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Carotenoids from persimmon juice processing

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ABSTRACT

The aim of this study was the use and revalorization of two persimmon by-products A and B generated in the juice production process. The by-product B resulting from a pectinase enzymatic treatment of peels and pulp to optimize juice extraction was especially suitable for recovery of valuable bioactive carotenoids. The extraction solvents and solvent combinations used were: ethanol, acetone, ethanol/acetone (50:50 v/v) and ethanol/acetone/hexane (25:25:50 v/v/v). HPLC-DAD analysis detected and identified a total of nine individual carotenoids namely violaxanthin, neoxanthin, antheraxanthin, lutein, zeaxanthin, β-cryptoxanthin 5,6-epoxide, β-cryptoxanthin, α-carotene, and β-carotene. β-cryptoxanthin and β-carotene represented 49.2% and 13.2% of the total carotenoid content (TCC) in the acetone extract from by-product B. TCC contributed greatly to antioxidant activity of acetone extract derived from this by-product. Pectinase enzymatic treatment of persimmon peels and pulp followed by absolute acetone extraction of carotenoids could be an efficient method to obtain a rich extract in these compounds that could be used as nutraceutical ingredient.

1. Introduction

The persimmon (*Diospyros kaki* Thunb.) is a tree of the Ebenaceae botanical family and is cultivated in subtropical climates (Martínez-Calvo, Badenes, & Llácer, 2012). It originates from China, Japan and Korea, but nowadays it has been extended to other countries such as Brazil, United States of America (USA), Australia and some countries present in the Mediterranean Sea coast such as Israel, Italy or Spain. After China and Korea, Spain is today the third largest world producer (404,131 t) ahead of Japan and Brazil (FAOSTAT, 2017). The western area of Andalucía mainly in the province of Huelva (specifically, in the municipalities of Cartaya, Lepe, Isla Cristina and Villablanca) and Sevilla, and Valencia Region highlight as the largest producers. According to Ministerio de Agricultura, Pesca y Alimentación (MAPA) estimates, in 2018 production of persimmon in Andalucía was 56,780 t from the variety Sharon or Triumph marketed under the Sharoni brand, while Valencia Region produced 425,075 t of the Rojo Brillante variety marketed under the protected denomination of origin (PDO) 'Kaki Ribera del Xúquer'.

It is remarkable that thousands tons of persimmon fruits (in particular, the Spanish Association of Persimmon currently estimates discarded fruits in the Valencia Region by about 18,000 t) are discarded every year due to a combination of the high quality standards of supermarkets, strict government regulations and the high expectations that consumers have when buying these fruits in terms of size, shape and color (Porter, Reay, Bomberg, & Higgins, 2018). Surplus fruits, damaged fruits and those fruits unacceptable to the consumers that result from prolonged storage and chemical treatments (Arnal & Del Río, 2003) require the development of new derivative products. In addition, fruits must be processed to facilitate their consumption and for commercial, logistic and economical reasons (Ayala-Zavala et al., 2011). This generates large quantities of by-products including peels, seeds and unused flesh in different steps of processing chain. These by-products are rich in valuable compounds which can be utilized in various industries as novel,

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economical and natural sources of dietary fiber, antioxidants, pectin, enzymes, organic acids, food additives, essential oils, and others using different methods of extraction, purification and fermentation (Kodagoda & Marapana, 2017; Lapornik, Prošek, & Wondra, 2005). The actions of reusing plant by-products agree with Sustainable Development Goal number 12 (SDG 12) of the 2030 Agenda for Sustainable Development of the United Unions (UN General Assembly, 2015).

Persimmon fruits are rich dietary source of bioactive compounds such as vitamin C, dietary fiber, polyphenols and carotenoids (Gorinstein et al., 2001; Pérez-Burillo, Oliveras, Quesada, Rufián-Henares, & Pastoriza, 2018; Veberic, Jurhar, Mikulic-Petkovsek, Stampar, & Schmitzer, 2010) which may act in concert to provide their antioxidant, anti-inflammatory and other health-related properties useful to protect against non-communicable chronic diseases (Aune et al., 2017; Hosseini et al., 2018). Nowadays, the carotenoids have a great interest in the industry. Extensive research is allocated to the recovery and production of these compounds because of their functional properties. They are used as feed additives in animal nutrition (Jamilah, Mohamed, Abbas, Abdul Rahman, & Karim, 2009), natural colorants in foods, nutraceuticals and cosmetics (Berman et al., 2015; Jaswir, Noviendri, Hasrini, & Octavianti, 2011), and aromatic compounds precursors (Crupi et al., 2010). On the other hand, carotenoids are also used for their beneficial effect in the prevention of diseases such as cancers (Bolhassani, 2015), cardiovascular diseases (Csepanyi et al., 2015), and degeneration of optical vision (Harrison, 2019).

The aim of this study was to obtain an extract rich in carotenoid pigments, using various solvents with different combinations such as ethanol, acetone, ethanol/acetone (1:1), and ethanol/acetone/hexane (25:25:50). As plant material, two by-products derived from the industrial production of persimmon juice were used. Various extracted carotenoids were identified and quantified by high-performance liquid chromatography (HPLC), using diode array detector (DAD) and analytical standards.

2. Material and methods

2.1. Chemicals

The carotenoid standards: violaxanthin (purity ≥ 95%), neoxanthin (purity ≥ 97%), antheraxanthin (purity ≥ 95%), lutein (purity ≥ 99%), zeaxanthin (purity ≥ 97%), β-cryptoxanthin (purity ≥ 97%), α-carotene (purity ≥ 97%) and β-carotene (purity ≥ 96%) were obtained from CaroteNature (Lupsingen, Switzerland). Methanol (MeOH), acetone (Ac), ethanol (EtOH), *n*-hexane (Hx), ethyl ether, chloroform, and potassium hydroxide (KOH, purity = 90%) were from Panreac Química SLU (Castellar del Vallès, Barcelona, Spain). All HPLC organic solvents were of analytical grade. Folin-Ciocalteu reagent, potassium persulfate, sodium carbonate, gallic acid, ABTS [2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)], and AAPH [2,2′-Azobis (2-amidinopropane) dihydrochloride] were purchased from Sigma-Aldrich Corp. (Saint Louis, Missouri, USA). Fluorescein (FL) and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were purchased from Fluka Chemika (Neu-Ulm, Germany). Ultrapure water was obtained from a purified water system Q-Gard® 1 from Merck Millipore (Darmstadt, Germany) with a resistivity of 18.0 MΩ·cm. Gas nitrogen has been obtained from Air Liquide (Madrid, Spain).

2.2. Plant material

Batches of the fresh by-products A and B discarded in different days of fruit processing were purchased from Mitra Sol Technologies (Elche, Spain). Both by-products derived from persimmon fruits of the Sharon or Triumph variety (non-astringent, seedless, and hard) and are composed of peels and pulp resulting from different stages of industrial processing of persimmon juice (Fig. 1). By-product B was obtained by pectinase enzymatic treatment of by-product A to optimize juice extraction. So by-product B had homogeneous granular appearance and orange color, while by-product A, which was obtained liquefying the edible parts of persimmon fruits using an industrial fruit juice machine, showed a more viscous and heterogeneous appearance.

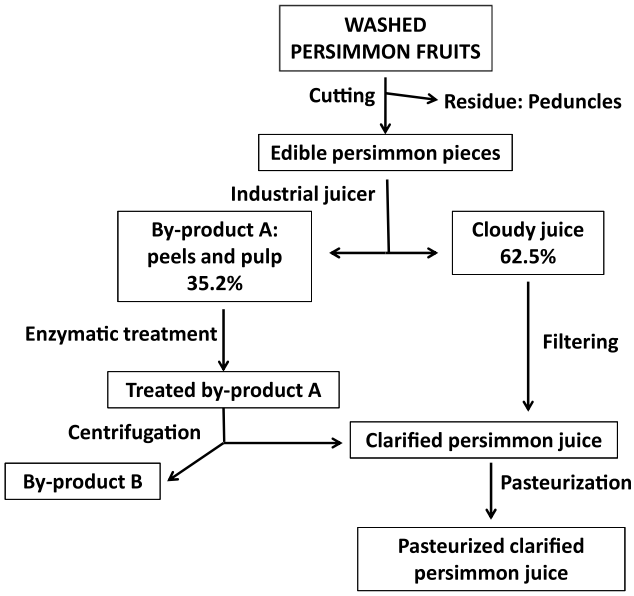

Fig. 1. Flow diagram of industrial production of persimmon juice.

Moisture content (%MC) for the two by-products was determined. 10 g samples were dried at 60 ± 1 °C in a drying oven (JP Selecta, Barcelona, Spain) and led to constant weight.

2.3. Solid-liquid extraction

The methodology for carotenoids extraction was described by Olives Barba, Cámara Hurtado, Sánchez Mata, Fernández Ruiz, and López Sáenz de Tejada (2006). Initially, fresh by-product A was washed with water (25:75 w/v) to eliminate residual sugars that could interfere with the subsequent drying process of extracts. Then, samples from both by-products A and B were divided into 4 portions and each of them were individually extracted three times with one of the solvents or solvent combinations assayed for 20 min at 40 °C and 150 rpm, until a colorless liquid was obtained. Extraction solvents and solvent combinations used were: ethanol, acetone, ethanol/acetone (50:50 v/v) and ethanol/acetone/hexane (25:25:50 v/v/v). The different extracts were reduced using a rotary evaporator low pressure (Series R-210, Buchi) at 40 °C in darkness. Residue was saponified with 5 mL of KOH (30%) during 1 h at 56 °C according to Müller (1997). Obtained solution was transferred to a funnel and mixed with 100 mL ethyl ether to separate the organic phase, which was subsequently washed three times with water according to Izuchi, Takahashi, and Inada (2009). Extracts were dried in a Genevac™ miVac Centrifugal Vacuum Concentrator (SP Scientific) at 40 °C for removing the residual water and lyophilized (Telstar Cryodos-80, Terrassa, Barcelona, Spain). Lyophilized extracts were stored at −80 °C. Extraction yield (EY) was expressed as mg/100 g dry weight (DW). The different extracts were obtained in triplicate.

2.4. Preparation of standards, calibration curve and HPLC-DAD analysis

All standard stock solutions were prepared and kept under nitrogen atmosphere at −20 °C until analysis. Violaxanthin, neoxanthin, antheraxanthin, lutein, zeaxanthin, β-cryptoxanthin, α-carotene, and β-carotene were separately dissolved in 1 mL of chloroform. Calibration curves were built with five concentrations (3.125, 6.25, 12.5, 25.0, and 50.0 mg/L) for each carotenoid standard, reaching R^2 values between

0.9279 and 0.9917. β-cryptoxanthin 5,6-epoxide was expressed as β-cryptoxanthin equivalents.

Lyophilized extracts were dissolved in acetone at a concentration of 20 mg/mL and were filtered through a 0.45 μm PVDF syringe filter. In each sample, carotenoids composition was analyzed by HPLC-DAD (Rivera & Canela-Garayoa, 2012). Injection volume was 10 μL. YMC Carotenoid HPLC Column, C30, 4.6 × 250 mm, 5 μm (Teknokroma Analítica, Sant Cugat del Vallès, Barcelona, Spain) was used. Analysis was performed at 25 °C. Mobile phase consisted in a gradient of 60:40 (v/v) methanol/acetone (A), and 60:40 (v/v) acetone/water (B). Flow rate was 0.5 mL/min. The gradient profile of the mobile phase was set as follows: decreasing from 60% B to 30% B in 3 min; 30% B for 19 min; decreasing from 30% B to 10% B in 4 min; 10% B for 15.5 min; and increasing from 10% B to 60% B in 3.5 min (total run time 45 min). Chromatograms were recorded at 450 nm. Carotenoids were identified by comparison of the retention times (RTs) with those of authentic standards; just β-cryptoxanthin 5,6-epoxide was identified by matching the observed versus literature RT under identical chromatographic conditions (Cano, Gómez-Maqueo, Welti-Chanes, & García-Cayuela, 2018).

2.5. Determination of total phenols

For total phenolic compounds (TPC) determination, 5 mg lyophilized extracts were dissolved in 1 mL ethanol. TPC were determined with Folin-Ciocalteu reagent in a SPECTROstar Omega UV/VIS absorbance microplate reader (BMG LABTECH GmbH, Offenburg, Germany) (González, Vegara, Martí, Valero, & Saura, 2015). 10 μL of extract solution, 50 μL Folin-Ciocalteu reagent, 100 μL of aqueous 20% Na_2CO_3 and 100 μL of distilled water were mixed. The mixture was kept for 30 min at room temperature before measuring absorbance at 750 nm. Gallic acid was used as calibration standard (concentrations: 100, 150, 200, 300, 350, 500, 750, and 1,000 mg/L; R^2 = 9884). Results were expressed as gallic acid equivalents (mg GAE/100 g extract).

2.6. Antioxidant activity

The antioxidant activity of the extracts was evaluated by both the ABTS radical scavenging and the oxygen radical absorbance capacity (ORAC) antioxidant assay. The radical cation was prepared by the reaction between a 7 mM solution of ABTS in water mixed with a 2.45 mM solution of potassium persulfate and incubated 24 h in the dark at room temperature (González et al., 2015). Then, this solution was diluted with ethanol to reach an absorbance of 0.7 ± 0.02 at 734 nm, measured in the microplate reader SPECTROstar Omega. To determine the antioxidant activity of extracts, 200 μL of the $ABTS^{\bullet+}$ solution was mixed with 20 μL of a 5 mg/mL ethanolic solution of extract and after 6 min the absorbance was measured at 734 nm. Trolox was used as calibration standard (concentrations: 20.60, 41.25, 82.50, 165, and 330 mM; R^2 = 9766) and results were expressed as Trolox equivalents (mM TE/100 g extract).

To assay the capacity of extracts to scavenge peroxyl radicals a validated ORAC method, which uses FL as the fluorescent probe ($ORAC_{FL}$), was utilized (Vegara et al., 2014). The automated ORAC assay was carried out on a FLUOstar Galaxy fluorescence microplate reader (BMG LABTECH GmbH). A freshly prepared AAPH water solution was used for each determination. The temperature of the incubator was set at 37 °C and the FL fluorescence was recorded every minute after the addition of AAPH. ORAC values were calculated by using a regression equation between the Trolox concentration and the net area of the FL decay curve (R^2 = 0.9729) and expressed as Trolox equivalents (mM TE/100 g extract).

2.7. Statistical analysis

Experiments were carried out in triplicate and results were expressed as mean ± standard deviation (SD). Analysis of variance (ANOVA) was performed with GraphPad Prism® version 6.0 (San Diego, California, USA) and differences between means were estimated by post-hoc Tukey's HSD (honestly-significant-difference) test. Regression analysis was used to describe the relationship between TPC and ABTS or ORAC, as well as between total carotenoid content (TCC) and ABTS or ORAC.

3. Results

3.1. %MC of persimmon by-products

The %MC of the two persimmon by-products varied greatly. Estimated %MC, expressed as g water per 100 g fresh weight (FW), were found to be 94.10 ± 0.73% and 69.23 ± 0.08% for by-product A and B, respectively. The high %MC of by-product A suggests a large sugars content that would explain its sticky appearance.

3.2. EY

The EY for both by-products (A and B) obtained by the different extraction solvents are shown in Fig. 2A. In general, solid–liquid extraction for by-product A was more effective than for by-product B. Regardless of the solvent used in the extraction process, there were statistically significant differences in the amount of final dry extract. The highest amount of dry matter was obtained using absolute acetone as organic solvent (10,413.66 ± 72.90 mg/100 g DW), followed by ethanol and the mixture of both solvents. The lowest EY was obtained using the solvent combination ethanol/acetone/hexane (3,537.01 ± 119.90 mg/100 g DW). Significant differences were also observed between the determined EYs for by-product B. The highest EY was obtained with the solvent combination ethanol/acetone (1,550.19 ± 76.29 mg/100 g DW) as well as ethanol alone (1,531.99 ± 50.56 mg/100 g DW). The lowest EY was obtained using acetone as solvent (902.82 ± 21.14 mg/100 g DW) (Fig. 2A).

3.3. Extracted carotenoid quantity

TCC from the two persimmon by-products A and B was determined by summing up the individual carotenoid concentrations measured in each extract. In all cases, TCCs were higher in by-product B than in by-product A (Fig. 2B). The lowest TCC values were recorded for by-product A with values varying from 2,444.54 ± 566.61 mg/100 g in the ethanol/acetone extract to 111.61 ± 13.39 mg/100 g in the ethanol/acetone/hexane extract. By-product B provided the largest TCCs, i.e., 33,970.25 ± 1,542.61 mg/100 g in the acetone extract and 13,953.20 ± 891.87 mg/100 g in the ethanol extract (Fig. 2B).

3.4. Identification and quantification of extracted carotenoids

Representative HPLC-DAD chromatograms of carotenoids in different extracts from the persimmon by-product B are shown in Fig. 3A-D. Nine carotenoids including violaxanthin, neoxanthin, antheraxanthin, lutein, zeaxanthin, β-cryptoxanthin 5,6-epoxide, β-cryptoxanthin, α-carotene, and β-carotene were identified by RTs (6.7, 7.4, 10.6, 14.5, 17.0, 25.9, 30.3, 34.9, and 38.4 min, respectively) coincident with the respective commercial standards or already reported values under identical chromatographic conditions (Cano et al., 2018).

As expected, the chromatographic profile of the main carotenoids found in all the extracts of persimmon by-products A and B was similar. On the contrary, individual concentration of all carotenoids present was clearly higher in by-product B than in by-product A (Fig. 4A and B). Moreover, the acetone extract derived from by-product B was the richest in carotenoids. The predominant carotenoid was β-cryptoxanthin (16,709.90 ± 518.70 mg/100 g extract) followed by β-carotene (4,479.07 ± 713.71 mg/100 g extract), zeaxanthin (4,250.83 ± 27.90 mg/100 g extract), and antheraxanthin (2,265.48 ± 19.37 mg/100 g extract) (Fig. 4A). The major carotenoid in the ethanol extract was

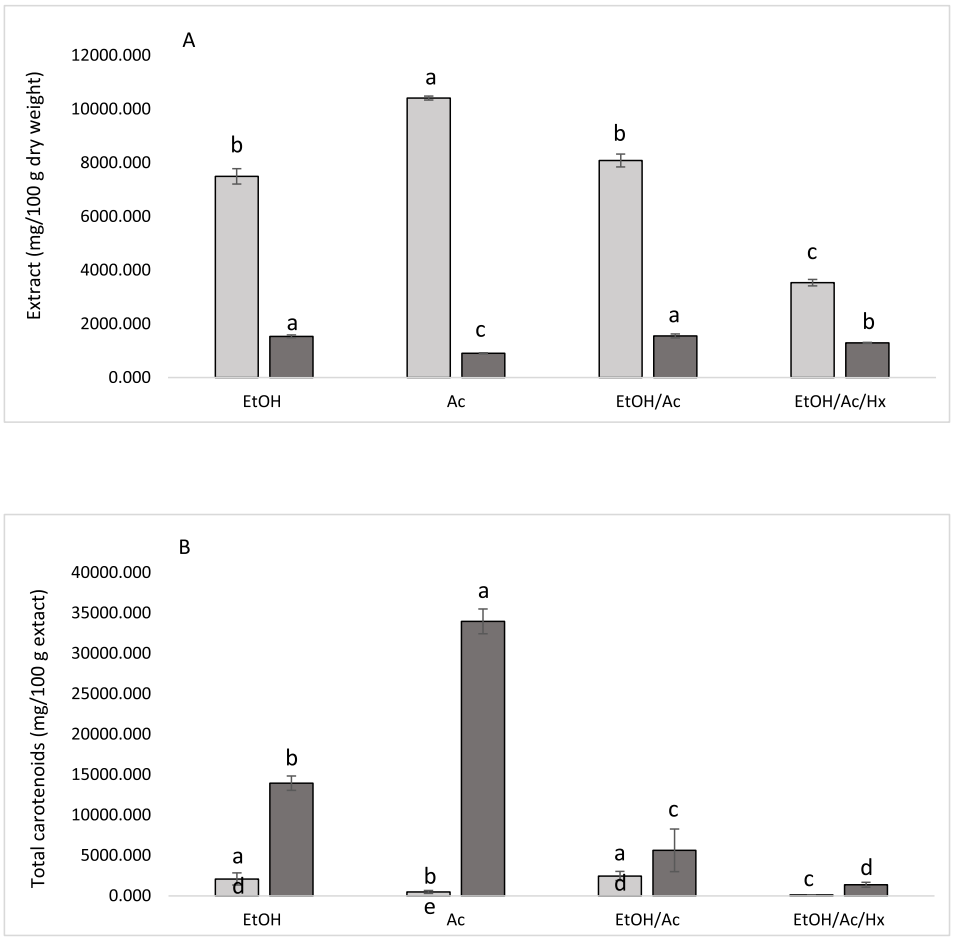

Fig. 2. (A) Extraction yields (mg/100 g dry weight) and (B) total carotenoid contents (mg/100 g extract) of persimmon extracts obtained from by-product A (▭) and B (▬) using different solvents or solvent combinations.

β-cryptoxanthin (6,436.06 ± 286.17 mg/100 g extract) followed by zeaxanthin (2,161.05 ± 160.04 mg/100 g extract). In comparison to previous both extracts, individual concentrations of all carotenoids were noticeably lower in the ethanol/acetone and the ethanol/acetone/hexane extracts from the same by-product B.

In by-product A, the highest individual carotenoid amounts were measured in the ethanol/acetone extract (Fig. 4B). The most abundant carotenoids in decreasing order were β-carotene (688.34 ± 45.20 mg/100 g extract), β-cryptoxanthin (645.65 ± 88.35 mg/100 g extract), antheraxanthin (443.94 ± 262.73 mg/100 g extract), and neoxanthin (411.43 ± 120.09 mg/100 g extract).

3.5. TPC

TPC determination indicated considerable variations among extracts from by-product B, which was selected for its higher concentration in carotenoids, since levels oscillated between ~49 and ~101 mg GAE/100 g extract (Table 1). The lowest value was recorded in the ethanol/acetone extract and the highest in acetone one, with 48.88 ± 3.84 and 101.28 ± 6.53 mg GAE/100 g extract, respectively.

3.6. Antioxidant activity

In this study, *in vitro* antioxidant activity of persimmon extracts was measured by two different analytical methods: ABTS and ORAC. Results are summarized in Table 1. Concerning ABTS, values varied significantly from ~3 to ~139 mM TE/100 g extract. According to TPC, the highest values were found in the acetone extract from by-product B, whilst the lowest ones were recorded both in the ethanol/acetone and the ethanol/acetone/hexane extracts. ORAC results were higher than those already remarked for ABTS with respect to the biological activity assayed (Table 1). The acetone extract displayed a moderately large antioxidant activity (~455 mM TE/100 g extract) while the ethanol, ethanol/acetone, and ethanol/acetone/hexane extracts showed values between ~210 and ~302 mM TE/100 g extract.

TPC results were correlated to ABTS ($r = 0.9783$; $R^2 = 95.7141$) and ORAC ($r = 0.9116$; $R^2 = 83.1099$) at the 95% ($P = 0.0217 < 0.05$) and 90% ($P = 0.0884 < 0.10$) confidence level, respectively. Since the confidence level of correlations is <99%, this suggests that TPC contributes only partially to the antioxidant activity of the extracts studied in this work. The initial washing with water of the fresh by-product drastically decreased the extractable amount of these compounds. In contrast, there was a statistically significant relationship between TCC in the different extracts from by-product B and ORAC values at the 99% ($r = 0.9999$; $R^2 = 99.9836$; $P = 0.0001 < 0.01$) confidence level, suggesting that the carotenoids contribute greatly to the antioxidant activity of the persimmon by-product extracts.

4. Discussion

Although production of persimmon fruits is mainly intended for fresh consumption, many unsuitable fruits must be processed annually to produce new derived products such as persimmon juice. González et al. (2015) reported for the first time an enzyme maceration process for production of persimmon juice at semi industrial scale which has been successfully tested in the juice industry. Jiménez-Sánchez et al. (2015)

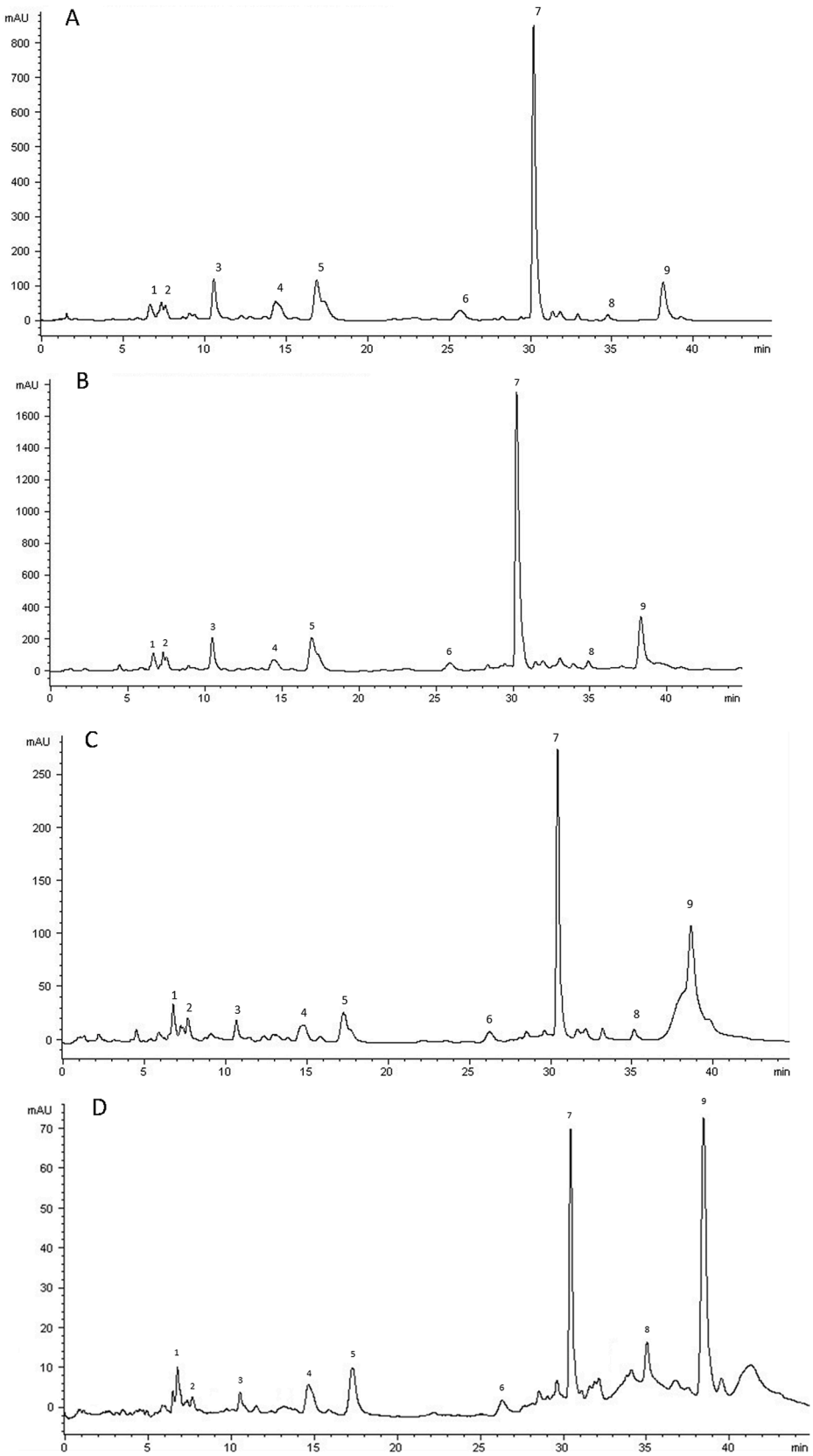

Fig. 3. HPLC-DAD Chromatograms of carotenoids in different extracts from persimmon by-product B: (A) Ethanol extract, (B) Acetone extract, (C) Ethanol/acetone (50:50 v/v) extract, and (D) Ethanol/acetone/hexane (25:25:50 v/v/v) extract. Peaks are labeled as follows: 1 = violaxanthin, 2 = neoxanthin, 3 = antheraxanthin, 4 = lutein, 5 = zeaxanthin, 6 = β-cryptoxanthin 5,6-epoxide, 7 = β-cryptoxanthin, 8 = α-carotene, and 9 = β-carotene.

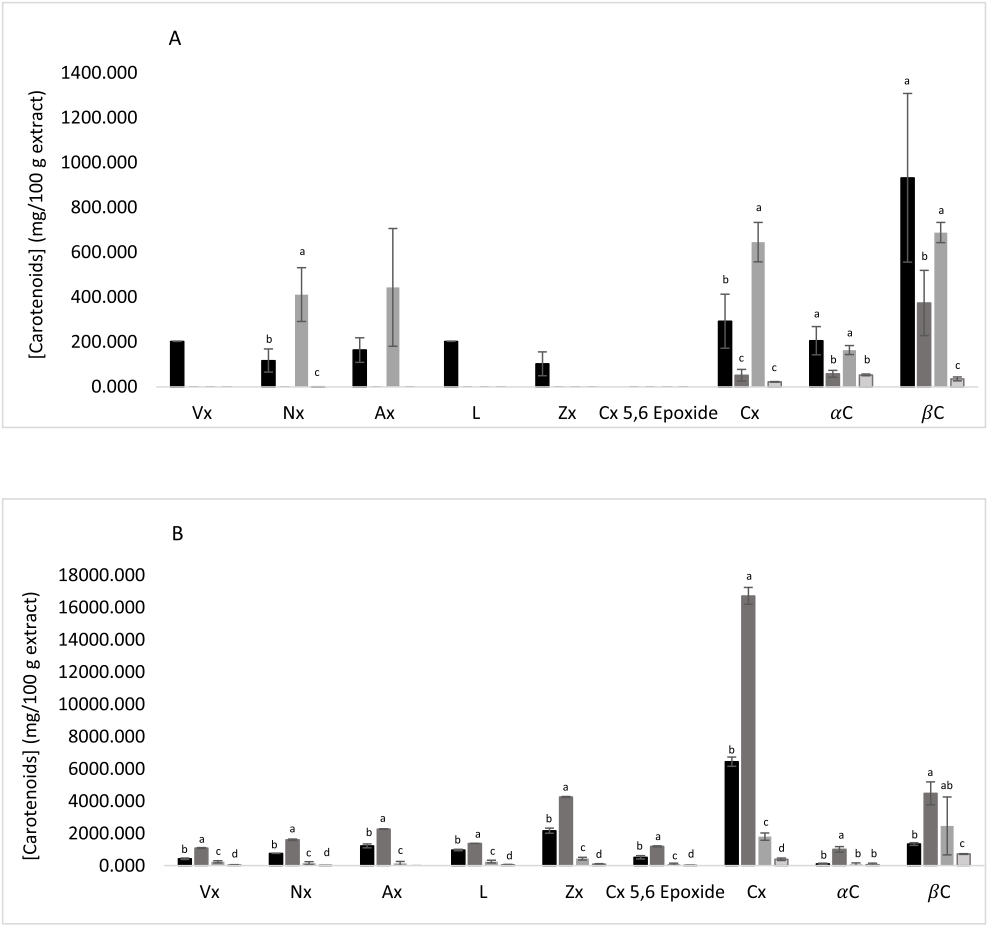

Fig. 4. Individual carotenoid contents (mg/100 g extract) of persimmon extracts obtained from (A) by-product A and (B) by-product B using different solvents or solvent combinations. Vx, violaxanthin; Nx, neoxanthin; Ax, antheraxanthin; L, lutein; Zx, zeaxanthin; Cx 5,6 Epoxide, β-cryptoxanthin 5,6 epoxide; Cx, β-cryptoxanthin; αC, α-carotene; and βC, β-carotene.

Table 1
Total phenolic compounds (TPC) and antioxidant activity assays (ABTS and ORAC) in different extracts from persimmon by-product B derived from juice processing.

Organic Solvents and Solvent Combinations	TPC (mg GAE/ 100 g extract)	ABTS (mM TE/ 100 g extract)	ORAC (mM TE/ 100 g extract)
Ethanol	86.64* ± 5.23b	123.37 ± 11.73 a	302.10 ± 8.77 b
Acetone	101.28 ± 6.53 a	138.98 ± 14.59 a	454.63 ± 11.65 a
Ethanol/Acetone	48.88 ± 3.84 c	11.61 ± 3.36 b	239.27 ± 10.60 c
Ethanol/Acetone/ Hexane	54.79 ± 5.76 c	3.36 ± 0.29 c	210.00 ± 3.73 d

* Values are reported as mean ± standard deviation (SD). Means followed by different lower-case letters are significantly different ($P < 0.05$) according to the Tukeýs multiple range test.

performed a complete qualitative analytical characterization through HPLC–DAD–ESI-TOF/MS of different persimmon juices produced under different technologies. Additionally, Martínez, Vegara, Martí, Valero, and Saura (2017) showed that production of persimmon beverages might open up new uses for discarded fruits. Unfortunately, juice industry generates a serious environmental problem of accumulation and waste disposal after processing. The new agro-industry should become a system where everything is used, trying to eliminate the generation of waste, according to the ZERI (zero emissions researches and initiatives) concept.

In relation to the use and revalorization of persimmon by-products generated in the juice production process, by-product B resulting from a macerating pectinase treatment to optimize juice extraction was especially suitable for recovery of valuable bioactive compounds like carotenoids. Furthermore, enzymatic pretreatment of a persimmon slurry was also effective in releasing carotenoids from complex food matrix, thus significantly improving the extraction yield in a similar way to an enzyme-assisted extraction (EAE) non-thermal method (Saini & Keum, 2018). In the other hand, by-product B showed the lowest %MC, what is generally considered favorable for the efficient extraction of carotenoids due to their hydrophobic nature. In summary both enzymatic treatment of by-product A and low %MC of by-product B influenced greatly the carotenoid yields obtained.

The main extraction methods of carotenoids reported using different persimmon tissues, as natural sources, are solid–liquid extraction (Izuchi et al., 2009; Veberic et al., 2010), accelerated solvent extraction (ASE) (Zaghdoudi et al., 2015), high-pressure treatment (HP) (De Ancos, González, & Cano, 2000), and supercritical fluid extraction (SFE) (Zaghdoudi et al., 2016). The choice of solvent is always the most critical factor for efficient extraction of carotenoids, and mainly depends on the carotenoid composition of the natural source (Saini & Keum, 2018). Usually, presence of carotenoids with varied levels of polarity makes their simultaneous extraction difficult. Polar solvents such as acetone and ethanol were chosen for extraction of dipolar (lutein and zeaxanthin) and monopolar (β-cryptoxanthin) carotenoids, whereas a mixture of ethanol/acetone/hexane was applied for the simultaneous extraction of polar and non-polar carotenoids (lycopene and carotenes). In general, solvent combinations provide synergistic effects on the

extraction of carotenoids. Our studies revealed the predominant presence of relatively polar xanthophylls in the persimmon by-products, which justified the high reached extraction efficiency using polar solvents such as ethanol and especially acetone.

Persimmon by-products A and B showed different EYs expressed as mg dry extract per 100 g DW. The highest EYs were exhibited by the by-product A and these were approximately 4.9, 11.5, 5.2, and 2.7 times higher than those from by-product B for the solvents ethanol, acetone or the solvent combinations ethanol/acetone and ethanol/acetone/hexane, respectively. Obtained EYs were higher than those from the agro-industrial by-products grape marc, mango bagasse and peanut skin (Braga et al., 2016). Intermediate EYs were obtained when extractions from persimmon seed powder were performed with ethanol (4,850 mg/100 g extract) and acetone (2,580 mg/100 g extract) as absolute solvents (Akter, Ahmed, & Eun, 2010). According to Babbar, Oberoi, Uppal, and Patil (2011), the type of plant residue would be more influential than the solvent system on extraction yield.

HPLC has become the method of choice for carotenoid analysis (Rivera & Canela-Garayoa, 2012) and reverse-phase columns like YMC Carotenoid C30 are the most widely used stationary phases for the analysis of these molecules. All of the identified carotenoids, except β-cryptoxanthin 5,6-epoxide and α-carotene, have been found in saponified extracts from flesh of persimmon fruits cv. Sharon grown in Spain (De Ancos et al., 2000). Carotenoid pattern changes in the peel of ripening persimmon fruits cv. Triumph were determined by Ebert and Gross (1985) using successive Thin-Layer Chromatographic (TLC) analytical methods. In this sense, the content of antheraxanthin, zeaxanthin, β-cryptoxanthin 5,6-epoxide, and β-cryptoxanthin increased in post-harvest intermediate ripe fruits (no fully ripe). The presence of lutein and β-carotene decreased drastically at the same ripening stage. In any case, β-cryptoxanthin and β-carotene represented 49.2% and 13.2% of the TCC determined in the acetone extract from by-product B. Additionally, the high content of both carotenoids give to this extract an important provitamin A value (De Ancos et al., 2000; Saini & Keum, 2018). Carotenoids which contain an unsubstituted β-ionone ring can be converted *in vivo* into vitamin A, developing the same biological effects.

Carotenoids have potential health benefits and some of them have been attributed to their antioxidant activity (Seifried, Harrison, & Seifried, 2017). According to Jomova and Valko (2013), carotenoids effectively scavenge peroxyl radicals and act predominantly as antioxidants. The growing interest in the substitution of synthetic food antioxidants by natural ones has fostered research on plant sources and the screening of inexpensive or residual materials from agricultural industries for extracting new antioxidants (Moure et al., 2001). Although polyphenols are the major plant compounds with antioxidant activity, various studies (González et al., 2015; Mena et al., 2011; Tezcan, Gültekin-Özgüven, Diken, Özçelik, & Erim, 2009) have reported differences relating to the phenolic compounds contribution to antioxidant capacity assays. Our data pointed out that TPC contributed only partially to the antioxidant activity of persimmon by-product extracts obtained herein whereas the TCC greatly did.

5. Conclusion

Two by-products derived from the industrialization of persimmon juice were used for carotenoid extraction in order to revalue these agro-industrial residues. This study showed that the by-product B resulting from a macerating pectinase enzyme treatment to optimize juice extraction was especially suitable for recovery of valuable carotenoids. The high content of carotenoids which contain an unsubstituted β-ionone ring gave to this extract an important provitamin A activity and capacity to develop similar biological effects. Sequence of processing persimmon peels and pulp by a macerating pectinase enzyme followed by the solid–liquid extraction of carotenoids from the resulting by-product using absolute acetone as solvent could be an efficient method to obtain a lyophilized extract that could be used as a nutraceutical ingredient.

CRediT authorship contribution statement

S. Gea-Botella: Investigation, Methodology, Formal analysis. **L. Agulló:** Investigation, Methodology. **N. Martí:** Conceptualization, Validation. **M.C. Martínez-Madrid:** Visualization, Supervision. **V. Lizama:** Methodology, Formal analysis. **F. Martín-Bermudo:** Conceptualization, Supervision. **G. Berná:** Investigation, Supervision. **D. Saura:** Conceptualization, Validation, Project administration. **M. Valero:** Conceptualization, Formal analysis, Data curation, Writing - original draft, Writing - review & editing, Project administration.

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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References

Akter, M. S., Ahmed, M., & Eun, J. (2010). Solvent effects on antioxidant properties of persimmon (*Diospyros kaki* L. cv. Daebong) seeds. *International Journal of Food Science & Technology, 45*(11), 2258–2264.

Arnal, L., & Del Río, M. A. (2003). Removing astringency by carbon dioxide and nitrogen-enriched atmospheres in persimmon fruit cv. "Rojo brillante". *Journal of Food Science, 68*(4), 1516–1518.

Aune, D., Giovannucci, E., Boffetta, P., Fadnes, L. T., Keum, N., Norat, T., … Tonstad, S. (2017). Fruit and vegetable intake and the risk of cardiovascular disease, total cancer and all-cause mortality—a systematic review and dose-response meta-analysis of prospective studies. *International Journal of Epidemiology, 46*(3), 1029–1056.

Ayala-Zavala, J. F., Vega-Vega, V., Rosas-Domínguez, C., Palafox-Carlos, H., Villa-Rodriguez, J. A., Wasim Siddiqui, Md., … González-Aguilar, G. A. (2011). Agro-industrial potential of exotic fruit byproducts as a source of food additives. *Food Research International, 44*, 1866–1874.

Babbar, N., Oberoi, H. S., Uppal, D. S., & Patil, R. T. (2011). Total phenolic content and antioxidant capacity of extracts obtained from six important fruit residues. *Food Research International, 44*(1), 391–396.

Berman, J., Zorrilla-López, U., Farré, G., Zhu, C., Sandmann, G., Twyman, R. M., … Christou, P. (2015). Nutritionally important carotenoids as consumer products. *Phytochemistry Reviews, 14*(5), 727–743.

Bolhassani, A. (2015). Cancer chemoprevention by natural carotenoids as an efficient strategy. *Anti-Cancer Agents in Medicinal Chemistry (formerly Current Medicinal Chemistry - Anti-Cancer Agents), 15*(8), 1026–1031.

Braga, G. C., Melo, P. S., Bergamaschi, K. B., Tiveron, A. P., Massarioli, A. P., & de Alencar, S. M. (2016). Extraction yield, antioxidant activity and phenolics from grape, mango and peanut agro-industrial by-products. *Ciência Rural, 46*(8), 1498–1504.

Cano, M. P., Gómez-Maqueo, A., Welti-Chanes, J., & García-Cayuela, T. (2018). Characterization of carotenoid and carotenoid esters of astringent persimmon tissues (*Diospyros kaki* Thunb. var. Rojo Brillante). Effects of thermal and high pressure non-thermal processing. *Preprints, 2018*. https://doi.org/10.20944/preprints201811.0548.v1.

Crupi, P., Coletta, A., Milella, R. A., Palmisano, G., Baiano, A., La Notte, E., & Antonacci, D. (2010). Carotenoid and chlorophyll derived compounds in some wine grapes grown in Apulian region. *Journal of Food Science, 75*, S191–S198.

Csepanyi, E., Czompa, A., Haines, D., Lekli, I., Bakondi, E., Balla, G., … Bak, I. (2015). Cardiovascular effects of low versus high-dose beta-carotene in a rat model. *Pharmacological Research, 100*, 148–156.

De Ancos, B., González, E., & Cano, M. P. (2000). Effect of high-pressure treatment on the carotenoid composition and the radical scavenging activity of persimmon fruit purees. *Journal of Agricultural and Food Chemistry, 48*(8), 3542–3548.

Ebert, G., & Gross, J. (1985). Carotenoid changes in the peel of ripening persimmon (Diospyros kaki) cv. Triumph. *Phytochemistry, 24*(1), 29–32.

Food and Agriculture Organization Corporate Statistical Database (FAOSTAT) (2017). Crops. Available in http://www.fao.org/faostat/en/#data/QC. Accessed on May 2, 2019.

González, E., Vegara, S., Martí, N., Valero, M., & Saura, D. (2015). Physicochemical characterization of pure persimmon juice: Nutritional quality and food acceptability. *Journal of Food Science, 80*(3), C532–C539.

Gorinstein, S., Zachwieja, Z., Folta, M., Barton, H., Piotrowicz, J., Zemser, M., … Martín-Belloso, O. (2001). Comparative contents of dietary fiber, total phenolics, and minerals in persimmons and apples. *Journal of Agricultural and Food Chemistry, 49*(2), 952–957.

Harrison, E. H. (2019). Mechanisms of transport and delivery of vitamin A and carotenoids to the retinal pigment epithelium. *Molecular Nutrition & Food Research, 2019*, 1801046.

Hosseini, B., Berthon, B. S., Saedisomeolia, A., Starkey, M. R., Collison, A., Wark, P. A. B., & Wood, L. G. (2018). Effects of fruit and vegetable consumption on inflammatory biomarkers and immune cell populations: A systematic literature review and meta-analysis. *American Journal of Clinical Nutrition, 108*, 136–155.

Izuchi, R., Takahashi, H., & Inada, Y. (2009). Preparing a carotenoid polyphenol-enriched extract from the peel of persimmon, *Diospyros kaki* L.f. *Bioscience, Biotechnology, and Biochemistry, 73*(12), 2793–2795.

Jamilah, B., Mohamed, A., Abbas, K., Abdul Rahman, R., & Karim, R. (2009). A review on the effect of animal diets and presence of selected natural antioxidants on lipid oxidation of meat. *Journal of Food, Agriculture & Environment, 7*(2), 76–81.

Jaswir, I., Noviendri, D., Hasrini, R. F., & Octavianti, F. (2011). Carotenoids: Sources, medicinal properties and their application in food and nutraceutical industry. *Journal of Medicinal Plants Research, 5*(33), 7119–7131.

Jiménez-Sánchez, C., Lozano-Sánchez, J., Marti, N., Saura, D., Valero, M., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2015). Characterization of polyphenols, sugars, and other polar compounds in persimmon juices produced under different technologies and their assessment in terms of compositional variations. *Food Chemistry, 182*, 282–291.

Jomova, K., & Valko, M. (2013). Health protective effects of carotenoids and their interactions with other biological antioxidants. *European Journal of Medicinal Chemistry, 70*, 102–110.

Kodagoda, K. H. G. K., & Marapana, R. A. U. J. (2017). Utilization of fruit processing by-products for industrial applications: A review. *International Journal of Food Science and Nutrition, 2*(6), 24–30.

Lapornik, B., Prošek, M., & Wondra, A. G. (2005). Comparison of extracts prepared from plant by-products using different solvents and extraction time. *Journal of Food Engineering, 71*(2), 214–222.

Martínez, A., Vegara, S., Martí, N., Valero, M., & Saura, D. (2017). Physicochemical characterization of special persimmon fruit beers using bohemian pilsner malt as a base. *Journal of the Institute of Brewing, 123*(3), 319–327.

Martínez-Calvo, J., Badenes, M. L., & Llácer, G. (2012). Descripción de variedades de caqui (Diospyros kaki Trunb.) del banco de germoplasma del IVIA. Monografías INIA: Serie Agrícola n° 28. Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid.

Mena, P., García-Viguera, C., Navarro-Rico, J., Moreno, D. A., Bartual, J., Saura, D., & Martí, N. (2011). Phytochemical characterisation for industrial use of pomegranate (*Punica granatum* L.) cultivars grown in Spain. *Journal of the Science of Food and Agriculture, 91*(10), 1893–1906.

Ministerio de Agricultura, Pesca y Alimentación (MAPA). (2018). https://www.mapa.gob.es/es/estadística/temas/estadísticas-agrarias/agricultura/superficies-producciones-anuales-cultivos/.

Moure, A., Cruz, J. M., Franco, D., Domínguez, J. M., Sineiro, J., Domínguez, H., … Parajó, J. C. (2001). Natural antioxidants from residual sources. *Food Chemistry, 72*(2), 145–171.

Müller, H. (1997). Determination of the carotenoid content in selected vegetables and fruit by HPLC and photodiode array detection. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung A, 204*(2), 88–94.

Olives Barba, A. I., Cámara Hurtado, M., Sánchez Mata, M. C., Fernández Ruiz, V., & López Sáenz de Tejada, M. (2006). Application of a UV–vis detection-HPLC method for a rapid determination of lycopene and β-carotene in vegetables. *Food Chemistry, 95*(2), 328–336.

Pérez-Burillo, S., Oliveras, M., Quesada, J., Rufián-Henares, J., & Pastoriza, S. (2018). Relationship between composition and bioactivity of persimmon and kiwifruit. *Food Research International, 105*, 461–472.

Porter, S. D., Reay, D. S., Bomberg, E., & Higgins, P. (2018). Avoidable food losses and associated production-phase greenhouse gas emissions arising from application of cosmetic standards to fresh fruit and vegetables in Europe and the UK. *Journal of Cleaner Production, 201*, 869–878.

Rivera, S., & Canela-Garayoa, R. (2012). Analytical tools for the analysis of carotenoids in diverse materials. *Journal of Chromatography A, 1224*, 1–10.

Saini, R. K., & Keum, Y.-S. (2018). Carotenoid extraction methods: A review of recent developments. *Food Chemistry, 240*, 90–103.

Seifried, R. M., Harrison, E. H., & Seifried, H. E. (2017). Antioxidants in health and disease. In A. M. Coulson, C. J. Boushey, M. G. Ferruzzi, L. M. Delahanty (Eds.) Nutrition in the Prevention and Treatment of Disease, 4th Edition, Chapter 16. Elsevier/Academic Press, Oxford, UK.

Tezcan, F., Gültekin-Özgüven, M., Diken, T., Özçelik, B., & Erim, F. B. (2009). Antioxidant activity and total phenolic, organic acid and sugar content in commercial pomegranate juices. *Food Chemistry, 115*(3), 873–877.

UN General Assembly, Transforming our world: the 2030 Agenda for Sustainable Development, 21 Oct. 2015, https://www.refworld.org/docid/57b6e3e44.htm.

Veberic, R., Jurhar, J., Mikulic-Petkovsek, M., Stampar, F., & Schmitzer, V. (2010). Comparative study of primary and secondary metabolites in 11 cultivars of persimmon fruit (*Diospyros kaki* L.). *Food Chemistry, 119*(2), 477–483.

Vegara, S., Martí, N., Lorente, J., Coll, L., Streitenberger, S., Valero, M., & Saura, D. (2014). Chemical guide parameters for *Punica granatum* cv. 'Mollar' fruit juices processed at industrial scale. *Food Chemistry, 147*, 203–208.

Zaghdoudi, K., Framboisier, X., Frochot, C., Vanderesse, R., Barth, D., Kalthoum-Cherif, J., … Guiavarc'h, Y. (2016). Response surface methodology applied to supercritical fluid extraction (SFE) of carotenoids from persimmon (*Diospyros kaki* L.). *Food Chemistry, 208*, 209–219.

Zaghdoudi, K., Pontvianne, S., Framboisier, X., Achard, M., Kudaibergenova, R., Ayadi-Trabelsi, M., … Guiavarc'h, Y. (2015). Accelerated solvent extraction of carotenoids from: Tunisian kaki (*Diospyros kaki* L.), peach (*Prunus persica* L.) and apricot (*Prunus armeniaca* L.). *Food Chemistry, 184*, 131–139.