistry*, *41*, 1616–1620.

Molina, M., Pardo-de-Santayana, M., Aceituno, L., Morales, R., & Tardío, J. (2011). Fruit production of strawberry tree (*Arbutus unedo* L.) in two Spanish forests. *Forestry*, *84*, 419–429.

Morales, P., Ferreira, I. C. F. R., Carvalho, A. M., Fernández-Ruiz, V., Sánchez-Mata, M. C., Cámara, M., Morales, R., & Tardío, J. (2013). Wild edible fruits as a potential source of phytochemicals with capacity to inhibit lipid peroxidation. *European Journal of Lipid Science and Technology*, *115*, 176–185.

Mulas, M., Cani, M.R., Brigaglia, N., & Deidda, P. (1998). Varietal selection in wild populations for the cultivation of myrtle and strawberry tree in Sardinia. *Rivista di Frutticoltura e di Ortofloricoltura*, *60*, 45–50.

Oliveira, I., Baptista, P., Bento, A., & Pereira, J. A. (2011). *Arbutus unedo* L. and its benefits on human health. *Journal of Food and Nutrition Research*, *50*, 73–85.

Oliveira, I., Coelho, V., Baltasar, R., Pereira, J. A., & Baptista, P. (2009). Scavenging capacity of strawberry tree (*Arbutus unedo* L.) leaves on free radicals. *Food and Chemical Toxicology*, *47*, 1507–1511.

Pallauf, K., Rivas-Gonzalo, J. C., del Castillo, M. D., Cano, M. P., & de Pascual-Teresa, S. (2008). Characterization of the antioxidant composition of strawberry tree (*Arbutus unedo* L.) fruits. *Journal of Food Composition and Analysis*, *21,* 273–281.

Pawlowska, A. M., de Leo, M., & Braca, A. (2006). Phenolics of *Arbutus unedo* L. (Ericaceae) fruits: Identification of anthocyanins and gallic acid derivates. *Journal of Agricultural and Food Chemistry*, *54*, 10234–10238.

Rivera, R. M., Christou, P., & Canela-Garayoa, R. (2013). Identification of carotenoids using mass spectrometry. *Mass Spectrometry Review*, doi: 10.1002/mas.21390.

Ruiz-Rodríguez, B.-M., Morales, P., Fernández-Ruiz, V., Sánchez-Mata, M.-C., Cámara, M., Díez-Marqués, C., Pardo-de-Santayana, M., Molina, M., & Tardío, J. (2011). Valorization of wild strawberry-tree fruits (*Arbutus unedo* L.) through nutritional assessment and natural production data. *Food Research International*, *44*, 1244–1253.

Ruiz-Rodríguez, B. M., Sánchez-Moreno, C., De Ancos, B., Cortes Sánchez-Mata, M. De, Fernández-Ruiz, V., Cámara, M., & Tardío, J. (2014). Wild *Arbutus unedo* L. and *Rubus ulmifolius* Schott fruits are underutilized sources of valuable bioactive compounds with antioxidant capacity. *Fruits*, *69(6)*, 435–448.

Sánchez-Mata, M. C., Loera, R. D. C., Morales, P., Fernández-Ruiz, V., Cámara, M., Marqués, C. D., Pardo-de-Santayana, M., & Tardío, J. (2012). Wild vegetables of the Mediterranean area as valuable sources of bioactive compounds. *Genetic Resources and Crop Evolution*, *59*, 431–443.

Schön, K. (1935). Studies on carotenoids. I. The carotenoids of diospyros fruits. II. The carotenoids of arbutus fruits (*Arbutus unedo*). *Biochemistry Journal*, *29*, 1779–1785.

Soufleros, E. H., Mygdalia, S. A., & Natskoulis, P. (2005). Production process and characterisation of the traditional Greek fruit distillate 'Koumaro' by aromatic and mineral composition. *Journal of Food Composition and Analysis*, *18*, 699–716.

Sulusoglu, M., Cavusoglu, A., & Erkal, S. (2011). *Arbutus unedo* L. (Strawberry tree) selection in Turkey Samanli mountain locations. *Journal of Medicinal Plants Research*, *5*, 3545–3551.

Takrouni, M. M., & Boussaid, M. (2010). Genetic diversity and population's structure in Tunisian strawberry tree (*Arbutus unedo* L.). *Scientia Horticulturae*, *126(3)*, 330–337.

Torres, J. A., Valle, F., Pinto, C., García-Fuentes, A., Salazar, C., & Cano E. (2002). *Arbutus unedo* L. communities in southern Iberian Peninsula mountains. *Plant Ecology*, *160*, 207–223.

Figure captions

Figure 1. C18 reversed-phase HPLC chromatogram obtained from direct (**A**) and saponified (**B**) carotenoid extracts from strawberry tree (*A. unedo* L.) fruits. Peak identities: 1, (all-*E*)-neoxanthin; 2, (9'*Z*)-neoxanthin; 3, neochrome; 4, (all-*E*)-violaxanthin; 5, (9*Z*)-violaxanthin; 6, luteoxanthin; 7, (all-*E*)-antheraxanthin; 8, auroxanthin; 9, lutein 5,6-epoxide; 10, (all-*E*)-zeaxanthin; 11, (all-*E*)-lutein; 12, (9*Z*)-lutein; 13, (13*Z*)-lutein; 14, (all-*E*)-β-cryptoxanthin; 15, (all-*E*)-β-carotene; 16, partially esterified xanthophylls; 17, totally esterified xanthophylls. The identity of the major peaks (referred by lowercase letters) in the partially and totally esterified xanthophyll fractions are detailed in **Table 3**. Detection wavelength was 450 nm. For other experimental conditions see *Materials and Methods* section.

Figure 2. Mass spectra obtained by LC-MS (APCI+) and fragmentation profile for (all-*E*)-violaxanthin monomyristate (peak ***c***) and (all-*E*)-violaxanthin dimyristate (peak ***p***) present in the direct extract of strawberry tree fruits. References for peaks as in **Figure 1** and **Table 3**. (Note: the loss of H_2O_{epox} refers to the water fragmentation from the 5,6-epoxide group).

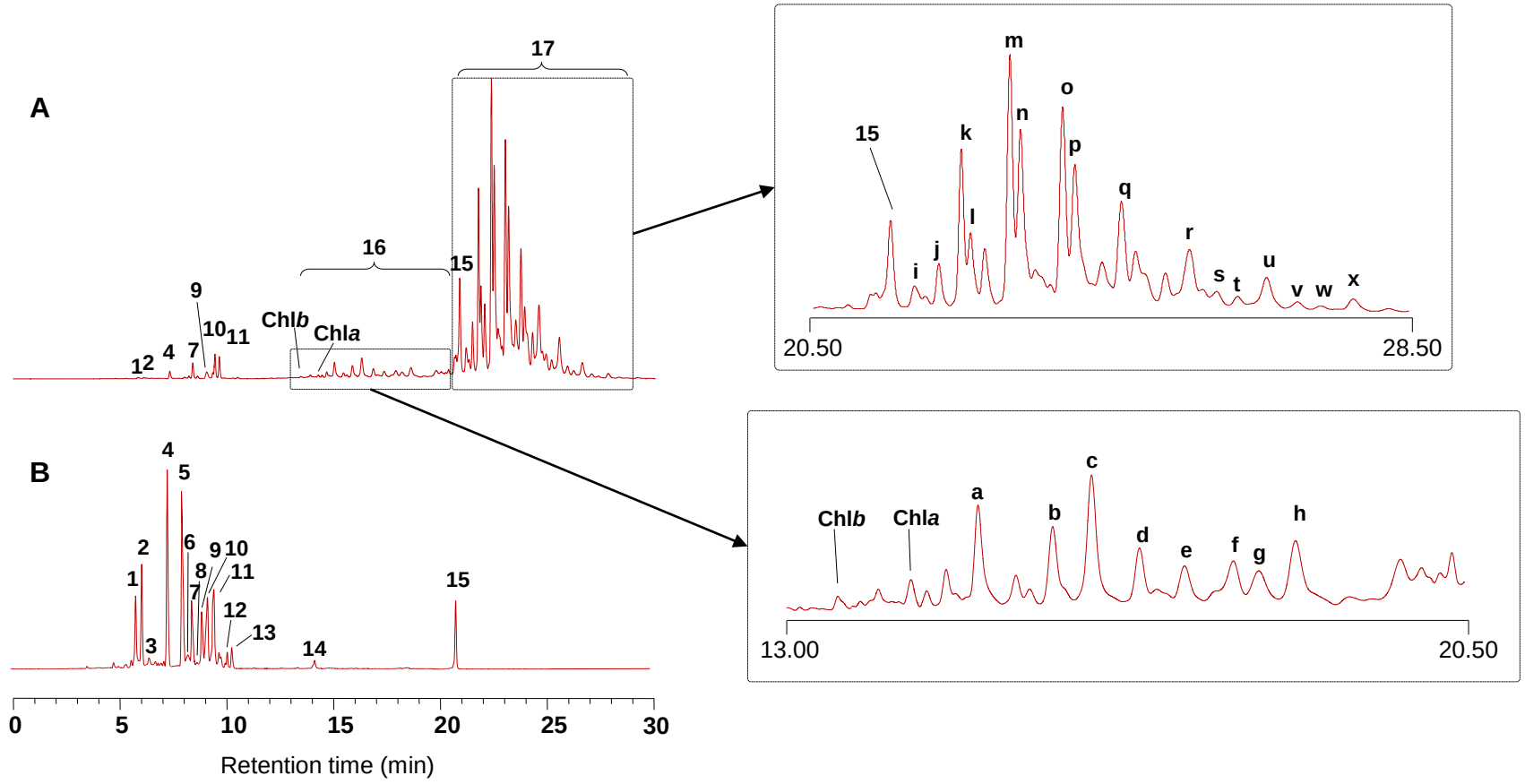
Supplementary material

Table S1. Fatty acids composition of the total lipids from strawberry tree (*Arbutus unedo* L.) fruits.

Figure S1. Fruits (A) and geographical distribution (B) of strawberry tree (*Arbutus unedo* L.).

Figure S2. Fully developed strawberry tree (*Arbutus unedo* L.) fruits (A) at different ripening stages according to external colour (green, yellow, orange and red) and place of harvesting at Natural Park "Sierra Norte de Sevilla", Spain (B).

Figure S3. Chemical structures of major carotenoids in strawberry tree (*Arbutus unedo* L.) fruits.


A
17
16
15
9
10
11
Chl*b*
Chl*a*
1
2
4
7
m
o
n
k
p
q
15
r
l
j
i
s
t
u
v
w
x
20.50
28.50
B
4
5
2
6
9
10
1
8
11
7
12
13
3
14
15
c
a
b
Chl*b*
Chl*a*
d
e
f
g
h
13.00
20.50
0
5
10
15
20
25
30
Retention time (min)

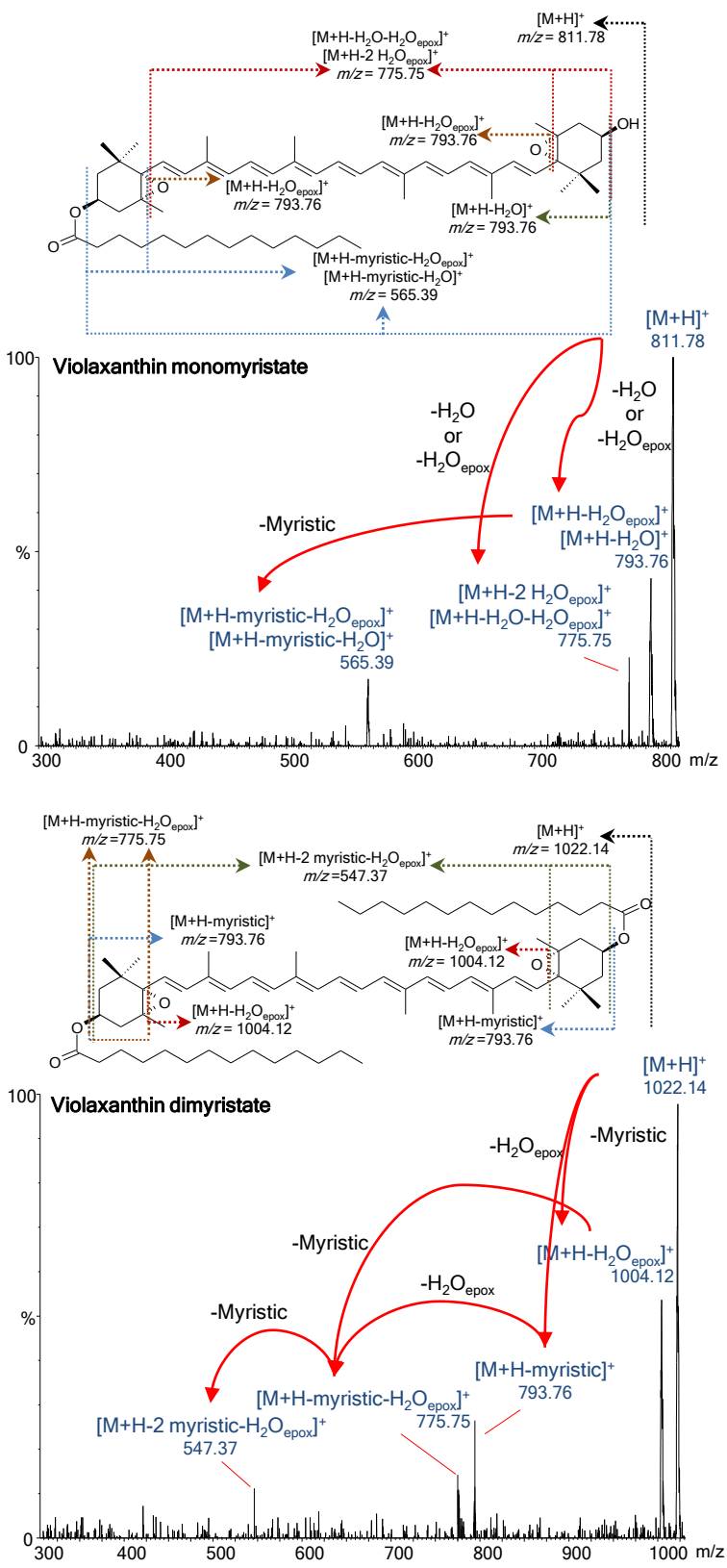

[M+H]+
m/z = 811.78
[M+H-H2O-H2Oepox]+
[M+H-2 H2Oepox]+
m/z = 775.75
[M+H-H2Oepox]+
m/z = 793.76
OH
O
[M+H-H2Oepox]+
m/z = 793.76
[M+H-H2O]+
m/z = 793.76
[M+H-myristic-H2Oepox]+
[M+H-myristic-H2O]+
m/z = 565.39
Violaxanthin monomyristate
100
%
0
300
400
500
600
700
800
m/z
[M+H]+
811.78
-H2O
or
-H2Oepox
-H2O
or
-H2Oepox
-Myristic
[M+H-H2Oepox]+
[M+H-H2O]+
793.76
[M+H-2 H2Oepox]+
[M+H-H2O-H2Oepox]+
775.75
[M+H-myristic-H2Oepox]+
[M+H-myristic-H2O]+
565.39
[M+H-myristic-H2Oepox]+
m/z =775.75
[M+H-2 myristic-H2Oepox]+
m/z =547.37
[M+H]+
m/z = 1022.14
[M+H-myristic]+
m/z =793.76
[M+H-H2Oepox]+
m/z = 1004.12
[M+H-H2Oepox]+
m/z = 1004.12
[M+H-myristic]+
m/z =793.76
Violaxanthin dimyristate
100
%
0
300
400
500
600
700
800
900
1000
m/z
[M+H]+
1022.14
-H2Oepox
-Myristic
-Myristic
[M+H-H2Oepox]+
1004.12
-H2Oepox
-Myristic
[M+H-myristic]+
793.76
[M+H-myristic-H2Oepox]+
775.75
[M+H-2 myristic-H2Oepox]+
547.37

Table 1. Characteristics of the strawberry tree (*Arbutus unedo* L.) fruits selected at four different ripening stages according to the external colour.

Stage of ripening	Colour		Weight (g) per fruit*	Moisture content (%)**
	External	Flesh		
Unripe	Greenish	Greenish/Yellowish	2.8 ± 0.1	68.7 ± 0.8
Unripe	Yellowish	Yellowish/Orange	3.2 ± 0.2	61.7 ± 1.7
Unripe	Orange	Yellowish/Orange	3.8 ± 0.3	49.0 ± 4.1
Ripe	Redish	Orange	4.7 ± 0.2	53.0 ± 0.1

* Data represent the average and standard deviation (n = 10).
** Data represent the average and standard deviation (n = 3).

Table 2. Chromatographic, UV-visible and mass (APCI+) spectroscopy properties, and quantitative composition (μg/g dry weight), of the carotenoid pigments identified in saponified extracts from strawberry tree (*A. unedo* L.) fruits.

Peak[a]	Carotenoid	Rt (min)	λ_{max} (nm)	λ_{max} (nm) according to bibliography in acetone[b]	%III/II	Epoxide test	HPLC/APCI(+) MS fragmentation pattern (*m/z*)		Concentration (μg/g dw)[d]
							$[M+H]^+$	Characteristic fragments	
1	(all-*E*)-Neoxanthin	5.81	419, 444, 472	418, 442, 471[c]	99	+	601	583 $[M+H-H_2O]^+$, 565 $[M+H-2H_2O]^+$, 547 $[M+H-3H_2O]^+$, 521 $[M+H-80]^+$	26.4 ± 1.8
2	(9'*Z*)-Neoxanthin	6.10	415, 439, 468	416, 440, 470	91	+	601	583 $[M+H-H_2O]^+$, 565 $[M+H-2H_2O]^+$, 547 $[M+H-3H_2O]^+$, 521 $[M+H-80]^+$	24.6 ± 1.9
3	Neochrome	6.53	402, 424, 452	398, 421, 448	98	-	601	583 $[M+H-H_2O]^+$, 565 $[M+H-2H_2O]^+$, 547 $[M+H-3H_2O]^+$	1.2 ± 0.1
4	(all-*E*)-Violaxanthin	7.28	418, 443, 472	421, 442, 473	98	+	601	583 $[M+H-H_2O]^+$, 565 $[M+H-2H_2O]^+$, 521 $[M+H-80]^+$	123.5 ± 5.9
5	(9*Z*)-Violaxanthin	8.03	411, 436, 468	411, 435, 465	89	+	601	583 $[M+H-H_2O]^+$, 565 $[M+H-2H_2O]^+$, 521 $[M+H-80]^+$	87.8 ± 5.7
6	Luteoxanthin	8.17	400, 424, 451	400, 423, 448	100	+	601	583 $[M+H-H_2O]^+$, 565 $[M+H-2H_2O]^+$, 521 $[M+H-80]^+$	1.5 ± 0.1
7	(all-*E*)-Antheraxanthin	8.33	424, 448, 476	422, 444, 474[c]	66	+	585	567 $[M+H-H_2O]^+$, 549 $[M+H-2H_2O]^+$, 505 $[M+H-80]^+$	18.9 ± 1.5
8	Auroxanthin	8.80	384, 403, 427	377, 398, 423	120	-	601	583 $[M+H-H_2O]^+$, 565 $[M+H-2H_2O]^+$	Traces

9	Lutein 5,6-epoxide	9.00	418, 441, 470	418, 441, 471	85	+	585	567 $[M+H-H_2O]^+$, 549 $[M+H-2H_2O]^+$, 505 $[M+H-80]^+$	20.5 ± 0.9
10	(all-*E*)-Zeaxanthin	9.35	428, 454, 481	430, 452, 479	38	-	569	551 $[M+H-H_2O]^+$, 533 $[M+H-2H_2O]^+$	9.1 ± 0.4
11	(all-*E*)-Lutein	9.56	428, 448, 476	424, 445, 474[c]	70	-	569	551 $[M+H-H_2O]^+$, 533 $[M+H-H_2O]^+$	18.9 ± 0.8
12	(9*Z*)-Lutein	10.12	330, 420, 444, 472	330, 420, 442, 471[c]	67	-	569	551 $[M+H-H_2O]^+$, 533 $[M+H-2H_2O]^+$	1.6 ± 0.1
13	(13*Z*)-Lutein	10.22	334, 418, 441, 470	332, 418, 441, 469[c]	45	-	569	551 $[M+H-H_2O]^+$, 533 $[M+H-2H_2O]^+$	2.1 ± 0.2
14	(all-*E*)-β-Cryptoxanthin	14.13	428, 454, 480	428, 450, 478	18	-	553	535 $[M+H-H_2O]^+$, 461 $[M+H-92]^+$	1.2 ± 0.1
15	(all-*E*)-β-Carotene	20.68	427, 454, 479	429, 452, 478	16	-	537	445 $[M+H-92]^+$	6.0 ± 0.4

[a] Peak numbers are according to Fig. 1; [b] Britton, Liaaen-Jensen, and Pfander, 2004; [c] Measured in ethanol; [d] mean ± standard deviation of a triplicate analysis.

Table 3. Spectroscopic properties (UV and MS(APCI+)) of the main xanthophyll esters in strawberry tree (*Arbutus unedo* L.) fruits.

Peak*	Xanthophyll ester	λ_{max} (nm)	%III/II	HPLC-MS/APCI(+) MS fragmentation pattern *m/z*	
				$[M+H]^+$	Characteristics fragments
	Partially esterified				
a	(all-*E*)-Violaxanthin monolaurate	418, 443,472	91	783.72	765.71 $[M+H-H_2O_{epox}]^+$, $[M+H-H_2O]^+$; 747.70 $[M+H-2\ H_2O_{epox}]^+$, $[M+H-\ H_2O-H_2O_{epox}]^+$; 565.39 $[M+H\text{-lauric-}H_2O_{epox}]^+$, $[M+H\text{-lauric-}H_2O]^+$
b	(9*Z*)-Violaxanthin monolaurate	411, 436, 468	92	783.72	765.71 $[M+H-H_2O_{epox}]^+$, $[M+H-H_2O]^+$; 747.70 $[M+H-2\ H_2O_{epox}]^+$, $[M+H-\ H_2O-H_2O_{epox}]^+$; 565.39 $[M+H\text{-lauric-}H_2O_{epox}]^+$, $[M+H\text{-lauric-}H_2O]^+$
c	(all-*E*)-Violaxanthin monomyristate	418, 443,472	91	811.78	793.76 $[M+H-H_2O_{epox}]^+$, $[M+H-H_2O]^+$; 775.75 $[M+H-2\ H_2O_{epox}]^+$, $[M+H-\ H_2O-H_2O_{epox}]^+$; 565.39 $[M+H\text{-myristic-}H_2O_{epox}]^+$, $[M+H\text{-myristic-}H_2O]^+$
d	(9*Z*)-Violaxanthin monomyristate	411, 436, 468	92	811.78	793.76 $[M+H-H_2O_{epox}]^+$, $[M+H-H_2O]^+$; 775.75 $[M+H-2\ H_2O_{epox}]^+$, $[M+H-\ H_2O-H_2O_{epox}]^+$; 565.39 $[M+H\text{-myristic-}H_2O_{epox}]^+$, $[M+H\text{-myristic-}H_2O]^+$
e	(all-*E*)-Lutein-3-*O*-caprate	428, 448, 476	65	723.68	705.67 $[M+H-H_2O]^+$; 533.40 $[M+H\text{-capric-}H_2O]^+$
f	(all-*E*)-Violaxanthin monopalmitate	418, 443,472	91	839.83	821.82 $[M+H-H_2O_{epox}]^+$, $[M+H-H_2O]^+$; 803.80 $[M+H-2\ H_2O_{epox}]^+$, $[M+H-\ H_2O-H_2O_{epox}]^+$; 565.39 $[M+H\text{-palmitic-}H_2O_{epox}]^+$, $[M+H\text{-palmitic-}H_2O]^+$
g	(9*Z*)-Violaxanthin monopalmitate	411, 436, 468	92	839.83	821.82 $[M+H-H_2O_{epox}]^+$, $[M+H-H_2O]^+$; 803.80 $[M+H-2\ H_2O_{epox}]^+$, $[M+H-\ H_2O-H_2O_{epox}]^+$; 565.39 $[M+H\text{-palmitic-}H_2O_{epox}]^+$, $[M+H\text{-palmitic-}H_2O]^+$
h	(9*Z*)-Lutein-3-*O*-caprate	330, 420, 444, 472	63	723.68	705.67 $[M+H-H_2O]^+$; 533.40 $[M+H\text{-capric-}H_2O]^+$
i	(all-*E*)-Violaxanthin monostearate	418, 443,472	91	867.89	849.87 $[M+H-H_2O_{epox}]^+$, $[M+H-H_2O]^+$; 831.86 $[M+H-2\ H_2O_{epox}]^+$, $[M+H-\ H_2O-H_2O_{epox}]^+$; 565.39 $[M+H\text{-stearic-}H_2O_{epox}]^+$, $[M+H\text{-stearic-}H_2O]^+$
	Totally esterified				
j	(9*Z*)-Violaxanthin dicaprate	411, 436, 468	92	909.92	891.91 $[M+H-H_2O_{epox}]^+$; 737.66 $[M+H\text{-capric}]^+$, 719.64 $[M+H\text{-capric-}H_2O_{epox}]^+$, 547.37 $[M+H\text{-2 capric-}H_2O_{epox}]^+$
k	(all-*E*)-Neoxanthin dilaurate	419, 444, 472	100	966.03	948.02 $[M+H-H_2O_{epox}]^+$; 765.71 $[M+H\text{-lauric}]^+$; 747.70 $[M+H\text{-lauric-}H_2O_{epox}]^+$; 547.37 $[M+H\text{-2 lauric-}H_2O_{epox}]^+$
l	(all-*E*)-Violaxanthin dilaurate	418, 443,472	91	966.03	948.02 $[M+H-H_2O_{epox}]^+$; 765.71 $[M+H\text{-lauric}]^+$; 747.70 $[M+H\text{-lauric-}H_2O_{epox}]^+$; 547.37 $[M+H\text{-2 lauric-}H_2O_{epox}]^+$
m	(9*Z*)-Violaxanthin dilaurate	411, 436, 468	92	966.03	948.02 $[M+H-H_2O_{epox}]^+$; 765.71 $[M+H\text{-lauric}]^+$; 747.70 $[M+H\text{-lauric-}H_2O_{epox}]^+$; 547.37 $[M+H\text{-2 lauric-}H_2O_{epox}]^+$

n	(all-*E*)-Violaxanthin laurate-myristate	418, 443,472	91	994.09	976.07 $[M+H-H_2O_{epox}]^+$; 775.75 $[M+H\text{-lauric-}H_2O_{epox}]^+$; 747.70 $[M+H\text{-myristic-}H_2O_{epox}]^+$; 565.39 $[M+H\text{-lauric-myristic}]^+$; 547.37 $[M+H\text{-lauric-myristic-}H_2O_{epox}]^+$
o	(9*Z*)-Violaxanthin laurate-myristate	411, 436, 468	92	994.09	976.07 $[M+H-H_2O_{epox}]^+$; 775.75 $[M+H\text{-lauric-}H_2O_{epox}]^+$; 747.70 $[M+H\text{-myristic-}H_2O_{epox}]^+$; 565.39 $[M+H\text{-lauric-myristic}]^+$; 547.37 $[M+H\text{-lauric-myristic-}H_2O_{epox}]^+$
p	(all-*E*)-Violaxanthin dimyristate	418, 443,472	91	1022.14	1004.12 $[M+H-H_2O_{epox}]^+$; 793.76 $[M+H\text{-myristic}]^+$; 775.75 $[M+H\text{-myristic-}H_2O_{epox}]^+$; 547.37 $[M+H\text{-2 myristic-}H_2O_{epox}]^+$
q	(9*Z*)-Violaxanthin dimyristate	411, 436, 468	92	1022.14	1004.12 $[M+H-H_2O_{epox}]^+$; 793.76 $[M+H\text{-myristic}]^+$; 775.75 $[M+H\text{-myristic-}H_2O_{epox}]^+$; 547.37 $[M+H\text{-2 myristic-}H_2O_{epox}]^+$
r	(all-*E*)-Violaxanthin myristate-palmitate	418, 443,472	91	1050.19	1032.18 $[M+H-H_2O_{epox}]^+$; 803.80 $[M+H\text{-myristic-}H_2O_{epox}]^+$; 775.75 $[M+H\text{-palmitic-}H_2O_{epox}]^+$; 565.39 $[M+H\text{-myristic-palmitic}]^+$; 547.37 $[M+H\text{-myristic-palmitic-}H_2O_{epox}]^+$
s	(9*Z*)-Violaxanthin myristate-palmitate	411, 436, 468	92	1050.19	1032.18 $[M+H-H_2O_{epox}]^+$; 803.80 $[M+H\text{-myristic-}H_2O_{epox}]^+$; 775.75 $[M+H\text{-palmitic-}H_2O_{epox}]^+$; 565.39 $[M+H\text{-myristic-palmitic}]^+$; 547.37 $[M+H\text{-myristic-palmitic-}H_2O_{epox}]^+$
t	(all-*E*)-Violaxanthin dipalmitate	418, 443,472	91	1078.25	1060.23 $[M+H-H_2O_{epox}]^+$; 821.82 $[M+H\text{-palmitic}]^+$; 803.80 $[M+H\text{-palmitic-}H_2O_{epox}]^+$; 547.37 $[M+H\text{-2 palmitic-}H_2O_{epox}]^+$
u	(all-*E*)-Lutein dimyristate	428, 448, 476	65	990.15	761.77 $[M+H\text{-myristic}]^+$; 533.40 $[M+H\text{-2 myristic}]^+$
v	(all-*E*)-β-Cryptoxanthin stearate	427, 454, 479	18	819.92	727.92 $[M+H\text{-92}]^+$; 535.43 $[M+H\text{-stearic}]^+$
w	(all-*E*)-Violaxanthin distearate	418, 443,472	91	1134.36	1116.34 $[M+H-H_2O_{epox}]^+$; 849.87 $[M+H\text{-stearic}]^+$; 831.86 $[M+H\text{-stearic-}H_2O_{epox}]^+$; 547.37 $[M+H\text{-2 stearic-}H_2O_{epox}]^+$
x	(all-*E*)-Lutein myristate-palmitate	428, 448, 476	65	1018.20	789.83 $[M+H\text{-myristic}]^+$; 761.77 $[M+H\text{-palmitic}]^+$; 533.40 $[M+H\text{-myristic-palmitic}]^+$
y	(all-*E*)-Zeaxanthin myristate-palmitate	428, 455, 481	18	1018.20	789.83 $[M+H\text{-myristic}]^+$; 761.77 $[M+H\text{-palmitic}]^+$; 533.40 $[M+H\text{-myristic-palmitic}]^+$
z	(all-*E*)-Lutein dipalmitate	428, 448, 476	65	1046.26	789.83 $[M+H\text{-palmitic}]^+$; 533.40 $[M+H\text{-2 palmitic}]^+$

* Peak numbers are according to Figure 1.

Table 4. Changes in the concentration (μg/g dw)* of carotenoid (free and esterified) and chlorophyll pigments in strawberry tree fruits at different stages of ripening selected according to external colour of the fruits.

Pigment	Stage of ripening (external colour of the fruits)			
	Green	Yellow	Orange	Red
Free carotenoids				
(all-*E*)-Neoxanthin	0.8 ± 0.1[a]	0.4 ± 0.0[b]	0.3 ± 0.0[b]	0.2 ± 0.0[b]
(9'*Z*)-Neoxanthin	3.1 ± 0.2[a]	1.1 ± 0.1[b]	0.6 ± 0.1[c]	0.1 ± 0.0[d]
(all-*E*)-Violaxanthin	14.2 ± 1.0[a]	9.1 ± 1.1[b]	7.6 ± 0.8[b]	1.4 ± 0.1[c]
(9*Z*)-Violaxanthin	1.6 ± 0.1[a]	0.5 ± 0.1[b]	0.4 ± 0.1[b]	n.d.[**]
(all-*E*)-Antheraxanthin	0.9 ± 0.1[a]	1.6 ± 0.1[b]	2.6 ± 0.3[c]	1.2 ± 0.1[a,b]
Lutein 5,6-epoxide	3.6 ± 0.1[a]	1.5 ± 0.1[b]	0.9 ± 0.1[c]	0.2 ± 0.0[d]
(all-*E*)-Zeaxanthin	0.7 ± 0.1[a]	0.8 ± 0.1[a]	1.5 ± 0.2[b]	2.0 ± 0.1[c]
(all-*E*)-Lutein	5.2 ± 0.2[a]	2.6 ± 0.3[b]	2.2 ± 0.3[b]	1.3 ± 0.1[c]
(9*Z*)-Lutein	0.2 ± 0.0[a]	0.1 ± 0.0[b]	0.1 ± 0.0[b]	n.d.
(13*Z*)-Lutein	0.4 ± 0.0[a]	0.2 ± 0.1[b]	0.1 ± 0.0[b,c]	0.1 ± 0.0[c]
(all-*E*)-β-Carotene	14.6 ± 0.3[a]	10.5 ± 1.1[b]	10.9 ± 1.2[b]	10.7 ± 0.9[b]
Xanthophyll esters				
Partially esterified	44.6 ± 1.2[a]	39.4 ± 4.2[b,c]	33.4 ± 3.8[c]	24.9 ± 2.1[d]
Totally esterified	275.9 ± 5.0[a]	281.9 ± 26.6[a]	279.9 ± 27.0[a]	312.9 ± 19.8[a]
Total free carotenoids	45.3 ± 1.7[a]	28.4 ± 2.8[b]	27.2 ± 2.7[b]	17.2 ± 1.3[c]
Total esterified carotenoids	320.5 ± 6.2[a]	321.3 ± 29.4[a]	313.3 ± 30.8[a]	337.8 ± 21.9[a]
Total carotenoids	365.8 ± 4.9[a]	349.7 ± 32.2[a]	340.4 ± 33.1[a]	355.0 ± 23.1[a]
Chlorophylls				
Chlorophyll *a*	165.5 ± 8.5[a]	52.4 ± 5.9[b]	21.8 ± 2.5[c]	4.3 ± 0.6[d]
Chlorophyll *b*	38.8 ± 1.2[a]	10.6 ± 1.1[b]	4.0 ± 0.5[c]	0.6 ± 0.1[d]
Total chlorophylls	204.3 ± 9.6[a]	63.0 ± 7.0[b]	25.8 ± 2.9[c]	4.9 ± 0.7[d]

[*] Data represent the mean and standard deviation (n = 3). For each pigment, concentration values marked with different superscript letter are significantly different ($p < 0.05$, Tukey's HSD test).
[**] n.d.: Not detected.

Highlights

- Carotenoids in strawberry tree fruits have been characterized.
- The detailed individual pigment characterization was carried out for the first time.
- (all-*E*)-Violaxanthin and 9*Z*-violaxanthin were the major carotenoid pigments (>60%).
- Xanthophylls were mostly (>90 %) esterified with saturated fatty acids.