Development and validation of a HPLC–DAD method for quantification of phenolic compounds in different sweet cherry cultivars

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Abstract

This work describes the validation of a new high-performance liquid chromatography method for the determination of nine phenolic compounds in six cultivars of sweet cherries. Phenolic compounds are a wide and heterogeneous set of secondary metabolites in plants, highly diversified and broadly distributed. These compounds are known for being directly related to many of the health promoting capabilities usually associated to sweet cherries. The samples were submitted to lyophilization, and the process' efficiency varied between 13.21 and 27.13%. The samples were extracted with the extraction directed to phenolic compounds, and the efficiency varied between 78.35 and 98.56%. The high performance liquid chromatography method with a diode array detector was validated, taking into account the guidelines of the Food and Drug Administration. The procedure was considered linear for concentrations ranging from 2 to 50 µg/mL, with mean R^2 value of 0.998 and with precision of the calibrators (mean relative error) within a range of $\pm 15\%$ except for the lower limit of quantification where the value was $\pm 20\%$. The herein described procedure was successfully applied for the determination of the studied analytes in six cherry cultivars, demonstrating its usefulness. The compound found in the greatest quantity in the different cultivars was quercetin-3-4'-di-O-glycoside (3.67 ± 0.12 – 132.07 ± 7.51 mg per 100 g of fresh fruit).

Keywords *Prunus avium* L. · Phenolic compounds · HPLC–DAD · Methanolic extraction

1 Introduction

Phenolic compounds are secondary plant metabolites, being considered one of the most extensive phytochemical groups. These compounds are responsible for a number of food characteristics, namely aspects such as colour, taste, aroma and astringency [8].

There are also multiple health promoting properties associated with this group of compounds. Indeed, it is evidenced that the consumption of phenolic compounds stimulates vasodilation, reduces vascularization and hyperlipidemia, leading to a regression of atherosclerosis [12, 14]. The risk of thrombosis can also be reduced with

the consumption of these compounds, leading to a lower incidence of myocardial infarction, heart disease and ischemia [14]. Phenolic compounds also perform have an important anticancer role [12, 14] and reduce the risk of diabetes [1]. In vivo studies have also shown that polyphenols lead to a decrease in lipid peroxidation resulting from the higher activity of antioxidant enzymes (SOD and Gpx) [6].

Sweet cherries are an excellent source of phytochemicals, namely phenolic compounds [6]. Amongst these, flavonoids are the most predominant group, constituting an important part of the diet and being, therefore, the most studied group [5]. Among the flavonoids, anthocyanins

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are the most relevant subgroup. The concentration of polyphenols can vary between different sweet cherry cultivars and in different parts thereof [19]. Studies have shown that the antioxidant potential is higher in the stem than in the fruit pulp, leading to the belief that this is due to the higher concentration of phenols also located here [3]. The levels of phenolic compounds can be affected by several factors, and this is associated to both climatic and agronomic conditions [4, 23]. Ambient temperature, light intensity and type of cultivation are crucial factors that dictate the amount and stability of polyphenols [6, 15]. The composition of the soil, the type of fertilization and the supply of water and nutrients required by the plant interfere with the nutritional composition of the fruit [6]. Also, the influence of light intensity on the amount of phenols has been described, allowing to conclude that in environments with higher temperatures (25–30 °C) and greater sunlight exposure, anthocyanins and the remaining phenolic content increase significantly [6, 15].

Due to their biological properties, interest in the study of phenolic compounds in different sweet cherry cultivars increased substantially. However, the number of reliable analytical methods is limited.

In Portugal, the Fundão region is well known for sweet cherry fruit farms, which are part of the local economy [16]. There are several sweet cherry cultivars, among them *Sweetheart*, *Earlise*, *Summit*, *Primulat*, *Brooks* and *Saco*. It should be noted that *Saco* is an autochthonous cultivar from the Fundão region and the oldest and most traditional variety [16].

The determination of phenolic compounds was reported in other food matrices as in olive oil [18], using a high performance liquid chromatography coupled to mass spectrometry (HPLC–MS/MS). Another study in acid cherry (*Prunus cerasus* L.) also allowed the phenolic composition to be determined using a high performance liquid chromatography coupled with fluorescence detection (HPLC–FLD) [20]. A study of several sweet cherry cultivars using high-performance liquid chromatography equipment coupled to ultraviolet (HPLC–UV), diode array (HPLC–DAD) and mass spectrometric (LC–MS) detectors is also described [4]. The sweet cherry (*Prunus avium* L.) from the region of Fundão, Portugal, has already been studied to quantify melatonin by Rosado et al. [16], applying high performance liquid chromatography coupled with electrochemical detection (HPLC–ECD).

However, HPLC–DAD equipment is one of the simplest and most popular analytical techniques. In addition, the equipment is relatively inexpensive, features easy maintenance [17]. Thus, the present work describes a fast, sensitive and fully validated method to determine polyphenols in six cherry cultivars of the Fundão region using the HPLC coupled to diode array detection technique. The obtained

values are compared to others present in the literature and, for the first time the present work reports the levels of the studied compounds in *Brooks* cultivars.

2 Materials and methods

2.1 Reagents and materials

The analytical standards quercetin-3,4'-di-O-glycoside, cyanidin-3-O-glycoside and cyanidin ($\geq 98\%$ purity grade) were purchased from Extrasynthese (Genay Cedex, France). The remaining standards, namely chlorogenic acid, gallic acid, *p*-coumaric acid, rutin, epicatechin and quercetin were obtained from Sigma-Aldrich (Sintra, Portugal).

Solvents and other reagents used were of analytical grade. Acetonitrile was purchased from Carlo Erba (Italy), methanol was obtained from Fisher Scientific (United Kingdom) and trifluoroacetic acid was purchased from Sigma-Aldrich (Sintra, Portugal). Deionised (DI) water was obtained from a Milli-Q System (Millipore, Billerica, MA, USA).

2.2 Stock and working solutions

Individual stock solutions of the analytes were prepared at 1 mg/mL in methanol. Working solutions were prepared by serial dilutions in methanol. All solutions were stored at –20 °C and protected from light.

2.3 Cherry cultivars specimens

In the present work, the five cherry cultivars studied from the region of Fundão were *Saco*, *Summit*, *Sweetheart*, *Primulat*, *Brooks* e *Earlise*. These specimens were kindly provided by “Cerfundão—Embalamento e comercialização de cerejas da Cova da Beira, Lda.”, and were properly stored at –20 °C until analysis.

2.4 Lyophilization of cherry samples

Two hundred gram of each cherry sample (fresh or after thawing) was weighed. After weighing, the fruit was frozen at –80 °C for at least 24 h before lyophilization for 60 h. This process was concluded after verifying the almost total loss of water present in the fruit. The freeze-drying yield was calculated according to the following equation:

$$\eta = \frac{m_{\text{lyophilized}}}{m_{\text{fresh}}} \times 100.$$

2.5 Extraction of phenolic compounds from cherry

To each gram of lyophilized fruit was added 20 mL of methanol acidified with 0.1% hydrochloric acid. The

mixture was placed in a thermostatic bath for 2 h at a temperature of 35 °C and agitation at 64 rpm. This extraction process was repeated twice. Subsequently, the extract was centrifuged for 20 min at 4000 rpm. The solvent was further evaporated under reduced pressure (130 mbar) and at 35 °C until the dried extract was obtained. The extraction yield was calculated according to the following equation:

$$\eta = \frac{m_{\text{extract}}}{m_{\text{lyophilized fruit}}} \times 100.$$

2.6 Instrumental and chromatographic conditions

The quantification of the phenolic compounds was performed on a high-performance liquid chromatography system (HPLC) 1290 with a binary pump coupled to diode array detector (DAD) from Agilent technologies (Soquímica, Lisboa, Portugal). The compounds were separated with an Eclipse Plus C18 (3.5 µm, 4.6 × 100 mm) analytical column from Agilent Technologies (Soquímica, Lisboa, Portugal). Gallic acid and epicatechin were detected at 280 nm, quercetin-3,4'-di-O-glycoside, *p*-coumaric and chlorogenic acids at 320 nm, rutin and quercetin at 360 nm and, cyanidin and cyanidin-3-O-glycoside at 520 nm. The mobile phase was composed of 0.1% trifluoroacetic acid in water (A) and acetonitrile (B). A gradient elution system was applied at a flow rate of 0.5 mL/min. The gradient elution process included 10% A (0–3 min), 15% A (3–15 min), 15% A (15–20 min), 18% A (20–25 min) and finally 30% (25–40 min). The injection volume was 20 µL. The column and sampler temperatures were set to 35 and 4 °C, respectively.

2.7 Analytical method validation

The analytical method developed was fully validated according to the guiding principles of the Food and Drug Administration (FDA) [7], which included a 5-day validation protocol and the study of parameters such as selectivity, linearity and limits, intra and inter-day precision and accuracy.

In order to investigate the presence of interferences of the food matrix at the retention times of the phenolic compounds, the selectivity of the analytical method was evaluated. The pure standards of cyanidin, cyanidin-3-glycoside, quercetin, gallic acid, *p*-coumaric acid, rutin, epicatechin, quercetin-3,4'-di-O-glycoside and chlorogenic acid were prepared in methanol at a final concentration of 100 µg/mL. The same compounds were injected for determination of the retention time and the wavelength at which absorption was maximum. The method would be considered selective if the analytes did not interfere with each other nor with other matrix constituents.

The linearity of the method was established in the range of 2–50 µg/mL (six calibrators evenly distributed). By tracing the area of the peaks of the phenolic compounds against their concentration, we obtained the calibration curves, and the acceptance criteria included a coefficient of determination (R^2) of at least 0.99 and accuracy of the calibrators within a ± 15% interval, except for the lower limit of quantification (LLOQ), where ± 20% was considered acceptable. Together with each calibration curve, a blank sample and three quality control samples (QC) at concentrations of 4, 15 and 40 µg/mL ($n = 3$) were also analysed. The lowest concentration measured with adequate accuracy and precision, with a coefficient of variation (CV%) of less than 20% and a relative error (RE%) within ± 20% of the nominal concentration was defined as LLOQ. The limit of detection (LOD) was not systematically evaluated, but was assumed to be the same as the LLOQ ($n = 5$). Consequently, values below this value would be considered negative.

Intra-day precision was assessed by analysing in the same day 6 replicates at the concentration levels of 2, 15 and 40 µg/mL. Inter-day precision consisted in prepared and analysed samples fortified with the study analytes at concentrations of 2; 5; 10; 20; 30 and 50 µg/mL within the 5 day period. The accuracy of the method was characterized in terms of the mean relative error (RE%) between the measured and the specified concentrations; the accepted limit was 15% for all concentrations, except for LLOQ, where 20% were accepted (Figs. 1, 2).

The carry-over effect was assessed by mobile phase injection immediately after analysis of the highest level calibrator (50 µg/mL).

3 Results and discussion

3.1 Validation of the analytical method

3.1.1 Selectivity

The ability of the analytical method to unequivocally differentiate the mentioned phenolic compounds in the presence of other compounds that exist in the sweet cherry matrix was evaluated. There was no evidence of interference or possible contribution at the retention times of the target compounds, and therefore the method was considered selective.

3.1.2 Linearity and limits

The method was considered linear from 2 to 50 µg/mL, presenting a R^2 value between 0.996 and 0.999, while the accuracy of the calibrators (mean relative error (bias)

Fig. 1 Chromatograms obtained for quercetin (a), gallic acid (b), *p*-coumaric acid (c), rutin (d), chlorogenic acid (e), epicatechin (f), cyanidin-3-*O*-glycoside (g), cyanidin (h), quercetin-3,4'-*O*-glycoside (i), at wavelengths of 280 nm, 320 nm, 360 nm and 520 nm

Fig. 1 (continued)

between measured and fortified concentrations) was within a range of $\pm 15\%$ for all concentrations. Table 1 shows calibration data.

The LLOQ obtained was 2 $\mu\text{g/mL}$. The LOD was not systematically evaluated and was considered the same as the LLOQ.

3.1.3 Intra- and inter-day precision and accuracy

Intra-day precision resulted in coefficients of variation (CVs) of less than 8% at the studied concentration levels, with an average relative error within $\pm 12\%$. Meanwhile, the assessment of inter-day precision and accuracy

Fig. 2 Chromatogram of the cultivar *Saco* at wavelengths 280 nm [gallic acid (**a**) and epicatechin (**b**)], 320 nm [chlorogenic acid (**c**), quercetin-3,4'-di-*O*-glycoside (**d**) and *p*-coumaric acid (**e**)], 360 nm [rutin (**f**) and quercetin (**g**)] and 520 nm [cyanidin-3-*O*-glycoside (**h**)]

Table 1 Linearity data (n = 5)

Analytes	Linear range (µg/mL)	Linearity		r^{2a}	LLOQ (µg/mL)
		Slope ^a	Intercept ^a		
Gallic acid	2–50	102.30 ± 5.27	− 18.21 ± 75.86	0.998 ± 0.0017	2
<i>p</i> -Coumaric acid		258.27 ± 2.23	− 270.85 ± 87.54	0.996 ± 0.0028	
Rutin		22.78 ± 1.50	− 14.97 ± 31.13	0.998 ± 0.0031	
Chlorogenic acid		74.93 ± 3.29	− 83.55 ± 18.42	0.995 ± 0.0046	
Epicatechin		26.99 ± 6.10	− 14.87 ± 16.87	0.998 ± 0.0003	
Cyanidin		69.01 ± 0.95	5.69 ± 11.13	0.998 ± 0.0019	
Quercetin-3-4'-di- <i>O</i> -glycoside		9.43 ± 2.75	− 7.73 ± 5.78	0.999 ± 0.0014	
Cyanidin-3- <i>O</i> -glycoside		46.31 ± 1.28	− 55.74 ± 36.41	0.999 ± 0.0013	
Quercetin		44.32 ± 1.11	− 81.25 ± 37.42	0.998 ± 0.0017	

^aMean values ± SD

resulted in CVs lower than 13% at all tested concentration levels, with accuracy within ± 10% range. All data are summarized in Table 2.

3.1.4 Carry over effect

The possible carry over that could occur and contribute to the signal of the phenolic compounds after the analysis of highly concentrated samples was evaluated. This effect may be caused by contamination of the sample which causes the sample peaks to reappear in subsequent runs and therefore, a blank sample was analysed after the injection of the most concentrated calibrator of the calibration curve (50 µg/mL).

3.2 Cherry cultivars from region of Fundão, Portugal

The developed method was used to determine cyanidin, cyanidin-3-glucoside, quercetin, gallic acid, *p*-coumaric acid, rutin, epicatechin, quercetin-3,4'-di-*O*-glycoside and chlorogenic acid present in six common sweet cherry cultivars from the region of Fundão, Portugal. The cultivars processed and analysed were those of *Sweetheart*, *Earlise*, *Summit*, *Primulat*, *Brooks* and *Saco*.

3.3 Lyophilization of *Prunus avium* L. fruit

The efficiency of lyophilization was determined by comparing fresh weight and lyophilized weight, thus allowing the percentage of water loss to be determined. The results are shown in Table 3.

3.4 Methanolic extraction of lyophilized fruit of *Prunus avium* L

The efficiency of the extraction was determined by comparing the weight of the extract and the weight of lyophilized fruit, thus allowing to determine the effectiveness of the process. The results are shown in Table 3.

One must take into account, however, that other compounds of non-phenolic nature may have been extracted from the lyophilized cherries as well; they were not detected by our selective method, but the values obtained for efficiency may be overestimated.

3.5 Characterization of the extract of six sweet cherry cultivars from Fundão, Portugal

The phenolic content of each sweet cherry cultivar was evaluated and is shown in Table 4. For these six cultivars, the absence of quercetin and cyanidin was verified, which is compatible with the literature, although there is at least one study that identifies trace amounts of cyanidin [10]. The cultivar that showed the lowest content of the studied compounds was *Earlise*, and the results were in accordance to the literature [9, 11, 13].

Quercetin-3-4'-di-*O*-glycoside is one of the compounds whose amount varies from 3.67 ± 0.12 mg/100 g fresh fruit (*Earlise*) to 132.07 ± 7.51 mg/100 g of fresh fruit (*Primulat*). This is the compound that is present at high concentrations. Also cyanidin-3-*O*-glycoside varies considerably between cultivars. This compound is one of the most frequent in sweet cherries, with a higher content in the cultivar *Saco* and lower in *Earlise*.

Rutin, chlorogenic acid and *p*-coumaric acid are common compounds and there are several examples of studies which focus on these compounds in the literature. The sweet cherry cultivars of Fundão have not been the subject of similar studies, however, it was possible to compare

Table 2 Inter-day and intra-day precision and accuracy

Analytes	Concentration (µg/mL)	Inter-day precision			Intra-day precision		
		Calculated Concentration (µg/mL)	CV (%)	RE (%)	Calculated Concentration (µg/mL)	CV (%)	RE (%)
Gallic acid	2	1.60	3.75	−20.15	1.61	5.59	−19.57
	5	5.12	1.95	2.37	—	—	—
	10	10.29	1.65	2.86	—	—	—
	15	—	—	—	16.37	4.76	9.11
	20	20.37	2.21	1.86	—	—	—
	30	29.42	4.93	−1.94	—	—	—
	40	—	—	—	37.78	4.82	−5.54
	50	50.06	1.06	0.13	—	—	—
<i>p</i> -Coumaric acid	2	2.29	2.18	14.31	1.46	18.18	−26.98
	5	5.48	4.74	9.59	—	—	—
	10	10.07	1.59	0.74	—	—	—
	15	—	—	—	16.04	2.24	6.95
	20	19.86	0.25	−0.68	—	—	—
	30	27.65	0.69	−7.83	—	—	—
	40	—	—	—	42.91	2.38	7.28
	50	50.91	1.22	1.82	—	—	—
Rutin	2	1.56	3.23	−22.21	1.82	6.59	−9.07
	5	5.36	3.73	7.30	—	—	—
	10	10.15	0.39	1.46	—	—	—
	15	—	—	—	15.78	2.72	5.23
	20	20.25	0.35	1.24	—	—	—
	30	30.04	0.27	0.12	—	—	—
	40	—	—	—	39.37	3.35	−1.58
	50	50.34	1.13	0.68	—	—	—
Chlorogenic acid	2	2.33	2.15	16.48	1.63	7.36	−18.46
	5	5.65	6.02	12.96	—	—	—
	10	10.33	0.68	3.26	—	—	—
	15	—	—	—	15.57	2.89	3.78
	20	19.63	0.71	−1.87	—	—	—
	30	27.06	1.03	−9.80	—	—	—
	40	—	—	—	39.38	5.21	−1.54
	50	51.24	1.42	2.47	—	—	—
Epicatechin	2	2.27	0.88	13.74	2.11	8.53	5.44
	5	5.69	1.76	13.87	—	—	—
	10	10.08	3.08	0.76	—	—	—
	15	—	—	—	14.67	9.75	−2.20
	20	19.07	0.37	−4.66	—	—	—
	30	29.02	0.14	−3.28	—	—	—
	40	—	—	—	36.25	3.09	−9.37
	50	50.87	1.04	1.73	—	—	—
Cyanidin	2	1.86	3.76	−6.91	1.95	1.03	−2.31
	5	5.00	5.00	0.06	—	—	—
	10	10.01	4.90	0.08	—	—	—
	15	—	—	—	14.37	5.36	−4.23
	20	18.84	14.49	−5.81	—	—	—
	30	28.31	5.65	−5.63	—	—	—
	40	—	—	—	41.35	4.86	3.38

Table 2 (continued)

Analytes	Concentration (µg/mL)	Inter-day precision			Intra-day precision		
		Calculated Concentration (µg/mL)	CV (%)	RE (%)	Calculated Concentration (µg/mL)	CV (%)	RE (%)
Quercetin-3-4'-di-O-glycoside	50	48.42	6.86	−3.16	–	–	–
	2	2.27	13.66	13.74	2.05	3.90	2.47
	5	5.69	8.44	13.87	–	–	–
	10	10.08	7.64	0.76	–	–	–
	15	–	–	–	16.76	14.32	−6.50
	20	19.07	6.40	−4.66	–	–	–
	30	29.02	3.31	−3.28	–	–	–
	40	–	–	–	41.37	6.89	−7.26
Cyanidin-3-O-glycoside	50	50.87	1.42	1.73	–	–	–
	2	2.27	14.98	13.74	1.68	8.93	−15.80
	5	5.69	4.04	13.87	–	–	–
	10	10.08	1.69	0.76	–	–	–
	15	–	–	–	14.68	4.09	−2.14
	20	19.07	3.83	−4.66	–	–	–
	30	29.02	2.07	−3.28	–	–	–
	40	–	–	–	42.19	3.01	5.49
Quercetin	50	50.87	0.85	1.73	–	–	–
	2	2.21	9.05	10.57	2.23	4.48	11.32
	5	5.44	2.02	8.75	–	–	–
	10	9.97	1.40	−0.34	–	–	–
	15	–	–	–	15.25	5.44	1.66
	20	19.59	0.36	−2.03	–	–	–
	30	29.23	1.44	−2.55	–	–	–
	40	–	–	–	42.13	4.30	5.32
	50	50.65	0.55	1.31	–	–	–

Table 3 Efficiency of lyophilization and methanolic extraction of the different sweet cherry cultivars (n=3)

Cultivar	Lyophilization (%)	Extraction (%)
<i>Saco</i>	18.39	93.89
<i>Primulat</i>	19.77	97.59
<i>Summit</i>	21.20	85.10
<i>Earlise</i>	13.21	78.35
<i>Sweetheart</i>	27.13	98.56
<i>Brooks</i>	22.53	96.98

the *Primulat* variety with respect to the rutin, chlorogenic acid and epicatechin contents, in relation to the work of Usenik et al. [22].

In the study, it was possible to verify that the determined amounts of rutin, epicatechin and chlorogenic acid were 4.52 ± 0.19 , 2.14 ± 0.16 and 2.78 ± 0.02 mg per

100 grams of fresh fruit, respectively. On the other hand, the amounts obtained by Usenik et al. were 2.81 ± 0.43 , 4.51 ± 0.00 and 0.60 ± 0.06 [22]. It is known that in the sweet cherry cultivars produced in the Fundão region the amounts of these compounds are considerably higher.

Ballistreri et al. [2] quantified the chlorogenic acid content of *Sweetheart* cultivar, which was 1.46 ± 0.10 mg per 100 g of fresh fruit. By contrast, in the present work the amount of the same compound was 3.94 ± 0.07 mg per 100 of fresh fruit for the same cultivar.

Another study, published by Serra et al. [19], studied the compounds epicatechin, chlorogenic acid, rutin and cyanidin-3-O-glycoside for the cultivars *Saco* and *Summit*, among others. It can be observed that, similarly to the results obtained in our study, the *Saco* cultivar shows a higher content of phenolic compounds. On the other hand, with the exception of rutin, present in the cultivar *Saco*, all other components are present in higher quantities.

When comparing the results obtained for sweet cherry cultivars in the Fundão region to those of sweet cherries

Table 4 Identification and quantification of phenolic compounds in sweet cherry cultivars

Phenolic compound	Concentrations (n = 6) ^a					
	<i>Primulat</i>	<i>Summit</i>	<i>Earlise</i>	<i>Sweetheart</i>	<i>Brooks</i>	<i>Saco</i>
Quercetin	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
Gallic acid	0.55 ± 0.04	0.46 ± 0.00	0.27 ± 0.01	0.61 ± 0.00	0.54 ± 0.01	0.51 ± 0.01
<i>p</i> -Coumaric acid	2.77 ± 0.03	2.49 ± 0.01	1.40 ± 0.01	3.50 ± 0.00	2.91 ± 0.02	2.28 ± 0.01
Rutin	4.52 ± 0.19	4.13 ± 0.21	3.17 ± 0.02	7.56 ± 0.38	5.20 ± 0.15	10.66 ± 0.07
Chlorogenic acid	2.78 ± 0.02	3.13 ± 0.06	2.16 ± 0.03	3.94 ± 0.07	3.30 ± 0.03	2.95 ± 0.10
Epicatechin	2.14 ± 0.16	2.65 ± 0.09	0.79 ± 0.00	4.43 ± 0.29	3.63 ± 0.14	1.51 ± 0.15
Cyanidin-3- <i>O</i> -glycoside	12.23 ± 0.79	3.54 ± 0.10	1.81 ± 0.02	4.76 ± 0.13	4.43 ± 0.06	22.03 ± 0.74
Cyanidin	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
Quercetin-3-4'-di- <i>O</i> -glycoside	132.07 ± 7.51	43.54 ± 1.32	3.67 ± 0.12	28.31 ± 4.89	64.59 ± 1.13	24.61 ± 0.42

Concentrations expressed in milligrams per 100 g of fresh fruit ± SD

^aMean values ± SD

from other regions, differences are observed. Tsao et al. [21] quantified phenolic compounds in various fruits, including sweet cherries. It was possible to verify that the compounds present in larger quantities are phenolic acids. By contrast, in the present work, flavonoids are present in greater amounts. These compounds, as already mentioned, have a high number of health benefits. The phenolic acids analysed are found in lower quantities in the sweet cherry cultivars of Fundão.

4 Conclusions

An analytical method using a fast extraction and a high performance liquid chromatography coupled to a diode array detector was developed for the simultaneous quantification of nine phenolic compounds in six cultivars of sweet cherries samples. The analytical method was linear in the range of 2–50 µg/mL for the referred compounds. The precision and accuracy were also adequate and LLOQ obtained was 2 µg/mL. Concentrations of 0.27–132.07 mg/100 g of fresh fruit were found in the cultivars analysed, where the cultivar *Primulat* presented the highest levels. In contrast, *Earlise* showed the lowest concentration of phenolic compounds. The present work reports for the first time the levels of the studied compounds in *Brooks* cultivars. In addition, this method has a reduced consumption of solvents, is fast and simple, constituting an alternative to traditional extraction techniques.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with animals or human participants performed by any of the authors.

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