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Original Article

# Sweet cherry phytochemicals: Identification and characterization by HPLC-DAD/ESI-MS in six sweet-cherry cultivars grown in Valle del Jerte (Spain)

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ABSTRACT

Individual anthocyanin pigments and phenolic compounds were isolated, identified and quantified in six different sweet-cherry cultivars (*Prunus avium* L.) grown in Valle del Jerte area (Spain). An extractive-chromatographic method has been optimized for one-step extraction and simultaneous determination of all the studied components by HPLC/DAD-MS. The highest levels of phytochemicals were found in the autochthonous sweet-cherry cultivars that belong to the Protected Designation of Origin (POD) *Cereza del Jerte*. Van cultivar showed the lowest level of anthocyanin pigments and phenolic compounds. The most abundant anthocyanin pigment in all the studied cultivars was cyanidin-3-rutinoside (105 mg/100 g fresh weight (fwt) in Pico Negro sweet-cherry cultivar). The most abundant phenolic compound was the flavanol *p*-coumaroylquinic acid (130 mg/100 g fwt in Pico Negro sweet-cherry cultivar). In addition, chemical attributes (antioxidant activity, soluble solid content and pH) were also evaluated.


## 1. Introduction

Health benefits associated with Mediterranean diets are due to the significantly large intake of functional plant foods and beverages containing a great quantity of bioactive phytochemicals or nutraceutical compounds (Iriti and Faoro, 2006). Epidemiological studies show a strong association between fruit and vegetable consumption and reduced risk of several degenerative and chronic diseases, such as coronary heart disease and certain cancers. This results from a diversified eating style, either in terms of food or of food components (Chaovanalikit and Wrolstad, 2004). The most bioactive plant substances are mainly secondary metabolites and natural antioxidants such as isoprenoids, phenylpropanoids and indolamines, among others.

The phenolic compounds present in foods show considerable diversity in their structure, and may be divided into several different classes of compounds. These compounds contribute to flavor, color and sensory properties such as bitterness and astringency (Lee, 2000). The simplest derivates are the monocyclic acids (benzoic acid derivates and cinnamic acid derivates). On the basis of these simple structures, more complex derivates may occur through the addition of sugar chains and/or different metabolic reactions. An important group of phenolic compounds found in foods are the phenylpropanoid derivates. These polyphenol derivates form a diverse range of compounds and can be classified into many groups (anthocyanin pigments, flavonoids, flavanols, and flavonols among others). These compounds may be present as the aglycone or in a glycoside form bound to various sugars, such as arabinose, glucose, galactose, rhamnose and xylose. In addition, indolamines and indole alkaloids constitute other important group of phytochemicals in edible fruit and plants (Reiter et al., 2001); they are generated through a tryptophan biosynthetic route.

Phenylpropanoids and the indolamine biosynthetic route contain the common primary metabolite shikimate (Fig. 1). The biosynthetic route leads to simple phenols or phenolic acids, flavonoids, stilbenes, anhtocyanidins, indole alkaloids, etc. (Kurkin, 2003). Shikimate serves as the precursor of tryptophan and phenylalanine through different metabolic routes. The reaction with indolamines and aldehydes in the fruit leads to all the phenolic compounds found in sweet cherries.

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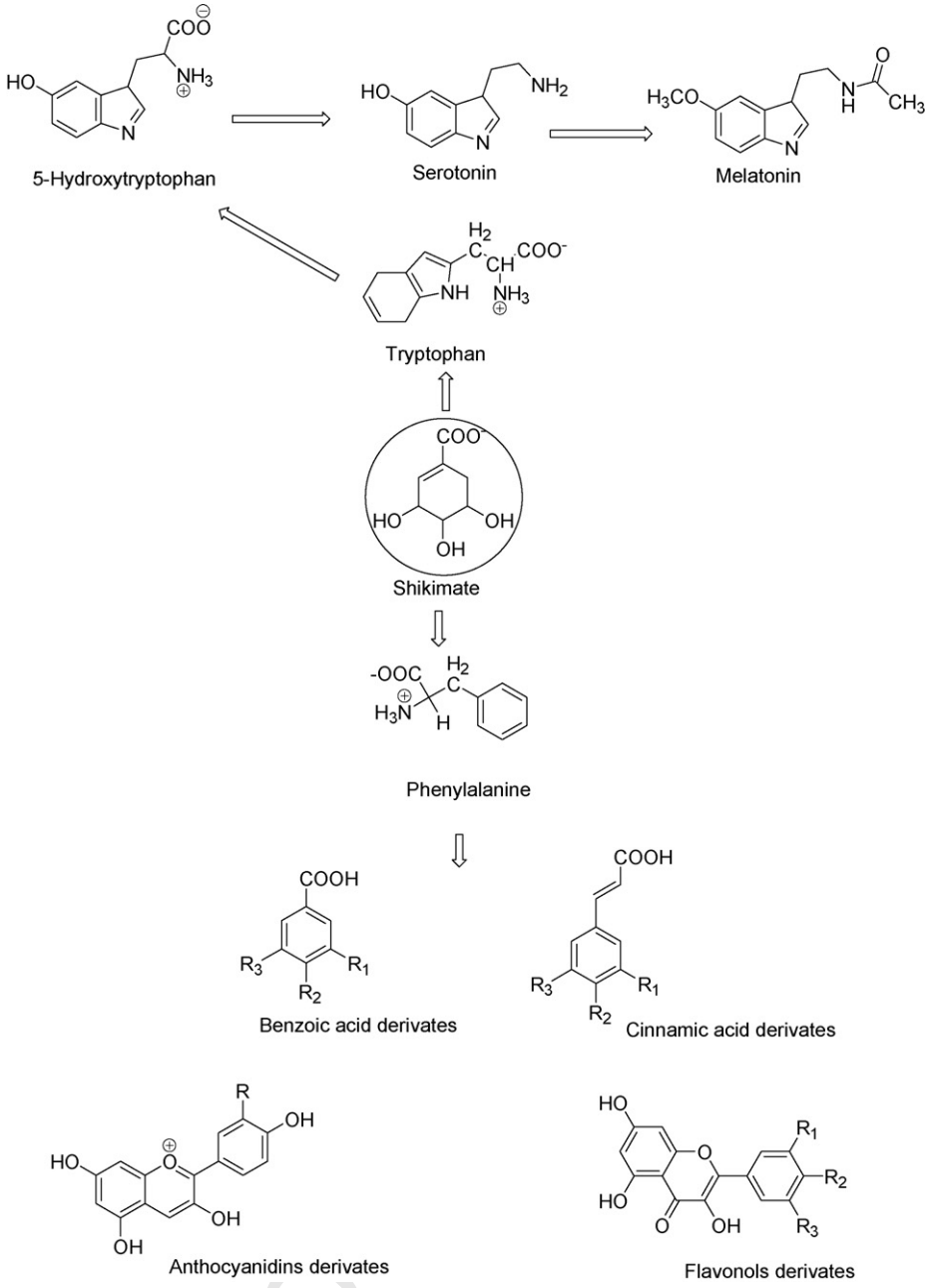


**Fig. 1.** The main phytochemical compounds present in sweet-cherry cultivars derive from shikimic acid via different metabolic routes.

Sweet cherries (*Prunus avium* L.) are important commercially as a table fruit. Consumption of sweet cherry has been associated with beneficial health effects, for instance Wang et al. (1999) reported that the consumption of sweet cherries alleviates arthritis and gout-related pain. Reduction of the proliferation of human colon cancer cells (Kang et al., 2003) has been specifically associated with the consumption of cherries (Serrano et al., 2005). Special interest has been shown for anthocyanins and polyphenolics because of their antioxidant properties.

It has been known since the beginning of the 20th century that sweet cherries contain substantial amounts of anthocyanins and polyphenolics (Gao and Mazza, 1995). Total and individual contents of phenolic and anthocyanin compounds in sweet-cherry cultivars have been previously reported (Bernalte et al., 1999; Bernalte et al., 2003; Gao and Mazza, 1995; Gonçalves et al., 2004a; Kim et al., 2005; Mozetic et al., 2006); these compounds contribute to total antioxidant activity (AA).

Different chromatographic methods have been described for the individual phenolic compound identification and determination in edible plants (Abad-García et al., 2007; Giusti et al., 1999; Lopes-da-Silva et al., 2002; Mertz et al., 2007; Wu and Prior, 2005). In sweet cherries, Usenik et al. (2008) established a chromatographic method to identify different anthocyanin pigments and phenolic compounds in several sweet-cherry cultivars.

In this paper individual anthocyanins and polyphenolic compounds have been identified and quantified in six sweet-cherry cultivars grown in Valle del Jerte (Spain), five of them belonging to the Protected Designation of Origin (PDO) *Cereza del Jerte*. Correlations between individual anthocyanins and polyphenolic compounds were evaluated, as well as other phytochemicals present in sweet-cherry cultivars, like the indolamines melatonin and serotonin (González-Gómez et al., in press, Research Institute of Food and Agriculture, Spain). All these metabolites have the shikimate as primary precursor in the

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biosynthetic metabolic route. For each one of the selected sweet-cherry cultivars, chemical attributes like solid soluble contents, antioxidant activity and acidity were evaluated, as well as the total contents of anthocyanin pigments and phenolic compounds.

## 2. Materials and methods

### 2.1. Apparatus

For all chromatographic studies, an Agilent 1100 series liquid chromatographic instrument equipped with a DAD/MS-ESI-quadrupole was used. Chromatographic data processing was done using Agilent ChemSation software.

### 2.2. Chemicals

LC-grade acetonitrile was acquired in Scharlau Chemie, Trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-caboxylic acid) and ABTS (2,2′-azinobis(3-ethylbenzoithiazolone 6-sulphonate) and phenolic standards were purchased at Sigma–Aldrich Spain. Anthocyanindin standards were obtained from Extrasynthese, France.

### 2.3. Sample selection and storing

For this research six sweet-cherry cultivars were studied: Ambrunés, Van, Pico Negro, Pico Negro Limón, Pico Colorado and Navalinda. The selection of the sweet-cherry cultivars was made in accordance with their harvesting time. Navalinda cultivar is one of the earliest to be harvested (late May). The other cultivars were harvested from May to July, following the sequence Van (12 days after Navalinda), Pico Negro Limón (25 days after Navalinda), Pico Negro, Ambrunés (31 days after Navalinda) and Pico Colorado (38 days after Navalinda). The cultivars Pico Colorado, Pico Negro, Pico Negro Limón, Navalinda and Ambrunés belong to *Cereza del Jerte* PDO.

All cherry cultivars used for this research were obtained in 2007 from local farmers of Valle del Jerte area (Extremadura, Spain). For each sweet-cherry cultivar, approximately 10 kg were harvested at commercial maturity from different trees at the same day. Three individual sets of samples were harvested for statistical analysis. For phenolic and anthocyanins studies, samples were frozen and vacuum packed in plastic bags and stored at −80 °C for further analysis. All the other determinations were carried out on fresh fruit.

### 2.4. Extraction of anthocyanin pigments and phenolic compounds

For anthocyanin pigments and phenolic compound extractions, 500 g of partially defrosted sweet cherry sample were pitted and a fruit homogenate was prepared using an Omni-Mixer homogenizer (Omni International, GA, USA). To avoid pigment and phenolic compound degradation, the homogenization was carried out in darkness and the sample was placed on ice throughout the entire process (∼5 min). A 10 g sample of homogenate was transferred to a volumetric flask and a 50 ml quantity of methanol solution (0.2% hydrochloric acid) was added. The sample was manually shaken and then stored at −20 °C. After 24 h, quantitative anthocyanins and phenolic compounds extraction was achieved. Fruit extracts were injected into the chromatographic system right after the extraction in order to avoid pigment degradation. Extractions were carried out in triplicate.

### 2.5. HPLC conditions for anthocyanin and phenolic compounds separation and quantification

Chromatographic separation was accomplished with a Phenomenex C18 HPLC column (150 mm × 4.6 mm, 5 μm) heated to 35 °C. The mobile phase used for the separation was composed of aqueous TFA 0.1% (A) and acetonitrile (B) in gradient mode set as follows: initial conditions 10% B; from 0 to 3 min 10% B; from 3 to 15 min 15% B; from 15 to 20 min the composition was kept constantly at 15% B; from 20 to 25 min 18% B and from 25 to 40 min 30% of B. A period of 5 min was necessary for column equilibration. The flow was fixed at 0.500 mL/min for all the experiments. The injected volume was 50 μL. The chromatographic system was coupled to an API-ESI single quadrupole mass spectrometer for analyte detection and quantification. The mass spectrometer was operated in the positive-ion mode. Peak identification was accomplished using mass spectrometer scan mode and retention time of standards. Once the compounds were identified, the SIM mode or DAD was used for the quantifications.

Quantitative calculations were conducted with the peak areas, and external standard method was applied for the respective mass chromatogram. Agilent ChemStation software was used for data acquisition and peak areas integration.

### 2.6. Determination of antioxidant activity

Antioxidant activity was evaluated according to the Cano et al. (1998) procedure, slightly modified. Briefly, 20 μL of sweet-cherry juice obtained from fruit homogenate was placed in a spectrometric cuvette, and 1 mL of the radical cation ABTS (2,2′-azinobis(3-ethylbenzoithiazolone 6-sulphonate) was added. The initial absorbance value at 730 nm was then compared with the absorbance obtained after 20 min of reaction. The AA was expressed as mg of Trolox per 100 g of fresh fruit.

### 2.7. Determination of total soluble solids content and pH

The total soluble solids content (SSC) expressed as °Brix was measured on juice squeezed from fresh fruit using a Mettler Toledo RE40 refractometer. An automatic pH titration system (Mettler Toledo Rondolino DL50) was used for pH determination.

### 2.8. Statistical analysis

For statistical studies, SPSS 13.0 software was used (SPSS Inc., Chicago, IL, USA). Correlations were estimated with the Pearson test at $p < 0.05$ significance level. All analyses were done in triplicate. Data are expressed as means ± S.D. and were analyzed using a one-way analysis of variance (ANOVA). When ANOVA detected significant differences between mean values, means were compared using ~~LSD~~ Tukey's test. ACOC software was used for calibration and analytical figure of merit calculations (Espinosa-Mansilla et al., 2005)

## 3. Results and discussion

### 3.1. Analytical parameters and method validation

Under the selected chromatographic conditions, calibration graphs were obtained by preparing standard samples of each compound in triplicate, with increasing concentration of each analyte. The main figures of merit were also calculated: the limit of detection (LOD) value and linearity. The precision of the method was determined by calculating the interday repeatability, expressed as standard deviation (S.D.) in terms of retention times and peak area ($w_{1/2}$), by injection of three different replicates of a standard sample in three different days. These data are summarized in Table 1.

### 3.2. Evaluation of sweet cherry chemical attributes

Antioxidant activity, acidity and total soluble solids contents were evaluated for all the studied sweet-cherry cultivars. Results



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**Table 1**
Statistical parameters for non-weighted least-squares regression and interday assay results using successive injections of three different standard samples of CY-3-GLU, CY-3-RUT, PEO, MV, CY, CHL, EPIC and QUER on three different days.

| | CY-3-GLU | CY-3-RUT | PEO | MV | CY | CHL | EPIC | QUER |
|---|---|---|---|---|---|---|---|---|
| Slope ± σ | 8.51 ± 0.06[a] | 2.44 ± 0.08[a] | 16.5 ± 0.9[a] | 15.2 ± 0.3[a] | 22.1 ± 0.54[a] | 8.76 ± 0.49[b] | 6.64 ± 0.05[b] | 10.3 ± 0.03[b] |
| Intercept ± σ | −0.05 ± 0.03[c] | 1.45 ± 0.54[c] | 2.99 ± 1.88[c] | 0.25 ± 0.18[c] | −0.69 ± 0.27[c] | 11.9 ± 3.6[d] | −1.33 ± 0.60[d] | 1.38 ± 1.14[d] |
| Regression coefficient ($R$) | 0.999 | 0.991 | 0.996 | 0.996 | 0.987 | 0.975 | 0.999 | 0.999 |
| Linearity (%) | 99.2 | 96 | 97 | 98 | 97 | 94 | 99 | 99 |
| LOD (ng/mL) | 11.6 | 17.6 | 34.2 | 35.6 | 36.8 | 12.3 | 27.2 | 33.0 |
| Interday assay | | | | | | | | |
| $Rt$ (min) ± σ | 21.875± 0.004 | 22.772 ± 0.004 | 37.755 ± 0.012 | 30.018 ± 0.002 | 37.35 ± 0.19 | 14.87 ± 0.22 | 19.72 ± 0.02 | 33.28 ± 0.09 |
| $w_{1/2}$ (min) ± σ | 0.327 ± 0.011 | 0.369 ± 0.001 | 0.193 ± 0.003 | 0.081 ± 0.003 | 0.012 ± 0.03 | 0.214 ± 0.004 | 0.206 ± 0.005 | 0.172 ± 0.021 |

Q3 CY-3-GLU: cyanidin-3*O*-glucoside, CY-3-RUT: cyanidin-3*O*-rutinoside, PEO: peonidin-3*O*-rutinoside, MV: malvidin, CY: cyanidin, CHL: chlorogenic acid, EPIC: epicatechin and QUER: quercetin-3-rutinoside.
[a] Slope expressed as peak area × mL $\mu g^{-1} \times 10^5$.
[b] Slope expressed as peak area × mL $\mu g^{-1}$.
[c] Intercept expressed as peak area × $10^5$.
[d] Intercept expressed as peak area.

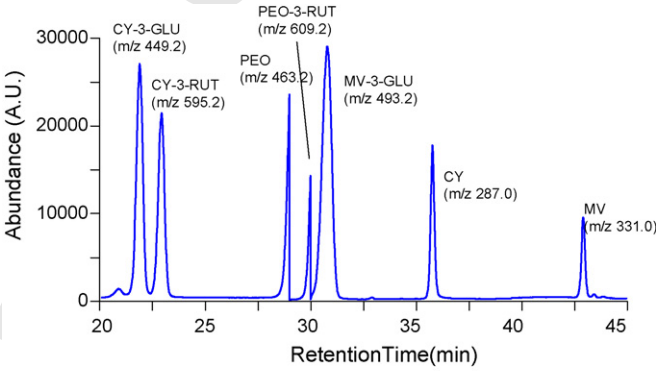
are listed in Table 2. According to ANOVA, significant differences were observed between the studied cultivars. Van was the cultivar showing the lowest value of SSC (12.3 °Brix). Less variation was found in acidity values. Van was the cultivar with the lowest pH value (4.0), while Pico Colorado was the cultivar with the highest pH value (4.4). Finally, the AA showed that Van and Ambrunés were the two cultivars with lowest AA (214 and 145 mg Trolox/100 g fresh weight (fwt), respectively), while the highest values of AA were observed in Pico Negro cultivar.

### 3.3. Phytochemical contents in sweet-cherry cultivars

This study was focused on the identification and quantification of anthocyanin pigments and phenolic compounds present in different sweet-cherry cultivars. A previously published liquid chromatographic procedure (de Pascual-Teresa et al., 2000) was modified in order to identify and quantify the maximum number of phenolic derivates. Identification and peak assignment of anthocyanins and phenolic compounds in the studied sweet-cherry cultivars was based on comparison of their retention times and mass spectral data to those of standards and published data.

A total of five different anthocyanin and five phenolic compounds have been identified and quantified after applying the extractive procedure and the chromatographic method described above. Fig. 2 shows the chromatogram of anthocyanin pigments standard solutions using MS in SIM mode for post-column detection.

Variations were found in the anthocyanin composition among cultivars (Table 3). Five different anthocyanin pigments were identified: cyanidin-3*O*-rutinoside (CY-3-RUT), cyanidin-3*O*-glucoside (CY-3-GLU), peonidin-3*O*-rutinoside (PEO) and the aglycones malvidin (MV) and cyanidin (CY). The most abundant anthocyanin pigment was CY-3-RUT (ranging between 14.2 and 105 mg/100 of fwt) followed by CY-3-GLU (0.34–21.2 mg/100 g of FW) and PEO in all of the studied cultivars. Fig. 3 represents the chromatogram for




CY-3-GLU: cyanidin-3*O*-glucoside, CY-3-RUT: cyanidin-3*O*-rutinoside, PEO: peonidin, PEO-3-RUT: peonidin-3*O*-rutinoside, MV-3-GLU: malvidin-3*O*-glucoside, CY: cyanidin, MV: malvidin

**Fig. 2.** HPLC and ESI-MS separation of anthocyanin standard pigments.

Pico Negro anthocyanin extract. Peak assignation was done according to standard previously identified (Fig. 2). The pigments in the lowest concentration were MV and CY in the aglycone form. Navalinda and Pico Negro were the sweet-cherry cultivars having the highest levels of anthocyanin pigments. In both cultivars CY-3-RUT represents 82% and 92% of the total of anthocyanin contents. In contrast, Van was the cultivar with the lowest anthocyanin content. MV was the only pigment detected in similar amounts in all the cultivars (i.e. no significant differences were found for this pigment). Usenik et al. (2008) found similar results in different sweet-cherry cultivars grown in Slovenia. As can be observed in Table 3, the sweet-cherry cultivars showing the highest amount of anthocyanin pigments belonged to the PDO *Cereza del Jerte*.

A total of five phenolic compounds were identified in the studied sweet-cherry cultivars (Table 4); *p*-coumaroylquinic acid (PCQ), chlorogenic acid (CHL) and neochlorogenic acid (NCHL)

**Table 2**
Chemical attributes of the studied sweet-cherry cultivars ($n$ = 3).

| | SSC (°Brix) | pH | AA (mg Trolox/100 g fwt) |
|---|---|---|---|
| VAN | 12.3 ± 0.1a | 4.0 ± 0.0a | 214 ± 11b |
| NAV | 15.4 ± 0.2b | 4.2 ± 0.1b | 418 ± 20d |
| P.L | 19.4 ± 0.1c | 4.3 ± 0.0b,c | 439 ± 22d |
| AMB | 20.7 ± 0.2d | 4.3 ± 0.0b,c | 145 ± 16a |
| P.N | 20.3 ± 0.0d | 4.2 ± 0.0b | 486 ± 43e |
| P.C | 23.5 ± 0.0e | 4.4 ± 0.0c | 387 ± 27c |

SSC: total soluble solids content, AA: antioxidant activity. Sweet-cherry cultivars: VAN: Van, NAV: Navalinda, P.L: Pico Negro Limón, AMB: Ambrunés, P.N: Pico Negro, P.C: Pico Colorado

**Table 3**
Anthocyanin pigments and indolamine contents o six sweet-cherry cultivars ($n$ = 3).

| | CY-3-GLU | CY-3-RUT | CY | MV | PEO |
|---|---|---|---|---|---|
| VAN | 0.34 ± 0.00a | 14.2 ± 1.10a | 0.04 ± 0.00a | 0.05 ± 0.00 ns | 0.40 ± 0.11a,b |
| NAV | 21.2 ± 0.32d | 100 ± 0.33e | 0.04 ± 0.00b | 0.05 ± 0.00 ns | 1.48 ± 0.20d |
| P.L | 3.49 ± 0.05b | 40.7 ± 0.63c | 0.05 ± 0.00b | 0.04 ± 0.01 ns | 1.05 ± 0.04c |
| AMB | 3.35 ± 0.32b | 65.8 ± 3.59d | 0.08 ± 0.00d | 0.05 ± 0.01 ns | 0.67 ± 0.02b,c |
| P.N | 4.45 ± 0.34c | 105 ± 2.35e | 0.07 ± 0.00c | 0.06 ± 0.00 ns | 3.93 ± 0.19e |
| P.C | 2.90 ± 0.11b | 25.9 ± 0.34b | 0.18 ± 0.00e | 0.05 ± 0.00 ns | 0.11 ± 0.02a |

CY-3-GLU: cyanidin-3*O*-glucoside, CY-3-RUT: cyanidin-3*O*-glucoside, CY: cyanidin, MV: malvidin, PEO: peonidin-3*O*-rutinoside. Sweet-cherry cultivars: VAN: Van, NAV: Navalinda, P.L: Pico Negro Limón, AMB: Ambrunés, P.N: Pico Negro, P.C: Pico Colorado. Amounts are expressed as mg/100 g fwt.

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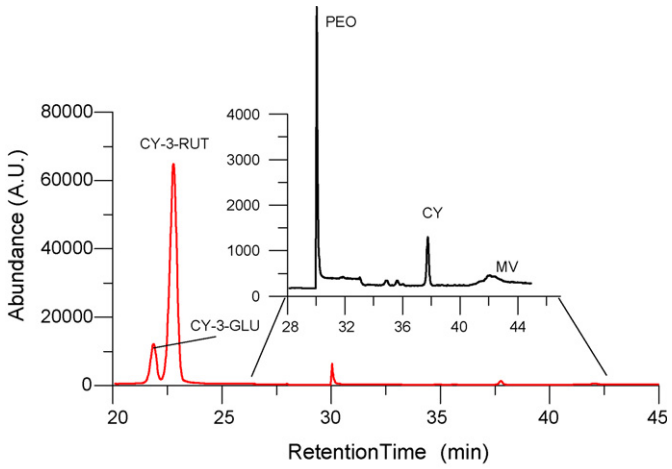


CY-3-GLU: cyanidin-3*O*-glucoside, CY-3-RUT: cyanidin-3*O*-rutinoside, PEO: peonidin, CY: cyanidin, MV: malvidin

**Fig. 3.** Chromatographic separation of anthocyanin pigments in Pico Negro sweet cherry. Peak assignments were based on mass spectra information and standard retention times.

(flavonoids derivates), epicatechin (EPIC) (flavanol derivates) and quercetin-3*O*-rutinoside (QUER) (flavonol derivates). In Fig. 4, chromatograms for extracts from a Pico Negro sweet-cherry cultivar using DAD detector are represented. Different wavelengths were used according to target compound. The most abundant phenolic compound was the flavanol PCQ, followed by the flavonoids NCHL and EPIC. The lowest amounts correspond to CHL and QUER. The five autochthonous sweet-cherry cultivars showed the highest amount of phenolic compounds, and PCQ was the most abundant in these cultivars. Kyou-Nam et al. (2007) had previously reported that the catechin derivate ((−)epicatechin gallate) shows strong biological activity, including apoptosis, or cell growing inhibition, and that it is involved in the membrane transport system. In addition, the cancer preventive activity of green tea was associated with diverse catechin derivates such as epigallocatechin, epicatechin-3-gallate and epicatechin (Yang et al., 2007). Pico Negro is the cultivar that showed the highest levels of phenolic compounds (225 mg/100 g fwt as sum of the five identified phenolic compounds). In this cultivar, PCQ represents 58% and NCHL 33%. The cultivar showing the lowest amount of phenolic compounds was Van (76.2 mg/100 of fwt as sum of the five identified phenolic compounds). For this cultivar, the flavonol NCHL was the most abundant phenolic compound (60.1 mg/100 of fwt). Our results for phenolic compound contents are similar to those reported by Serrano et al. (2005), and slightly higher than those reported by Usenik et al. (2008). These differences could be due to the fact that the cultivars studied in this research and those in Serrano's research were grown in a southern area (Spain), while Usenki's were grown in a northern area (Slovenia).

**Table 4**
Phenolic compound contents of the studied six sweet-cherry cultivars ($n = 3$).

| | NCHL | PCQ | CHL | EPIC | QUER |
|---|---|---|---|---|---|
| VAN | 60.1 ± 1.35b | 13.5 ± 0.47a | 0.25 ± 0.06a | 0.66 ± 0.04a | 1.84 ± 0.05a |
| NAV | 37.4 ± 0.11a | 89.7 ± 0.30c | 1.04 ± 0.02a | 13.38 ± 0.26f | 4.06 ± 0.04c |
| P.L | 55.6 ± 0.41b | 62.2 ± 0.22b | 1.01 ± 0.11a | 7.85 ± 0.43c | 2.56 ± 0.09b |
| AMB | 51.8 ± 0.40b | 60.5 ± 0.28b | 0.85 ± 0.12a | 5.83 ± 0.07b | 4.28 ± 0.04d |
| P.N | 74.1 ± 5.05c | 130 ± 0.45d | 5.74 ± 0.51b | 9.14 ± 0.01e | 5.54 ± 0.01e |
| P.C | 81.3 ± 4.47c | 89.3 ± 2.14c | 11.9 ± 0.40c | 12.2 ± 0.15d | 4.31 ± 0.03d |

NCHL: neochlorogenic acid, PCQ: *p*-coumaroylquinic acid, CHL: chlorogenic acid, EPIC: epicatechin and QUER: quercetin-3*O*-rutinoside. Sweet-cherry cultivars: VAN: Van, NAV: Navalinda, P.L: Pico Negro Limón, AMB: Ambrunés, P.N: Pico Negro, P.C: Pico Colorado. Amounts are expressed as mg/100 g fwt.

In our previous research (González-Gómez et al., in press, Research Institute of Food and Agriculture, Spain) the contents of two indolamines (melatonin and serotonin) in different sweet-cherry cultivars were studied. In this work, an inverse relation between the contents of these two indolamines was observed.

### 3.4. Relation between the studied components in sweet cherry

The relation between the studied phytochemicals (indolamines, anthocyanin pigments and phenolic compounds) has been analyzed. In Table 5 the Pearson correlation coefficient are listed at 0.01 and 0.05 significance level.

Our results show correlations between the concentrations of the studied compounds. In general terms, the five studied anthocyanin pigments show correlations with all the other phytochemicals found in these sweet-cherry cultivars. Strong correlations ($r > 0.900$) and medium correlations ($r > 0.600$) between anthocyanin pigments and phenolic compounds (phenylpropanoids) were observed. The strongest correlations were found between cyanidine and chlorogenic acid ($r > 0.904$). Peonidin was the only pigment correlated with both of the identified indolamines. In our earlier work (González-Gómez et al., in press Research Institute of Food and Agriculture, Spain), an inverse correlation was found between melatonin and serotonin for the same sweet-cherry cultivars ($r = -0.619$).

Correlation within the same family of compounds was also studied. The content of CY-3-RUT was correlated with CY-3-GLU and PEO. In addition, medium-strong correlations ($r > 0.750$) were observed among some of the studied phenolic compounds.

Fig. 1 represents the biosynthetic route for these phytochemical compounds. All the studied compounds arise from shikimate as the common primary metabolite. Tryptophan and phenylalanine are generated directly from shikimate, and then each metabolite follows different metabolic pathways to generate the indolamines and phenilpropanoid compounds present in the fruit (Iriti and Faoro, 2006). The correlations found between the studied phytochemical compounds may be due to the fact that all of them derive from the same primary metabolite.

Correlations between the antioxidant activity and the studied phytochemicals were also evaluated. We found significant correlations ($p < 0.01$) between PCQ and AA ($r = 0.710$), and between EPIC and AA ($r = 0.725$). As can be observed from Table 4, these two phenolic compounds were the most abundant in all of the autochthonous sweet-cherry cultivars. Similar results were reported by Gonçalves et al. (2004b), who found a correlation between the antioxidant activity of cherries and *p*-coumaroylquinic acid and also with catechin concentration. In addition, we found that PEO and MLT concentration was slightly correlated to

**Table 5**
Correlations coefficients ($r$) between the studied phytochemical compounds.

| | CY-3-RUT | PEO | NCHL | PCQ | CHL | EPIC | QUER |
|---|---|---|---|---|---|---|---|
| CY-3-GLU | 0.647* | | −0.660* | | | 0.679* | |
| CY-3-RUT | | 0.581* | | 0.745** | | 0.784** | 0.732** |
| CY | | | 0.676* | | 0.904** | | |
| MV | | | 0.609* | | | | |
| PEO | | | | 0.642* | | | |
| NCHL | | | | | 0.819** | | |
| PCQ | | | | | | 0.902** | 0.905** |
| EPIC | | | | | | | 0.742** |

CY-3-GLU: cyanidin-3*O*-glucoside, CY-3-RUT: cyanidin-3*O*-glucoside, PEO: peonidin-3*O*-rutinoside, NCHL: neochlorogenic acid, PCQ: *p*-coumaroylquinic acid, CHL: chlorogenic acid, EPIC: epicatechin and QUER: quercetin-3*O*-rutinoside, CY: cyaniding and MV: malvidin.

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).



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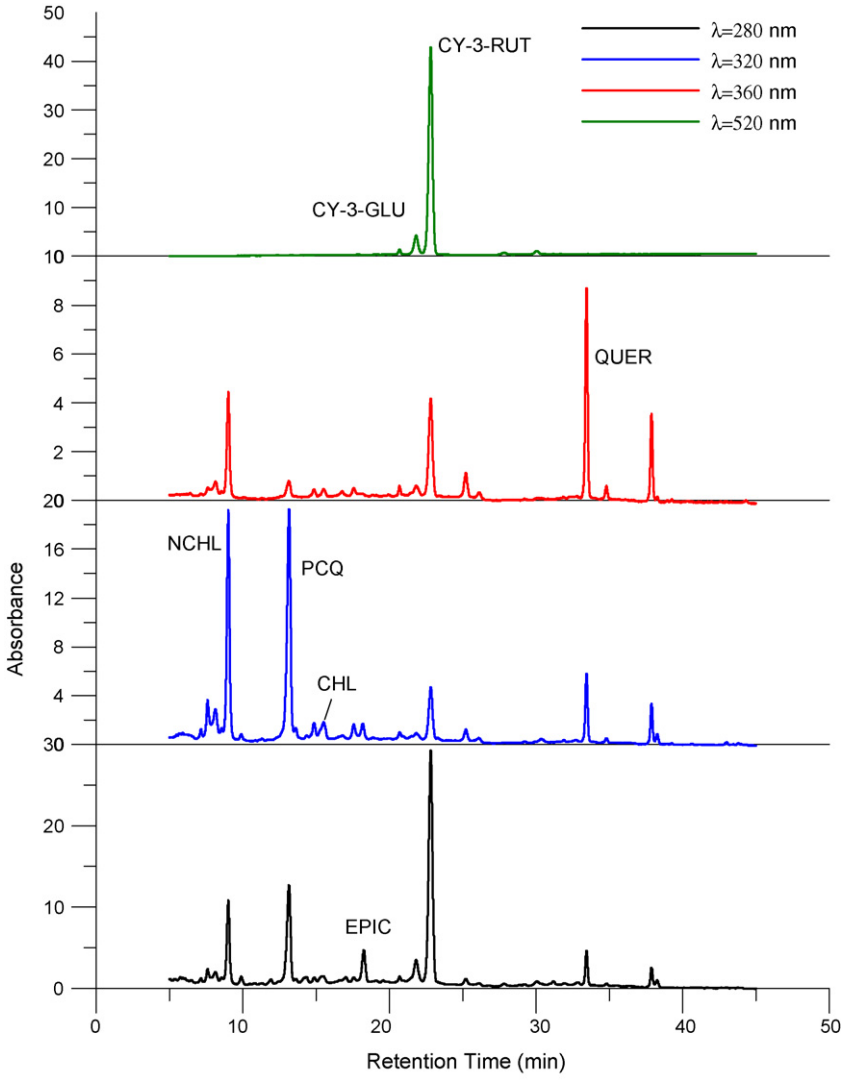


CY-3-GLU: cyanidin-3*O*-glucoside, CY-3-RUT: cyanidin-3*O*-rutinoside, QUER: quercetin, NCHL: neochlorogenic acid, PCQ: p-coumaroylquinic acid ,CHL: chlorgenic acid and EPIC: epicatechin

**Fig. 4.** HPLC separation of polyphenols (anthocyanin pigments and phenolic compounds) from Pico Negro sweet-cherry cultivar, detected at different wavelengths.

the antioxidant activity ($r = 0.639$ and $r = 0.652$, $p < 0.05$, respectively).

## 4. Conclusions

According to the results obtained, the highest concentrations of anthocyanin pigments and phenolic compounds were found in the autochthonous sweet-cherry cultivars. Statistically significant correlations were observed among phytocompound concentration. The high correlation could be due to the fact that all the studied phytochemicals derive from the shikimate biosynthetic route.

The concentrations of PCQ (flavonoid derivate) and EPIC (flavanol derivate) were correlated to the antioxidant activity. EPIC was among the most abundant of the phenolic compounds found in the PDO *Cereza del Jerte* sweet-cherry cultivars. This finding has particular relevance, because EPIC and other catechin derivates are strongly related to tumor suppression processes.

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