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Research Article

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Thinned stone fruits are a source of polyphenols and antioxidant compounds

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Abstract

BACKGROUND: Thinned fruits are agricultural by-products that contain large quantities of interesting compounds due to their early maturity stage. In this work, the phenolic **profile** and the antioxidant activity of six thinned stone fruits (apricot, cherry, **fl**at peach, peach, plum and nectarine) have been investigated, focussing on proanthocyanidins.

RESULTS: Thinned nectarine had the highest content of total phenols [67.43 mg gallic acid equivalents (GAE) g^{-1} dry weight (DW)] and total **fl**avonoids (56.97 mg CE g^{-1} DW) as well as the highest antioxidant activity measured by DPPH scavenging (133.30 mg [Trolox equivalents (TE) g^{-1} DW] and FRAP assay (30.42 mg TE g^{-1} DW). Proanthocyanidins were very abundant in these by-products, and the main phenolic group **quantified** in cherry (10.54 mg g^{-1} DW), **fl**at peach (33.47 mg g^{-1} DW) and nectarine (59.89 mg g^{-1} DW), while hydroxycinnamic acids predominate in apricot, peach and plum (6.67, 22.04 and 23.75 mg g^{-1} DW, respectively). The low, mean degree of polymerisation of proanthocyanidins suggests that their bioavailability could be very high.

CONCLUSIONS: This study shows that thinned stone fruit extracts might be used as antioxidants in foods or as a source of compounds with health-related **benefits** that can be used in the pharmaceutical, cosmetic and food industries.
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Keywords: thinned stone fruits; by-products; proanthocyanidins; hydroxycinnamic acids; antioxidant activity

INTRODUCTION

Industrial processing of fruit and vegetables generates substantial quantities of waste/by-products. In recent years it has been amply demonstrated that waste and by-products of fruit and vegetables may be an abundant source of antioxidant polyphenols and other phytochemicals and health-promoting compounds such as terpenoids (carotenoids, essential oils, steroids, etc.), nitrogen and sulfur-containing compounds, etc.[1–3] The biological activity of these compounds is often related to their antioxidant capacity or their ability to neutralise free radicals that are the origin of many diseases.[4] Most studies have focused on the use of industrial by-products, mainly for their application in the pharmaceutical, cosmetic and food industries. Examples are food supplements with high antioxidant contents based on resveratrol from grape pomace or on proanthocyanidins from grape seeds and apple pomace, or body and facial creams based on oils from both peach and apricot seeds. However, there are some agricultural practices, such as pruning or thinning, which also generate substantial quantities of waste whose contents have not yet been studied.

Stone fruit trees generally set more fruit than can be grown to a marketable size. Therefore, it is necessary to thin some fruits, thereby reducing their total number and increasing both their final size and the value of the crop. Thinning also relieves the tree of excess loads, removes the undesirable fruit (doubled, misshapen, scarred, injured, or undersized), and improves the formation of fruit buds for the next season's crop.[5] However, these small fruits are abandoned in the field generating large quantities of waste or, even worse, being incinerated with the environmental problems which that entails.[6] Moreover, thinning has both economic and time costs which have been calculated at 3.43–4.11 euro $tree^{-1}$ and 200–300 h $hectare^{-1}$.[7]

Some studies have shown the influence of the maturity and ripening stage on the phytochemical content in fruits and veg- etables. It has been demonstrated that the phenolic content is higher in immature fruits at an early stage.[6,8–12] These compounds decrease during typical fruit ripening whereas levels of colourful anthocyanidins increase.[13] As thinned fruits have a very early maturity stage, the concentration of phenolic compounds should be very high. They might therefore be considered as a rich source of bioactive compounds which may be extracted for use as supplements in the food, pharmaceutical and cosmetics industries.[6] The exploitation of thinned fruits for the extraction of compounds of both nutritional and technological importance[14] may be considered to have considerable economic and environmental benefits.

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Table 1. Physico-chemical parameters of the six thinned stone fruits

Species	Cultivar	Date of thinning	Equatorial diameter (mm)	Polar diameter (mm)	Weight (g)	TSS (° Brix)	TA (g malic acid kg^{-1})	Water content (%)
Apricot	Pink Cot	27 April 2013	25.9 ± 1.0	28.2 ± 0.9	11.7 ± 1.7	7.1 ± 0.3	26.4 ± 0.8	86.9 ± 0.9
		29 April 2014	26.0 ± 0.9	27.9 ± 0.9	10.9 ± 1.4	7.8 ± 0.4	27.5 ± 0.6	87.6 ± 0.6
Cherry	20-09	30 April 2013	13.8 ± 0.8	15.2 ± 0.8	2.1 ± 0.2	7.7 ± 0.3	11.7 ± 0.6	87.1 ± 1.6
		29 April 2014	13.8 ± 1.0	14.9 ± 0.7	1.9 ± 0.3	7.2 ± 0.5	13.3 ± 0.3	86.4 ± 2.8
Flat peach	UFO-3	27 April 2013	20.8 ± 1.2	12.1 ± 1.1	3.1 ± 0.6	7.7 ± 0.3	10.0 ± 0.2	84.4 ± 0.9
		28 April 2014	19.8 ± 1.3	11.5 ± 1.4	3.0 ± 0.6	7.7 ± 0.2	9.9 ± 0.4	83.2 ± 2.0
Peach	Royal Glory	29 April 2013	21.4 ± 1.7	28.0 ± 3.1	3.9 ± 0.2	8.3 ± 0.5	8.1 ± 0.3	86.1 ± 1.8
		26 April 2014	17.4 ± 1.9	21.1 ± 2.5	2.8 ± 0.2	8.1 ± 0.3	7.8 ± 0.1	88.4 ± 2.0
Plum	Tolosa	07 May 2013	19.9 ± 0.9	24.6 ± 1.6	5.6 ± 0.4	7.2 ± 0.3	19.0 ± 0.5	88.1 ± 1.9
		06 May 2014	20.0 ± 1.3	26.4 ± 1.8	5.1 ± 0.5	7.7 ± 0.2	20.8 ± 0.2	86.2 ± 1.1
Nectarine	Laura	30 April 2013	21.1 ± 1.7	26.8 ± 2.3	4.4 ± 1.0	7.5 ± 0.1	7.4 ± 0.6	87.1 ± 1.7
		27 April 2014	22.3 ± 2.5	25.4 ± 3.1	4.3 ± 1.0	7.6 ± 0.3	8.4 ± 0.2	86.8 ± 1.4

Values are the mean ± standard deviation of 2 years.

Phenolic compounds represent a large and important group of abundant secondary metabolites in fruit and vegetables. Polyphenols in plants contribute to several sensory properties and defence mechanisms, and their role in human health protection, related to antioxidant and anti-radical activities, has been repeatedly suggested.[15] Many classes of polyphenols, classically distinguished as flavonoids and non-flavonoids, are known to be present in many plant tissues. Among these compounds, proanthocyanidins have attracted considerable attention in recent years due to their human health benefits such as reducing cardiovascular diseases, carcinogenesis, neurodegeneration, skin deterioration, diabetic or anti-hyperglycaemic problems, as well as their anti-tyrosinase activities.[16,17] Proanthocyanidins (PAs) are composed of flavan-3-ol monomer units (catechin or epicatechin) linked mainly through C4–C8 or C4–C6 interflavan bonds,[18] which form oligomeric proanthocyanidins and polymeric proanthocyanidins.[19] PAs have properties such as forming stable complexes with metal ions and proteins, and act as good reducing agents. For example, they are able to scavenge reactive oxygen species (ROS), which include radical and non-radical oxygen species such as O_2^-, HO, H_2O_2, $^1O_2$, HOCl, as well being able to generate RO and ROO free radicals such as those derived from low-density lipoprotein, proteins, and oligonucleic acids (DNA and RNA).[20]

In this study, the potential of six thinned stone fruits (apricot, cherry, flat peach, peach, plum and nectarine) as a natural source of phenolic compounds with high antioxidant activity was examined. Special attention has been focused on proanthocyanidins due to their important health benefits. To the best of our knowledge, this is the first report concerning the identification and quantification of bioactive compounds in thinned stone fruits.

EXPERIMENTAL

Fruit samples

Apricots (*Prunus armeniaca* cv. Pink Cot), cherries (*Prunus avium* cv. 13S-20-09), flat peaches (*Prunus persica* cv. UFO-3), peaches (*Prunus persica* cv. Royal Glory), plums (*Prunus domestica* cv. Tolosa) and nectarines (*Prunus persica* cv. Laura) were hand-thinned in two seasons at a commercial orchard in Nonaspe (Zaragoza, Spain) located at coordinates 41° 13′ 24.78″ north (latitude) and 0° 13′ 4.76″ east (longitude) on different days in April or May, but in all cases 42 days after full bloom in 2013 and 48 days after full bloom in 2014 (Table 1).

The experiment involved 20 trees randomly located with the same growth vigour and tree age for each species. For each species, 800 fruits (40 samples per tree) of similar size, colour and an absence of any defect were randomly and manually picked and transferred immediately to the laboratory. One hundred fruits were used for measurement of the fruit size, weight, total soluble solids (TSS), titratable acidity (TA) and water content immediately after picking. Table 1 shows a physico-chemical description of the different fruits and Fig. 1 their visual appearance. Each fruit was weighed on a precision scale to 0.01 g confidence level and the equatorial and polar diameters were measured using a digital calliper (Mitutoyo, Tokyo, Japan). The soluble solids content (SSC) was determined by crushing the flesh and transferring the intact juice of 25 samples to a digital refractometer (Atago, Tokyo, Japan). Titratable acidity (TA) was measured using an automatic titrator (Crison, Barcelona, Spain). Ten grams of juice of 20 fruits (in triplicate) were diluted with 90 mL of distilled H_2O and titrated with 0.1 mol L^{-1} NaOH solution up to pH 8.1, expressing the results as grams malic acid per kilogram. The water content of 15 whole fruits was determined using a halogen moisture-meter, model HR73 (Mettler Toledo, New York, NY, USA).

The rest of the fruits were used for the determination of phe- nolic and flavonoid contents and antioxidant activities. The sam- ples were frozen in liquid nitrogen, freeze-dried (LyoBeta Telstar, Barcelona, Spain), ground using a pestle and mortar, vacuum packed and maintained in a desiccator at room temperature and darkness until analysis.

Preparation of extracts

The extracts were obtained by mixing 1 g of freeze-dried sample with 100 mL of a methanol/water solution (80:20; v:v) and homogenised with an ultraturrax during 30 s. They were then centrifuged at 4000 rpm for 10 min and at 4° C and the supernatant was filtered through a 45 μm nylon filter membrane. The extraction was done twice and both supernatants were mixed and stored at −18° C prior to further use and analysed within a month from extraction.

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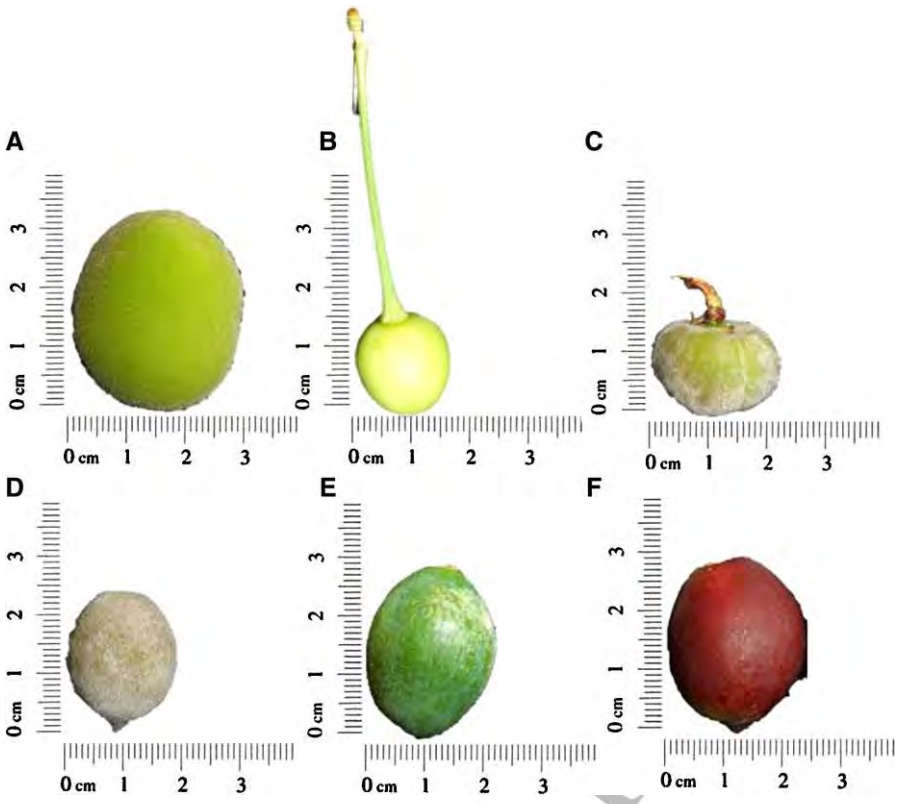

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Figure 1. Visual appearance of the stone fruits on the date of thinning. (A) apricot cv. Pink Cot; (B) cherry cv. 20–09; (C) flat peach cv. UFO-3; (D) peach cv. Royal Glory; (E) plum cv. Tolosa; (F) nectarine cv. Laura.

Phenolic compounds

Total phenolic content

The TPC was determined by the Folin–Ciocalteu method[21] with some modifications. An aliquot (1 mL) of extract or standard solution (0–250 mg L^{-1}) of gallic acid (Sigma, St. Louis, MO, USA) was added to a 10 mL volumetric flask and mixed with 1 mL of Folin–Ciocalteu reagent. After 5 min, 1 mL of 7.5% sodium carbonate solution was added and the solution diluted to 10 mL with deionised water. After incubation for 60 min at room temperature in darkness, the absorbance was determined at 760 nm with a spectrophotometer (Unicam, Waltham, MA, USA). TPC was expressed as mg gallic acid equivalents (GAE) per 100 g dry weight (DW).

Total flavonoid content

The TFC of the extracts was determined using a colorimetric assay[22] with some modifications. Briefly, at zero time 0.1 mL of 5% $NaNO_2$ (w/v) was added to 0.5 mL of extract or standard solution. After 5 min, 0.1 mL of 10% $AlCl_3$ (w/v) was added and after 6 min, 0.6 mL of 1 mol L^{-1} NaOH was added and immediately diluted with 1.7 mL of distilled water. A calibration curve was constructed with different concentrations (0–100 mg L^{-1}) of catechin (Sigma) as the standard. Absorbance of the pink mixture samples was measured with a spectrophotometer at 510 nm and the TFC was expressed at mg catechin equivalents (CE) per 100 g DW.

Identification of phenols by HPLC-DAD-MS^n/ESI

For the identification of phenolic compounds, 0.1 g of lyophilised fruit powder was extracted with 1 mL of methanol/water/formic acid (80:19:1, v/v) by sonication for 30 min. The resulting extract was centrifuged and filtered through a 0.22 μm PVDF filter. Chromatographic analyses were carried out on a LiChrocart C18 column (250 × 4 mm, 5 μm particle size; Merck, Darmstadt, Germany). The mobile phase was composed of two solvents: water with formic acid (1%) (A) and methanol (B) starting with 5% B and using a gradient to obtain 50% B at 22 min and 90% B at 27 min, using this isocratic solution for 1 min. The flow rate was 500 μL min^{-1} and the injection volume was 5 μL. Spectral data from all peaks were accu- mulated in the range 200 – 400 nm. Chromatograms were recorded at 320 and 360 nm. The HPLC-DAD-MS^n/ESI analyses were carried out in an Agilent 117 HPLC 1200 series (Agilent Technologies, Wald- bronn, Germany) equipped with a binary pump (model G1376A), an autosampler (model G1377A) refrigerated at 4 ° C (model G1330B), a degasser (model G1379B), and a photodiode array detector (model 120 G1315D). The HPLC system was controlled by ChemStation software (Agilent, v.B.01.03-SR2). The mass detector was a Bruker ion trap spectrometer (model HCT Ultra) equipped

with an electrospray ionisation interface controlled by software (LCMSD, Agilent, v. 6.1). The ionisation conditions were 300 ° C and 4.0 kV for capillary temperature and voltage, respectively. The nebuliser pressure and flow rate of nitrogen were 5.0 psi and 3L min^{-1}, respectively. The full scan mass covered the range from *m*/*z* 100 up to *m*/*z* 1200 and the target mass was adjusted to 350. Collision-induced fragmentation experiments were performed in the ion trap using helium as the collision gas, with voltage ramping cycles from 0.3 up to 2 V. Mass spectrometry data were acquired in the negative mode and the MS^n was carried out in the automatic mode. The identification of the peaks was carried out by the extracted ion-chromatograms of the ion current at *m*/*z* values corresponding to the $[M - H]^-$ ions of the individual inves- tigated compounds, as well as their fragmentation. Quantification

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Table 2. Total phenol content, total flavonoid content, DPPH scavenging and FRAP assay of six thinned stone fruits

Species	Cultivar	TPC (mg GAE g^{-1})	TFC (mg CE g^{-1})	DPPH scavenging (mg TE g^{-1})	FRAP (mg TE g^{-1})
Apricot	Pink Cot	9.32 ± 0.84^{e}	7.72 ± 1.13^{f}	31.93 ± 2.30^{d}	20.73 ± 1.01^{b}
Cherry	20-09	13.29 ± 1.42^{d}	10.24 ± 1.38^{e}	18.83 ± 2.59^{e}	17.50 ± 1.61^{c}
Flat peach	UFO-3	35.03 ± 1.58^{b}	33.04 ± 2.48^{b}	80.44 ± 4.88^{b}	21.33 ± 1.35^{b}
Peach	Royal Glory	15.79 ± 1.65^{d}	12.62 ± 1.99^{d}	22.72 ± 3.94^{e}	22.10 ± 1.47^{b}
Plum	Tolosa	25.70 ± 2.85^{c}	22.82 ± 3.33^{c}	63.13 ± 5.28^{c}	19.49 ± 2.07^{bc}
Nectarine	Laura	67.43 ± 3.54^{a}	56.97 ± 2.56^{a}	133.30 ± 4.48^{a}	30.42 ± 3.44^{a}

The samples were analysed in triplicate and the results are presented as mean values ± standard deviation of 2 years. Values are given on a dry weight (DW) basis.
Different letters in the same column indicate significant differences ($P < 0.05$).

of the identified analytes was performed by HPLC-DAD using the external standard methods with calibration graphs, as a function of concentration based on peak area, detected at the wavelength corresponding to the maximum absorbance (280 for flavan-3-ols, 320 for hydroxycinnamic acids and 360 for flavonols). Flavan-3-ols were quantified as catechin (Sigma), hydroxycinnamic acids as chlorogenic acid (5-*O*-caffeoylquinic acid) (Sigma) and flavonoids as quercetin-3-rutinoside (Sigma). The identification and quantifi- cation of phenols was performed only in the samples of 2014.

Determination of proanthocyanidin using phloroglucinol

The procedure[23] used was started by preparing a 0.1 mol L^{-1} HCl (37%) methanol solution (solution A). Solution B was then prepared by dissolving 120 mg of phloroglucinol in 2.4 mL of solution A. Finally, solution C was prepared dissolving 20 mg of ascorbic acid in 2 mL of solution B. The reaction started adding 800 μL of solution C to 50 mg of the lyophilised samples. They were vortexed to completely dissolve the powder and then incubated at 50 ° C for 20 min. The reaction was stopped by placing the samples in an ice bath and by diluting the reaction medium with 1 mL of a 40 mmol L^{-1} sodium acetate solution. The samples were centrifuged at 4000 rpm during 10 min at 5 ° C and then filtered with 45 μm nylon filter membrane. The samples (10 μL) were then analysed by the reversed phase on an 1100 series HPLC-DAD system (Agilent Technologies). This was equipped with a G1312A binary pump, a G1313A autosampler, a G1315B photodiode array detector, controlled by the Agilent software v. A.08.03, and a G1322A degasser. The column was an Atlantis dC18 (particle size 5 μm, 4.6 × 250 mm) purchased from Waters (Barcelona, Spain). The HPLC was coupled to an ion-trap mass spectrometer equipped with an electrospray ionisation system (ESI). The heated capillary

and voltage were maintained at 350 C and 4 kV, respectively. Mass scan (MS) and MS/MS daughter spectra were measured from *m*/*z* 100 to 1200. Collision-induced fragmentation experiments were performed in the ion trap using helium as the collision gas, and the collision energy was set at 50%. Mass spectrometry data were acquired in the negative ionisation modes. The mobile phase was a water/acetic acid (97.5:2.5 v/v) (A) and acetonitrile (B) mixture. The flow rate was 1.0 mL min^{-1} and the linear gradient applied was: 3% B at 0 min, 9% B at 5 min, 16% B at 15 min, 50% B at 45 min, the same gradient until 52 min, followed by washing and reconditioning the column with 3% B until 57 min. A chromatogram was recorded at 280 nm. The external standard was epicatechin (Sigma) and catechin (Sigma). The results were expressed as mg g^{-1} DW and the apparent mean degree of polymerisation (mDP) was also determined.[24] The quantification of proanthocyanidins was performed only in the samples of 2014.

Antioxidant activity

2,2-Diphenyl-1-picrylhydrazyl scavenging

DPPH is a stable azo free radical. Its colour changes from violet to yellow when it is reduced by the electron donation process.[25] Briefly, 900 μL of diluted extract were mixed with 900 μL of DPPH (133 μmol L^{-1} in methanol; Sigma). The free radical scavenging activity was evaluated by measuring the variation in absorbance at 515 nm after 150 min of reaction and the results were expressed as mg of Trolox equivalents (TE) 100 g^{-1} DW.

Ferric reducing antioxidant power assay

The FRAP assay is based on the ability of Fe^{3+} to form a Fe^{2+}-TPTZ complex, and measuring the blue colour generated in the sample.[26] The FRAP solution was prepared by mixing 25 mL acetate buffer (300 mmol L^{-1}, pH 3.6), 2.5 mL TPTZ solution (2,4,6-tripyridyl-*s*-triazine, 10 mmol L^{-1} in 40 mmol L^{-1} HCl) and 2.5 mL $FeCl_3 \cdot 6H_2O$ (20 mmol L^{-1}). Then, 150 μL of FRAP solution was allowed to react with 20 μL of each extract in the well of a 96-well polypropylene plate (MIDSCI, Valley Park, MO, USA). Absorbance at 595 nm was measured after 30 min in a microplate reader (Tecan Trading AG, Männedorf, Switzerland). The standard solution (0 – 1000 μmol L^{-1}) was made with Trolox (Sigma) and the results were expressed as mg TE 100 g^{-1} DW.

Statistical analysis

All samples were analysed in triplicate per year and the results were presented as mean values ± standard deviation of the two years. Statistical analyses were performed using a one-way ANOVA test and the significance of the difference between means was determined by Duncan's multiple range test ($P < 0.05$). Corre- lations were calculated according to Pearson's test at $P \leq 0.01$. Statistical analyses were performed using the Statistical Pack- age for the Social Science (SPSS) software version 22.0 (SPSS, Chicago, IL, USA).

RESULTS AND DISCUSSION

Total polyphenols and identification of individual phenols

Table 2 shows the total phenol and flavonoid contents of thinned fruits. Nectarine was the fruit with the highest content of total phenols (67.43 mg GAE g^{-1} DW), followed by flat peach (35.03 mg GAE g^{-1} DW). The apricot and cherry samples had the lowest content (9.32 and 13.29 mg GAE g^{-1} DW, respectively). This pattern was very similar for the TFC (R^2=0.995). The highest values were obtained for nectarine (56.97 mg CE g^{-1} DW) while the lowest were for apricot (7.72 mg CE g^{-1} DW).

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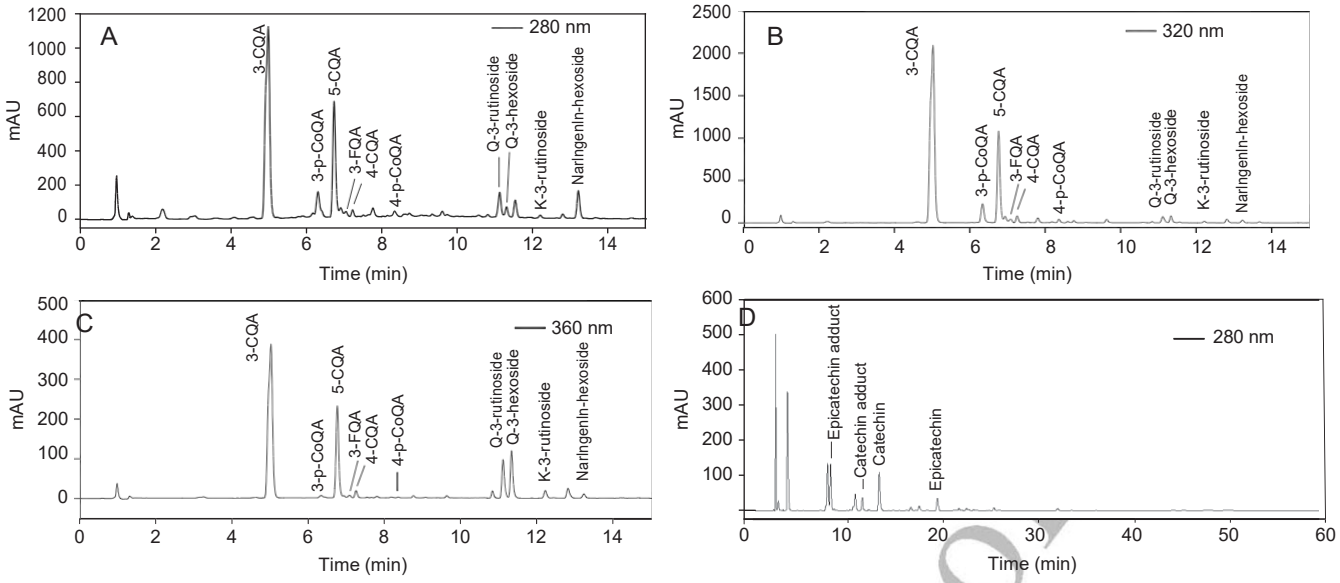

Figure 2. Example of chromatograms of thinned nectarine obtained by HPLC-MS at (A) 280 nm, (B) 320 nm and (C) 360 nm after 80% methanol extraction for quantification of individual phenols and at (D) 280 nm after acid catalysis with phloroglucinol for quantification of proanthocyanidins. 3-CQA, neochlorogenic acid; 3-*p*-CoQA, 3-*p*-coumaroylquinic acid; 5-CQA, chlorogenic acid; 3-FQA, 3-feruloylquinic acid; 4-CQA, 4-caffeoylquinic acid; 4-*p*-CoQA, 4-*p*-coumaroylquinic acid; Q-3-rutinoside, quercetin-3-rutinoside; Q-3-hexoside, quercetin-3-hexoside; K-3-rutinoside, kaempherol-3 rutinoside; narigenin-hesoxide.

It is difficult to compare these results with those found in the literature because this study is the first report concerning compounds in thinned stone fruits. The results have therefore been compared with the amounts present in other unripe fruits and by-products. The TPC and TFC obtained for thinned fruits are similar to those observed in fruits at an immature development stage such as Brazilian cherries (*Eugenia uniflora* L.), with 41.4 and 51.8 mg GAE g^{-1} DW for red and purple cultivars, respectively,[12] and calamondin peel with 25.7 mg GAE g^{-1} DW and 7.0 mg CE g^{-1} DW.[11] For industrial by-products, the optimisation of the extraction of total phenols and flavonoids in grape stems (20 min at 60 °C with ethanol 40%) led to 68.8 mg GAE g^{-1} DW and 68.2 mg CE g^{-1} DW, respectively.[27] However, the majority of the fruit by-products had lower contents than ours, for example apple pomace (4.8 mg GAE g^{-1} DW), orange bagasse (8.6 mg GAE g^{-1} DW), passion fruit peel (6.9 mg GAE g^{-1} DW),[28] banana peel (9.3 mg GAE g^{-1} DW),[2] onion by-products (4.1 mg GAE g^{-1} DW),[29] rice bran (3.5 mg GAE g^{-1} DW),[30] etc. Only certain by-products from tropical fruits had high TFC such as avocado (82.0 mg GAE g^{-1} DW) and mango (117.0 mg GAE g^{-1} DW) seeds.[2]

Apart from the TPC and TFC, it is obviously of interest to identify the individual phenolic content. Preliminary studies with different solvents were carried out in order to choose the best option to extract the highest amounts of individual phenols. Finally, two extractions were used separately: 80% methanol for flavonols, phenolic acids and hydroxycinnamic acids whereas proanthocyanidins were better extracted after acid catalysis (Fig. 2).

As can be seen in Table 3, the sum of total phenolic acids was the highest in nectarine (75.92 mg g^{-1} DW), followed by flat peach (55.95 mg g^{-1} DW), plum (33.59 mg g^{-1} DW), peach (29.59 mg g^{-1} DW) and cherry (15.46 mg g^{-1} DW), while apricot was the lowest (9.97 mg g^{-1} DW). These results, although higher, are very similar to the values obtained for the TFC using the Folin–Ciocalteu reagent.

Flavan-3-ols

No proanthocyanidins were detected when a general method for the identification of phenols was used. However, with an acid catalysis in the presence of an excess of phloroglucinol, an increase in the quantitative conversion of proanthocyanidins into their constitutive sub-units was achieved. This could be the reason why the TFC was lower than the amounts of phenols identified, since in the latter case the concentration of proanthocyanidins after acid catalysis has been taken into account.

The terminal sub-units were flavanol-3-ol monomers, while the extension sub-units reacted with phloroglucinol giving phloroglucinol adducts.[24] The products formed and identified after acid-catalysed cleavage of proanthocyanidins from stone fruits were catechin and epicatechin as terminal sub-units and epicatechin–phloroglucinol as an extension unit.

Proanthocyanidins after acid catalysis were by far the most abun- dant compounds identified in thinned fruits, ranging from 3.04 in apricot to 59.89 mg g^{-1} DW in nectarine (Table 4). These results are consistent with those reported by other authors[31,32] in which the proanthocyanidins were the compounds with the highest contents. As indicated in Table 4, catechin was the compound which had the highest concentration in the studied fruits (ranging from 1.03 in apricots to 20.52 mg g^{-1} DW in nectarines) except for flat peaches in which case it was epicatechin (12.67 mg g^{-1} DW). However, high amounts of epicatechin extension units (between 20.1% and 72.1% of total proanthocyanidins) were also detected.

A comparison of the content of PAs is difficult due to both the lack of research into the content of these compounds in thinned fruits and the different methodologies used to quantify the com- pounds. Some of these under-estimate the results because only monomers, dimers and trimers are detected and no conversion into sub-units is done. Some authors[33] have studied the con- centrations of proanthocyanidins in common foods, detecting the highest contents in cinnamon (89.6 mg g^{-1} DW), sorghum bran (39.6 mg g^{-1} DW) and grape seeds (35.3 mg g^{-1} DW). Among fruits, plums (17.1 mg g^{-1} DW), red delicious apple (9.5 mg g^{-1}

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Table 3. Identification and quantification of phenolic compounds in six thinned stone fruits (mg g^{-1} DW)

Phenolic compound	Apricot cv. Pink Cot	Cherry cv. 20-09	Flat peach cv. UFO-3	Peach cv. Royal Glory	Plum cv. Tolosa	Nectarine cv. Laura
Flavan-3-ols						
Catechin	ND	ND	ND	ND	ND	ND
Epicatechin	ND	ND	ND	ND	ND	ND
Proanthocyanidins†	3.04 ± 0.08^{f}	10.54 ± 0.19^{d}	33.47 ± 4.16^{b}	13.79 ± 0.62^{c}	9.69 ± 1.87ed	59.89±3.64^{a}
Total flavan-3-ols	3.04	10.54	33.47	13.79	9.69	59.89
Flavonols						
Quercetin-3-hexoside	ND	ND	0.17 ± 0.01^{a}	ND	ND	0.22±0.05^{a}
Quercetin 3-rutinoside	0.23 ± 0.01^{a}	0.09 ± 0.01^{c}	0.18 ± 0.02^{b}	0.06 ± 0.01^{d}	0.15 ± 0.02^{b}	0.18 ± 0.04^{ab}
Kaempherol-3-hexoside	ND	ND	ND	0.04 ± 0.01^{a}	ND	ND
Kaempherol-3-rutinoside	0.03 ± 0.00^{c}	0.03 ± 0.00^{c}	0.09 ± 0.01^{a}	0.05 ± 0.01^{b}	ND	0.04±0.01^{b}
Total flavonols	0.26	0.12	0.44	0.15	0.15	0.44
Flavanones						
Naringenin-hexoside	ND	ND	*	ND	ND	*
Total flavanones	–	–	–	–	–	–
Hydroxycinnamic acids						
Neochlorogenic acid	1.65 ± 0.08^{d}	1.88 ± 0.33^{d}	13.00 ± 1.00^{ab}	8.43 ± 1.22^{c}	15.00 ± 1.52^{a}	10.75 ± 2.38^{bc}
Chlorogenic acid	4.34 ± 0.08^{c}	0.31 ± 0.02^{e}	7.04 ± 0.53^{a}	5.63 ± 0.77^{b}	3.62 ± 0.44^{d}	3.54±0.76^{d}
Isochlorogenic acid	ND	0.04 ± 0.00^{c}	0.23 ± 0.02^{a}	0.09 ± 0.03^{b}	0.07 ± 0.01^{b}	ND
4-*p*-Coumaroylquinic acid	0.18 ± 0.03^{b}	0.14 ± 0.01^{c}	0.19 ± 0.01^{b}	0.17 ± 0.02^{b}	0.15 ± 0.02^{bc}	0.84±0.19^{a}
4-Caffeoylquinic acid	0.22 ± 0.01^{c}	0.11 ± 0.01^{d}	0.14 ± 0.02^{d}	0.63 ± 0.09^{b}	3.64 ± 0.47^{a}	0.22±0.05^{c}
3-Feruloylquinic acid	0.16 ± 0.01^{c}	0.03 ± 0.00^{d}	0.27 ± 0.03^{a}	0.18 ± 0.02^{bc}	0.21 ± 0.03^{ab}	0.13±0.03^{c}
3-*p*-Coumaroylquinic acid	0.12 ± 0.01^{e}	2.29 ± 0.17^{a}	1.17 ± 0.08^{b}	0.52 ± 0.08^{d}	1.06 ± 0.03^{c}	0.11±0.05^{e}
Total hydroxycinnamic acids	6.67	4.80	22.04	15.65	23.75	15.59
Total polyphenols identified	9.97	15.46	55.95	29.59	33.59	75.92

The samples were analysed in triplicate and the results are presented as mean values ± standard deviation of the year 2014. Different letters in the same line indicate significant differences ($P < 0.05$).
ND, not detected; *detected but not quantified.
†Determination after acid catalysis in the presence of excess of phloroglucinol.

Table 4. Proanthocyanidins content in samples after acid catalysis in the presence of phloroglucinol, mean degree of polymerisation (mDP) and catechin and epicatechin content

Species	Cultivar	Proanthocyanidin content (mg g^{-1} DW)	mDP	Catechin (mg g^{-1} DW)	Epicatechin (mg g^{-1} DW)
Apricot	Pink Cot	3.04 ± 0.08^{e}	2.07±0.01^{dc}	1.03 ± 0.02^{f}	0.44 ± 0.02^{d}
Cherry	20-09	10.54 ± 0.19^{d}	1.25 ± 0.02^{f}	7.71 ± 0.21^{c}	0.71 ± 0.02^{c}
Flat peach	UFO-3	33.47 ± 4.16^{b}	1.49 ± 0.04^{e}	9.80 ± 0.56^{b}	12.67 ± 2.82^{a}
Peach	Royal Glory	13.79 ± 0.62^{c}	3.59 ± 0.08^{a}	3.47 ± 0.08^{e}	0.38 ± 0.01^{e}
Plum	Tolosa	9.69 ± 1.87^{d}	2.22 ± 0.11^{c}	4.37 ± 0.63^{d}	Traces
Nectarine	Laura	59.89 ± 3.64^{a}	2.78 ± 0.11^{b}	20.52 ± 1.38^{a}	0.99 ± 0.07^{b}

The samples were analysed in triplicate and the results are presented as mean values ± standard deviation of the year 2014. Different letters in the same column indicate significant differences ($P < 0.05$).

DW), peaches (5.8 mg g^{-1} DW), nectarines (2.1 mg g^{-1} DW), apricots (1.1 mg g^{-1} DW) and cherries (0.4 mg g^{-1} DW) were analysed. These contents are lower than those detected in our study, although no acid catalysis was used. The number of studies with acid catalysis is scarce, although proanthocyanidin contents of 1.7–5.3 mg g^{-1} DW have been reported in apples,[32] 0.12 mg mL^{-1} in American cranberry juice[34] and 120 mg g^{-1} DW in black chokeberry industrial by-products.[35]

The bioavailability of proanthocyanidins is largely influenced by their degree of polymerisation (mDP).[36,37] The oligomeric and polymeric forms pass intact through the gastrointestinal tract and reach the colon, where they must be transformed by the intestinal microbiota before absorption. Only monomeric flavanols are readily absorbed in the small intestine. Therefore, a low mDP is desirable.[38] The mDP in our thinned fruits was between 1.25 in cherry and 3.59 in peach (Table 4). These values are lower than those reported in other fruits such as apples (5.7 – 7.1),[39] grapes (4.8 – 22.1)[40] or brown soybean seeds (30).[41] Thus, thinned fruits that have proanthocyanidins with small mDP might be used to obtain extracts with high bioavailability.

Flavonols

A total of four flavonols (kaempherol-3-hexoside, kaempherol-3-rutinoside, quercetin-3-hexoside and quercetin 3-rutinoside)

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were identified in the different thinned fruits (Table 3). Quercetin 3-rutinoside was found in all the fruits tested, with the highest concentrations in apricot (0.23 mg g^{-1} DW) and nectarine (0.18 mg g^{-1} DW). These values are similar to those obtained in immature peaches (0.01–0.26 mg g^{-1} DW)[10] and grape by-products (skin, 0.11 mg g^{-1} DW; pomace, 0.08 mg g^{-1} DW; stems, 0.03 mg g^{-1} DW; seeds, traces).[42] Kaempherol-3-rutinoside was identified in all the fruits except in plum. The highest content was found in flat peach (0.09 mg g^{-1} DW), being 10 times higher than the ones detected in the skin of different.[23] Meanwhile, quercetin-3-hexoside was only identified in nectarine (0.22 mg g^{-1} DW) and flat peach (0.17 mg g^{-1} DW) and kaempherol-3-hexoside only in flat peach (0.17 mg g^{-1} DW). The greatest content of flavonols was found in flat peach and nectarine, both with 0.44 mg g^{-1} DW. The flavonols (particularly kaempferol and quercetin) are considered to be antioxidant, anti-inflammatory, anticarcinogenic, anti-thrombotic and antiviral compounds.[13,43] 18

Flavanones

Although not quantified, naringenin-hexoside was identified in two fruits, the flat peach and nectarine. Flavanones occur almost exclusively in citrus fruits and are the major flavonoids in oranges and mandarins, although they have also been detected in grapefruit and tomato peel.[44] Therefore, flat peach and nectarine thinned fruits might be considered as a new source of flavanones. These compounds have been shown to inhibit chemically induced mammary, urinary bladder, and colon carcinogenesis in laboratory animals. They also act as antioxidants, regulate apolipoprotein B secretion by HepG2 cells, possibly through the inhibition of cholesterol ester synthesis, decrease low-density lipoprotein levels and hepatic cholesterol levels in plasma rabbits, and increase high-density lipoprotein levels in hypercholesterolaemic human subjects.[44]

Hydroxycinnamic acids

A total of seven hydroxycinnamic acids (neochlorogenic acid, chlorogenic acid, isochlorogenic acid, 4-*p*-coumaroylquinic acid, 4-caffeoylquinic acid, 3-feruloylquinic acid and 5-*p*-coumaroylquinic acid) were identified in the thinned fruits (Table 3). The total hydroxycinnamic acids identified ranged from 75.92 mg g^{-1} DW for nectarine to 9.97 mg g^{-1} DW for apricot. All of them were quantified in cherry, flat peach, peach and plum but isochlorogenic acid was not detected in apricot or nectarine. The main individual phenols identified (without acid catalysis) were neochlorogenic acid, ranging from 1.65 mg g^{-1} DW for apricot to 15.00 mg g^{-1} DW for plum, and chlorogenic acid, from 0.44 mg g^{-1} DW for apricot to 7.04 mg g^{-1} DW for flat peach. These concentrations are higher than those reported by other authors in fruit by-products. Thus, in the pulp of immature peaches the values ranged from 0.64 to 7.64 mg g^{-1} DW for neochlorogenic acid and from 1.59 to 5.48 mg g^{-1} DW for chlorogenic acid[10] while in the skin of nectarines 1.47 mg g^{-1} DW for chlorogenic acid and 0.27 mg g^{-1} DW for neochlorogenic acid were quantified.[45] Among the other compounds highlighted were isochlorogenic acid in flat peach (0.23 mg g^{-1} DW), 4-*p*-coumaroylquinic acid in nectarine (0.84 mg g^{-1} DW), 4-caffeoylquinic acid in plum (3.64 mg g^{-1} DW), 3-feruloylquinic acid in flat peach (0.27 mg g^{-1} DW) and 5-*p*-coumaroylquinic acid in cherry (2.29 mg g^{-1} DW). These compounds are very important for human health because they may exhibit antioxidative, antihypertensive, antibacterial, anti-tumour and anti-inflammatory properties. They may also be promising precursor compounds for the development of medical products that can resist HIV-1 RNase.[46]

Antioxidant activity

The antioxidant activity of the thinned fruit extracts was mea- sured by two different methods: DPPH scavenging and FRAP assay (Table 2). For DPPH scavenging, the behaviour was very similar to that obtained in TPC, with a high correlation between both assays (R^2 = 0.965). The highest values were obtained for nectarine (133.30 mg TE g^{-1} DW) while the lowest were for cherry (18.83 mg TE g^{-1} DW). For the FRAP assay, although the highest and lowest values were also achieved for nectarine (30.42 mg TE g^{-1} DW) and cherry (17.50 mg TE g^{-1} DW), respectively, the differences between the other fruits were not significant (P < 0.05), although the corre- lation between the FRAP assay and TPC was high (R^2=0.826).

These variations between the two different antioxidant assays could be due to the existence of numerous radicals, the different physical and chemical characteristics of the oxidants and the different reaction mechanisms. The same effect has been found by other authors in papaya, pineapple and tamarind,[47] guava,[26] apple, apricot, mandarin, oat, peach, plum, rice and wheat.[48]

Similar conclusions can be obtained when comparing the antiox- idant activity of thinned fruits with other by-products. DPPH scav- enging of two varieties of immature cherries has shown equal or lower concentrations (45.0 and 42.6 mg TE g^{-1} DW)[12] than the majority of our thinned fruits. The activity obtained with the FRAP assay in passion fruit peel (4.4 mg TE g^{-1} DW)[49] was much lower than that in our thinned samples. Therefore, if all the above authors conclude that the by-products studied represent a source of antioxidant compounds, it seems clear that thinned fruits must be an important source of interesting compounds that may be used in the food, chemical and pharmaceutical industries as antioxidants[16] or anti-browning agents.[14]

CONCLUSIONS

All the thinned stone fruits analysed in this study are clearly a potential source of polyphenols (>9.0 mg GAE g^{-1} DW and > 7.0 mg CE g^{-1} DW) and antioxidant compounds (>18.0 mg TE g^{-1} DW by DPPH scavenging and > 17.0 mg TE g^{-1} DW by FRAP assay). Nectarine had the highest content of total phe- nols (67.43 mg GAE g^{-1} DW), total flavonoids (56.97 mg CE g^{-1} DW, respectively) and antioxidant activity revealed by both methods, DPPH scavenging (133.30 mg TE g^{-1} DW) and FRAP assay (30.42 mg TE g^{-1} DW). The main individual phenols iden- tified were catechin (cherry and nectarine), epicatechin (flat peach), chlorogenic acid (apricot) and neochlorogenic acid (flat peach, peach and plum), although 5-*p*-coumaroylquinic acid and 4-caffeoylquinic acid were also significant in cherry and plum, respectively. Proanthocyanidins are very abundant in these by-products and, due to their low mean degree of polymerisation, their bioavailability could be very high. Thus, thinned fruits might be used as antioxidants in foods or as a source of compounds with health related benefits that can be used in the pharmaceutical, cosmetic and food industries. Significant economic benefits could thus be obtained from these by-products.

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