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Effect of Ripeness and Postharvest Storage on the Phenolic Profiles of Cherries (*Prunus avium* L.)

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The phenolic compounds hydroxycinnamates, anthocyanins, flavonols, and flavan-3-ols of sweet cherry cultivars Burlat, Saco, Summit, and Van harvested in 2001 and 2002 were quantified by HPLC-DAD. Phenolics were analyzed at partially ripe and ripe stages and during storage at 15 ± 5 °C (room temperature) and 1−2 °C (cool temperature). Neochlorogenic and *p*-coumaroylquinic acids were the main hydroxycinnamic acid derivatives, but chlorogenic acid was also identified in all cultivars. The 3-glucoside and 3-rutinoside of cyanidin were the major anthocyanins. Peonidin and pelargonidin 3-rutinosides were the minor anthocyanins, and peonidin 3-glucoside was also present in cvs. Burlat and Van. Epicatechin was the main monomeric flavan-3-ol with catechin present in smaller amounts in all cultivars. The flavonol rutin was also detected. Cultivar Saco contained the highest amounts of phenolics [227 mg/100 g of fresh weight (fw)] and cv. Van the lowest (124 mg/100 g of fw). Phenolic acid contents generally decreased with storage at 1−2 °C and increased with storage at 15 ± 5 °C. Anthocyanin levels increased at both storage temperatures. In cv. Van the anthocyanins increased up to 5-fold during storage at 15 ± 5 °C (from 47 to 230 mg/100 g of fw). Flavonol and flavan-3-ol contents remained quite constant. For all cultivars the levels of phenolic acids were higher in 2001 and the anthocyanin levels were higher in 2002, which suggest a significant influence of climatic conditions on these compounds.

KEYWORDS: Cherry; *Prunus avium;* ripeness; storage; HPLC-DAD; phenolics; anthocyanins; flavan-3-ols; flavonols; hydroxycinnamates.

INTRODUCTION

High fruit and vegetable consumption is associated with a reduced risk of major chronic diseases such as cancer, atherosclerosis, and cardiovascular disease (*1*−*4*). Phenolic compounds have been widely identified in fruits and vegetables and partly related to those protective effects (*5*−*7*).

The phenolic composition of plant foods is dependent on plant genetic information (*8*) and environmental factors during growth and postharvest. Cherries are considered to be a major source of phenolic compounds, which are also responsible for their color and taste and presumably also their antioxidant properties. Cherry phenols include flavonoids such as anthocyanins, flavan-3-ols, and flavonols in addition to the nonflavonoid compounds hydroxycinnamic acids and hydroxybenzoic acids (*9*, *10*).

Fruit tissues are able to synthesize phenolic compounds, and changes in this content can be induced by biotic and abiotic stress conditions (*8*, *11*). Water availability and soil composition (mineral and organic nutrients) have a marked effect on the phenolic content of plants (*12*) and on the ability of plant products to suffer browning and other phenolic-related physiological disorders that appear during the maturity stage and postharvest (*13*). Storage at low temperatures might have positive or negative effects on phenolics and in turn on fruit quality, depending on the commodity and the storage temperature (*13*, *14*).

Cherries are nonclimacteric fruits that are usually picked at peak maturity for optimal taste and appearance. However, in Portugal, and in other countries that produce sweet cherries for fresh consumption, the cherries are often stored for up to 3−4 weeks at cold temperatures to increase the seasonal supply. To our knowledge, information about the changes in the phenolic content of cherries during maturity and postharvest storage is limited; however, this information is relevant to the understanding of the parameters that affect fruit color and stability and the potential health protective effects of the phenolics. The aims of this study were to (1) identify and quantify individual phenolic compounds in sweet cherries at partially ripe and ripe

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Table 1. Quality Indices of Cherries at Two Ripeness Stages (Partially Ripe and Ripe)[a]

cv.	stage	wt (g)	skin color *a** value	soluble solids (°Brix)	titratable acidity (meq/L)	pH
			Year 2001			
Burlat	partially ripe	6.2 ± 0.6 d	36.4 ± 6.8 bc	10.4 ± 0.1 a	79.3 ± 7.6 b	3.70 ± 0.02 b
	ripe	7.4 ± 0.9 e	36.2 ± 5.9 bc	13.4 ± 0.1 c	70.3 ± 13.9 ab	3.74 ± 0.01 c
Saco	partially ripe	4.3 ± 0.4 a	42.0 ± 2.1 bc	14.7 ± 0.2 d	89.3 ± 1.2 c	4.21 ± 0.02 f
	ripe	5.0 ± 0.6 b	35.8 ± 10.3 e	15.1 ± 0.1 e	68.7 ± 1.2 a	4.27 ± 0.01 g
Summit	partially ripe	6.6 ± 0.8 d	38.5 ± 3.0 ab	12.5 ± 0.0 b	112.3 ± 0.6 e	3.82 ± 0.02 d
	ripe	9.0 ± 1.2 f	34.2 ± 10.2 cd	18.6 ± 0.1 g	99.3 ± 1.2 d	4.16 ± 0.00 e
Van	partially ripe	5.6 ± 0.6 c	40.9 ± 2.3 a	15.2 ± 0.2 e	133.7 ± 1.5 f	3.64 ± 0.01 a
	ripe	7.4 ± 0.7 e	32.8 ± 11.3 de	16.5 ± 0.1 f	143.3 ± 2.1 f	3.66 ± 0.01 a
			Year 2002			
Burlat	partially ripe	4.2 ± 0.6 a	43.2 ± 4.8 f	11.9 ± 1.4 a	58.7 ± 1.6 b	3.92 ± 0.01 d
	ripe	7.2 ± 0.8 c	18.5 ± 6.5 a	16.3 ± 2.1 cd	67.2 ± 1.7 c	3.91 ± 0.04 cd
Saco	partially ripe	4.3 ± 0.8 a	36.3 ± 7.8 e	15.8 ± 1.1 c	76.2 ± 0.6 d	3.95 ± 0.03 d
	ripe	5.2 ± 0.8 b	26.6 ± 6.6 c	17.6 ± 0.9 e	81.4 ± 1.8 ef	3.84 ± 0.02 ab
Summit	partially ripe	5.3 ± 0.7 b	41.0 ± 4.7 f	13.6 ± 1.4 b	74.3 ± 2.3 d	3.87 ± 0.02 bc
	ripe	7.0 ± 0.8 c	33.5 ± 4.6 d	16.7 ± 2.1 cde	79.9 ± 0.3 e	3.87 ± 0.02 bc
Van	partially ripe	5.5 ± 0.6 b	23.4 ± 12.9 b	17.3 ± 1.3 de	48.7 ± 2.2 a	4.16 ± 0.04 e
	ripe	7.0 ± 1.2 c	21.5 ± 5.4 b	19.2 ± 2.0 f	83.6 ± 1.4 f	3.80 ± 0.02 a

[a] Means of each year followed by the same letter are not significantly different at $P < 0.05$ (Duncan's test). Mean values ± SD ($n = 20$) for the year 2001 and 2002.

stages, (2) evaluate the effects of postharvest storage on the phenolic composition of sweet cherries, and (3) assess the variations caused by natural harvest fluctuations in different harvest years.

MATERIALS AND METHODS

Sample Preparation. Sweet cherries from the cultivars Burlat, Saco, Summit, and Van, grown in Vila Real, Portugal, were randomly harvested by hand in 2001 and 2002, at two different stages of ripeness: partially ripe and ripe. Skin color is the main criterion used for indicating maturity for cherry picking. For each cultivar the maturity was assessed for 20 fruits by the following indices: weight, skin color (by a Minolta colorimeter), soluble solids content (°Brix by a refractometer), titratable acidity (by an automatic titration system), and pH (by a pH-meter). The ranges of these indices for each cultivar are shown in **Table 1**.

The phenolic analyses were made on days 0, 5, 10, 15, 20, 25, and 30 in the fruits under storage at cold treatment [1−2 °C and 90% relative humidity (RH)] and on days 0, 3, and 6 for fruits subjected to room temperature treatment (15 ± 5 °C). Cherries were cut in half (the stone was taken off), and the cherry halves were frozen in liquid nitrogen, crushed, and freeze-dried prior to analysis.

Extraction of Phenolic Compounds. Pitted and freeze-dried cherry samples (0.5 g) were mixed in 5 mL of 60% MeOH, flushed with N_2, and extracted during shaking using a thermostated (25 ± 1 °C) water bath, at 200 rpm for 10 min. The suspension was initially filtered under vacuum through one layer of Whatman no. 1 filter paper. The extract was then filtered through a 0.45 μm hydrophilic Durapore filter (Millipore Corp., Bedford, MA), flushed with N_2, and the filtrate was injected into the HPLC, after a period not exceeding 24 h, for the separation and quantification of phenolic compounds. The cherry samples were submitted to a second and a third extraction and analyzed by HPLC, and the total content was determined as the sum of the three values.

HPLC-DAD Analyses. Samples of 10 μL of extracts were analyzed using an HPLC system equipped with a diode array detector (DAD) (Hewlett-Packard 1100 system, Waldbronn, Germany) operated by HP ChemStation software with a Nova-Pak C18 column (3.9 × 150 mm, Waters) at 40 °C. The mobile phase was made of three solvents delivered in a gradient system at a flow rate of 0.5 mL/min essentially as described by Lamuela-Raventós and Waterhouse (*15*).

Identification and Quantification of Phenolic Compounds. The phenolic compounds in cherry extracts were identified by their spectral and retention time characteristics, recorded with a diode array detector, and, wherever possible, by spectral chromatographic comparisons with authentic markers (*16*).

The quantities of the different phenolic compounds were assessed from peak areas and calculated as equivalents of seven representative standard compounds (from standard, linear regression curves of authentic standards) as follows: at 280 nm (flavan-3-ols), catechin and epicatechin, respectively; at 316 nm (hydroxycinnamates), neochlorogenic acid, chlorogenic acid, and other hydroxycinnamaic acids as chlorogenic acid equivalents and *p*-coumaroylquinic acid as *p*-coumaric acid equivalents; at 365 nm (flavonols), quercetin glucosides as rutin equivalents; at 520 nm (anthocyanins), cyanidin 3-glucoside and cyanidin 3-rutinoside, respectively, and other anthocyanins as cyanidin 3-rutinoside. Coefficients of variation on the HPLC quantifications were <5%. Concentrations were expressed as milligrams per 100 g of fresh weight (fw).

Determination of Total Phenolics. The concentration of total phenolics in cherry extracts was determined on the same extract used for HPLC analysis, according to the Folin−Ciocalteu procedure (*17*). The content was expressed as milligrams per liter gallic acid equivalents (GAE), being transformed into milligrams of gallic acid/100 g of fw.

Statistics. The analyses of data were performed as analysis of variance using the Super ANOVA software (1.11 Abacus Concepts Inc., 1991). Significances of differences were established from a Duncan's test ($P < 0.05$).

RESULTS

Phenolic Content. The total phenolic content obtained by using the Folin−Ciocalteu procedure of the different sweet cherry cultivars is shown in **Table 2**. The analysis of variance revealed significant differences ($P < 0.001$) in the total content of phenolic compounds among the four cultivars. The levels of total phenols in cv. Saco were consistently higher than the levels in the other cultivars, which were at the relatively same levels (**Table 2**). However, levels depended on ripeness stage, harvest year, and storage conditions ($P < 0.001$). Higher values of total phenolics were always observed in ripe cherries; for instance, 264 mg/100 g of fw was observed in cv. Saco versus cv. Van in the partially ripe stage, which contained only 69 mg/100 g of fw (**Table 2**), independent of the years. Between years, levels in 2001 were always greater than those of 2002 ($P < 0.001$), except for cv. Burlat cherries after storage at 15 ± 5 °C. Regarding the influence of storage on total phenolic contents, it was observed that in partially ripe cherries levels increased with storage at low temperature. During cool storage, the total

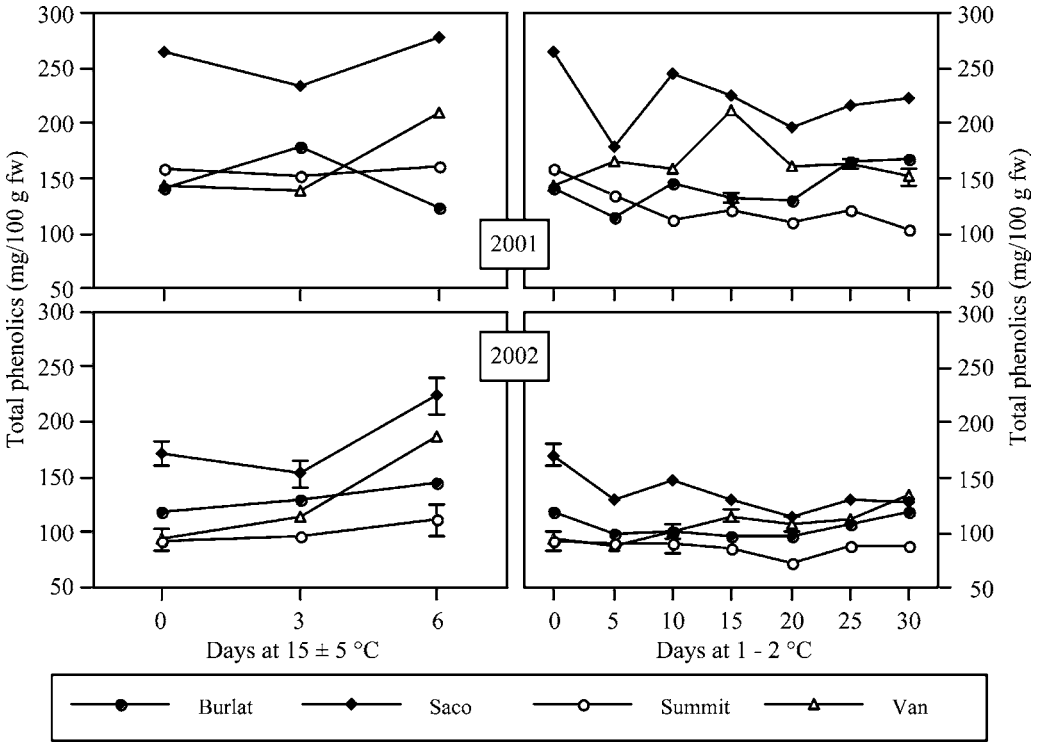

Figure 1. Total phenolics (by Folin–Ciocalteu's procedure) of the four sweet cherry cultivars in mg/100 g of fresh weight during storage at room temperature (15 ± 5 °C) and cool temperature (1–2 °C) in 2001 and 2002. Values are the mean ± SD (n = 3). SD bars are not shown if they are smaller than the symbols.

Table 2. Total Phenolic Content of Cherry Cultivars Determined According to Folin–Ciocalteu's Method at Two Ripeness Stages (Partially Ripe and Ripe), after Storage at Room (15 ± 5 °C) and Cool Temperature (1–2 °C)[a]

cv./stage	storage conditions	total phenolics (mg/100 g of fresh wt) year 2001	year 2002
Burlat			
partially ripe	day 0	108 ± 2.2 a	91.6 ± 1.0 a
partially ripe	1–2 °C; day 30	135 ± 2.5 c	92.5 ± 0.2 a
ripe	day 0	141 ± 2.0 d	119 ± 0.7 b
ripe	15 ± 5 °C; day 6	124 ± 1.8 b	144 ± 1.1 c
ripe	1–2 °C; day 30	167 ± 1.3 e	118 ± 1.7 b
Saco			
partially ripe	day 0	169 ± 0.8 a	123 ± 1.2 a
partially ripe	1–2 °C; day 30	*b*	123 ± 7.6 a
ripe	day 0	264 ± 0.9 c	171 ± 10.6 b
ripe	15 ± 5 °C; day 6	278 ± 2.0 d	223 ± 16.7 c
ripe	1–2 °C; day 30	222 ± 3.9 b	129 ± 1.3 a
Summit			
partially ripe	day 0	120 ± 1.6 b	74.7 ± 2.2 a
partially ripe	1–2 °C; day 30	132 ± 1.0 c	84.5 ± 4.4 a
ripe	day 0	159 ± 2.1 d	92.7 ± 9.4 ab
ripe	15 ± 5 °C; day 6	160 ± 0.6 d	110 ± 14.6 b
ripe	1–2 °C; day 30	104 ± 2.8 a	88.2 ± 0.6 a
Van			
partially ripe	day 0	115 ± 3.4 a	69.0 ± 0.5 a
partially ripe	1–2 °C; day 30	158 ± 3.0 c	111 ± 0.4 c
ripe	day 0	144 ± 2.3 b	94 ± 2.4 b
ripe	15 ± 5 °C; day 6	209 ± 3.8 d	187 ± 1.4 e
ripe	1–2 °C; day 30	152 ± 7.0 bc	134 ± 0.3 d

[a] Means of each cultivar and year followed by the same letter are not significantly different at $P < 0.05$ (Duncan's test). Mean values ± SD (n = 3) for the year 2001 and 2002. [b] Data not available.

phenolic levels in the ripe cv. Saco decreased slightly (both years $P < 0.001$), and in 2001 they also decreased in the cv. Summit ($P < 0.001$). The phenolic levels of the other cultivars showed only small variations during cool storage, although the contents in cv. Van apparently increased during the cool storage, especially for the 2002 harvest ($P < 0.001$; **Figure 1**). At 15 ± 5 °C, a slight increase in phenolics was noted in cvs. Van and Saco in the two years of study ($P < 0.001$ in all cases). In contrast, cv. Burlat showed a slight but significant decrease in the total phenols between days 3 and 6 at ambient storage in 2001. The others did not differ between the sampling dates for the two years (**Figure 1**).

The data presented here as well as those in the literature suggest that cultivar, ripeness stage, harvest year, and storage conditions have a major influence on the quantitative individual phenolic composition of cherries. It is important to note, however, that during short-term storage at ambient temperature, the phenolics increase significantly in certain cultivars, whereas cold storage can induce small decreases in the phenols of certain cherry cultivars.

HPLC-DAD Analysis of Cherry Phenolics. The methanol extracts of cherries were analyzed by HPLC-DAD, and example chromatograms recorded at 280 nm are shown in **Figure 2**. Generally, the same phenolic compounds were present in all cultivars, but the relative levels varied among the four cultivars. Four groups of phenolic compounds were identified: the hydroxycinnamic acids, anthocyanins, flavan-3-ols, and flavonols (**Figure 2**).

The chromatograms showed three peaks (**Figure 2**) with UV spectra characteristic of caffeic acid derivatives. In cvs. Saco and Van, the main hydroxycinnamic acid was identified as neochlorogenic acid (3-*O*-caffeoylquinic acid) (**Table 3**, peak 1 in **Figure 2**), followed by *p*-coumaroylquinic acid (**Table 3**, peak 2 in **Figure 2**); note that in the cv. Burlat, the levels of neochlorogenic acid and *p*-coumaroylquinic acid were at approximately the same levels (**Table 3**). The third compound was identified as chlorogenic acid (5-*O*-caffeoylquinic acid) (peak 3, **Figure 2**). Other minor compounds with the charac-

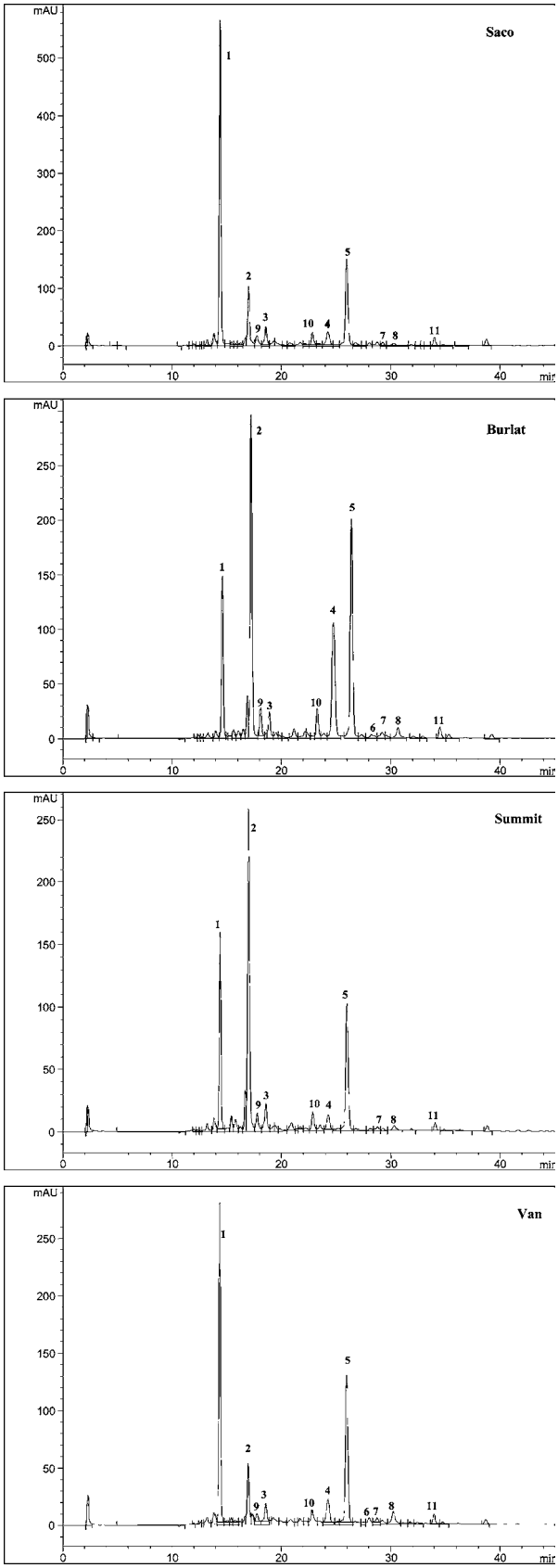

Figure 2. HPLC chromatograms of the four sweet cherry cultivar extracts recorded at 280 nm. Hydroxycinnamic acid derivative peaks: (1) neochlorogenic acid; (2) *p*-coumaroylquinic acid; (3) chlorogenic acid. Anthocyanin peaks: (4) cyanidin 3-glucoside; (5) cyanidin 3-rutinoside; (6) peonidin 3-glucoside; (7) pelargonidin 3-rutinoside; (8) peonidin 3-rutinoside. Flavan-3-ols peaks: (9) catechin; (10) epicatechin. Flavonol peak: (11) rutin.

teristic spectra of hydroxycinnamic acid derivatives were detected, but their exact identities could not be ascertained by DAD analysis, which is why they are designated "other hydroxycinnamic acids" in **Table 3**.

The chromatograms revealed that the cherries of the four cultivars contained at least five different anthocyanins (**Figure 2**). They had similar UV−vis spectra with a maximum around 515 nm in the spectra recorded with the diode array detector. The main pigments were identified as cyanidin 3-rutinoside (peak 5), followed by cyanidin 3-glucoside (peak 4). Other minor anthocyanins, peonidin 3-glucoside (peak 6), pelargonidin 3-rutinoside (peak 7), and peonidin 3-rutinoside (peak 8), were also detected. In addition were identified two peaks (**Figure 2**) with UV spectra of flavan-3-ols (maximum at 280 nm). The major compound of this phenolic group was identified as epicatechin (peak 10), followed by catechin (peak 9). One flavonol peak (**Figure 2**) was detected, and the UV spectra suggest it to be rutin (quercetin 3-*O*-rutinoside) (peak 11) glycosylated at the hydroxyl in the 3-position (*18*).

Phenolic Acids Contents. The hydroxycinnamic acids content of the cherry cultivars is shown in **Table 3**. The levels of phenolic acids differed between cultivars, ripeness stage, year, and storage conditions ($P < 0.001$). On average, hydroxycinnamic acids in cv. Saco (156 mg/100 g of fw) were higher than in the other cultivars, the lowest being in cv. Burlat (53 mg/100 g of fw). However, levels depended on maturity stage, year, and storage conditions ($P < 0.001$). At harvest, cv. Saco partially ripe cherries presented the highest values of phenolic acids (147 mg/100 g of fw in 2001 and 140 mg/100 g of fw in 2002), and the lowest values were observed in cv. Burlat (51 mg/100 g of fw) in 2001 and in cv. Van (55 mg/100 g of fw) in 2002 (**Table 3**). When picked at the ripe stage, cv. Saco cherries also had the highest values (222 and 137 mg/100 g of fw in 2001 and 2002, respectively), and cv. Burlat showed the lowest values (52 and 53 mg/100 g of fw, in 2001 and 2002, respectively) (**Table 3**). The majority of the sampling dates revealed higher levels of hydroxycinnamic acids in 2001. Storage period induced some variations in phenolic acids, although the final tendency was a reduction of these levels in cherries storage at 1−2 °C and an increase in cherries stored at 15 ± 5 °C except for cv. Burlat (**Table 3**).

With regard to the individual hydroxycinnamic acid derivatives, it was noted that neochlorogenic acid was the major compound, varying from 22 to 190 mg/100 g of fw in ripe fruits, and represented 19 and 71% of the phenolics, respectively, and from 19 to 126 mg/100 g of fw in partially ripe fruits representing 24−72% of the phenolics. The contents of *p*-coumaroylquinic and chlorogenic acids presented similar values during ripeness and storage for the same cultivar (**Table 3**). The *p*-coumaroylquinic acid content varied from 4 to 34 mg/100 g of fw and represented 4% of the total phenolics in cherry extract in cv. Van and 27% in cv. Summit, respectively (**Table 3**). The chlorogenic acid content ranged from 3 to 12 mg/100 g of fw and represented 2% of the total phenolics in cherry extract cv. Van and 4% in cv. Saco, respectively. Other hydroxycinnamic acids represented <2% of the total phenolics (**Table 3**).

Anthocyanins Content. The anthocyanin levels differed among the cherry cultivars, ripeness stage, year of study, and storage conditions ($P < 0.001$). Levels of anthocyanins in partially ripe cherries were very low (from 5 to 23 mg/100 g of fw) when compared to ripe fruits (19 to 96 mg/100 g of fw) (**Table 4**). At harvest, cv. Burlat ripe cherries showed the largest content, totaling 96 mg/100 g of fw in 2002, and cv. Van showed

Table 3. Hydroxycinnamic Acid Derivative Levels (Milligrams per 100 g of Fresh Weight) in Cherry Cultivars at Two Ripeness Stages (Partially Ripe and Ripe), after Storage at Room (15 ± 5 °C) and Cool Temperature (1–2 °C) in 2001 and 2002

cv./stage	storage conditions	neochlorogenic acid		*p*-coumaroyl-quinic acid		chlorogenic acid		other hydroxy-cinnamic acids		total phenolic acids	
		2001	2002	2001	2002	2001	2002	2001	2002	2001	2002
Burlat											
partially ripe	day 0	18.7 (24.5)	26.2 (24.1)	29.0 (37.5)	25.9 (23.9)	3.27 (4.4)	4.20 (3.6)	nd[a]	0.65 (0.2)	51.0	57.0
partially ripe	1–2 °C; day 30	29.6 (30.9)	24.1 (21.9)	29.4 (30.3)	20.9 (19.7)	4.59 (4.8)	4.30 (3.5)	2.33 (1.2)	0.39 (0.1)	65.9	49.7
ripe	day 0	21.7 (18.6)	25.9 (13.6)	26.3 (19.9)	23.1 (12.4)	3.65 (3.1)	3.96 (1.8)	nd	0.33 (0.1)	51.6	53.2
ripe	15 ± 5 °C; day 6	18.1 (19.1)	25.5 (8.6)	25.4 (27.9)	23.2 (8.4)	2.34 (2.5)	3.83 (1.2)	1.04 (0.4)	0.32 (0.1)	46.9	52.8
ripe	1–2 °C; day 30	34.1 (21.6)	23.3 (11.3)	32.3 (20.4)	20.0 (10.0)	5.39 (3.2)	3.33 (1.4)	2.54 (0.9)	0.35 (0.1)	74.4	47.0
Saco											
partially ripe	day 0	126 (72.1)	121 (74.1)	12.6 (7.9)	11.5 (7.4)	7.99 (4.6)	6.49 (4.0)	nd	0.63 (0.2)	147	140
partially ripe	1–2 °C; day 30	*b*	122 (68.6)	*b*	13.5 (8.9)	*b*	6.06 (3.4)	*b*	4.23 (1.7)	*b*	145
ripe	day 0	190 (70.5)	117 (52.8)	15.2 (5.5)	9.16 (4.2)	12.0 (4.2)	7.56 (3.3)	5.27 (1.3)	3.48 (1.1)	222	137
ripe	15 ± 5 °C; day 6	186 (73.7)	132 (40.0)	16.9 (5.2)	12.5 (4.0)	11.1 (3.5)	7.28 (2.2)	7.35 (0.8)	5.54 (1.2)	221	158
ripe	1–2 °C; day 30	136 (46.5)	93.4 (47.7)	12.2 (4.4)	8.45 (4.7)	9.73 (3.5)	5.58 (2.9)	6.39 (1.5)	3.49 (0.8)	164	111
Summit											
partially ripe	day 0	33.3 (35.6)	35.0 (37.9)	27.5 (30.2)	26.0 (30.4)	8.58 (9.0)	5.84 (5.9)	2.91 (1.2)	1.61 (0.7)	72.3	68.4
partially ripe	1–2 °C; day 30	37.2 (37.2)	31.3 (32.2)	28.9 (30.7)	28.3 (31.9)	7.63 (7.5)	5.64 (5.0)	4.99 (2.3)	2.29 (0.9)	78.7	67.5
ripe	day 0	40.4 (31.6)	28.3 (25.8)	34.0 (27.3)	20.9 (20.4)	9.73 (9.9)	4.60 (4.1)	1.86 (0.5)	2.21 (0.8)	86.0	56.0
ripe	15 ± 5 °C; day 6	62.5 (42.8)	30.7 (19.2)	27.7 (18.2)	23.5 (16.8)	9.81 (6.3)	4.80 (2.8)	nd	2.19 (0.6)	100	61.2
ripe	1–2 °C; day 30	24.4 (24.9)	26.7 (25.1)	18.5 (19.4)	22.0 (21.6)	5.57 (5.6)	4.38 (3.7)	2.06 (0.8)	0.93 (0.3)	50.5	54.1
Van											
partially ripe	day 0	59.2 (70.0)	47.3 (56.1)	5.27 (6.8)	2.83 (4.3)	5.07 (5.9)	3.81 (4.2)	0.87 (0.4)	0.65 (0.3)	70.4	54.6
partially ripe	1–2 °C; day 30	96.5 (65.6)	76.3 (63.4)	8.29 (6.3)	6.15 (6.8)	7.96 (5.3)	5.35 (3.8)	2.82 (0.8)	1.82 (0.6)	116	89.6
ripe	day 0	84.9 (64.2)	46.2 (37.5)	7.25 (6.2)	3.97 (3.5)	6.32 (4.6)	3.19 (2.4)	2.02 (0.9)	0.75 (0.3)	101	54.2
ripe	15 ± 5 °C; day 6	98.4 (48.0)	72.5 (19.2)	9.89 (5.3)	5.33 (1.6)	7.08 (3.7)	4.67 (1.0)	1.98 (0.4)	1.21 (0.1)	117	83.8
ripe	1–2 °C; day 30	81.2 (53.7)	49.1 (25.5)	7.54 (5.9)	3.72 (2.2)	5.80 (3.7)	3.38 (1.7)	1.14 (0.3)	0.56 (0.1)	95.7	56.8

[a] nd: not detected. [b] Data not available. The relative amount of each compound in each type of cherry sample is shown in parentheses.

Table 4. Anthocyanin Levels (Milligrams per 100 g of Fresh Weight) in Cherry Cultivars at Two Ripeness Stages (Partially Ripe and Ripe), after Storage at Room (15 ± 5 °C) and Cool Temperature (1–2 °C) in 2001 and 2002

cv./stage	storage conditions	cyanidin 3-glucoside		cyanidin 3-rutinoside		peonidin 3-glucoside		pelargonidin 3-rutinoside		peonidin 3-rutinoside		total anthocyanins	
		2001	2002	2001	2002	2001	2002	2001	2002	2001	2002	2001	2002
Burlat													
partially ripe	day 0	1.23 (1.6)	4.27 (3.7)	4.21 (5.5)	18.3 (18.2)	nd[a]	0.02 (0.01)	nd	0.04 (0.01)	0.31 (0.27)	0.56 (0.54)	5.75	23.18
partially ripe	1–2 °C; day 30	2.56 (2.6)	5.06 (4.9)	13.0 (13.7)	29.6 (29.4)	nd	0.02 (0.01)	0.02 (0.01)	0.10 (0.1)	0.47 (0.32)	1.09 (1.13)	16.08	35.84
ripe	day 0	14.5 (12.6)	31.94 (19.0)	28.5 (24.6)	60.6 (36.5)	nd	0.26 (0.1)	nd	0.27 (0.1)	1.67 (1.56)	2.61 (1.67)	44.67	95.68
ripe	15 ± 5 °C; day 6	9.85 (10.3)	48.5 (18.6)	30.6 (31.0)	125 (50.4)	0.13 (0.1)	0.40 (0.1)	0.15 (0.1)	0.59 (0.2)	2.06 (2.16)	5.63 (2.37)	42.83	180.00
ripe	1–2 °C; day 30	11.2 (6.9)	27.2 (14.6)	43.0 (28.7)	86.1 (48.4)	0.15 (0.06)	0.26 (0.1)	0.17 (0.1)	0.48 (0.3)	1.92 (1.20)	4.33 (2.30)	56.41	118.40
Saco													
partially ripe	day 0	0.25 (0.1)	0.33 (0.3)	6.22 (3.2)	5.97 (3.6)	nd	nd	nd	0.02 (0.01)	nd	0.05 (0.01)	6.47	6.37
partially ripe	1–2 °C; day 30	*b*	1.36 (0.4)	*b*	16.7 (8.9)	*b*	nd	*b*	0.05 (0.01)	*b*	0.55 (0.21)	*b*	18.60
ripe	day 0	1.94 (0.6)	8.23 (3.8)	24.5 (8.5)	52.6 (23.9)	nd	nd	0.09 (0.01)	0.23 (0.1)	nd	0.80 (0.23)	26.51	61.86
ripe	15 ± 5 °C; day 6	3.14 (0.9)	14.4 (4.2)	81.7 (22.8)	122 (39.4)	nd	nd	0.57 (0.1)	0.69 (0.24)	0.84 (0.02)	4.16 (1.38)	86.25	141.65
ripe	1–2 °C; day 30	11.2 (3.8)	8.58 (4.4)	62.9 (22.1)	55.8 (29.1)	nd	nd	0.43 (0.1)	0.28 (0.10)	0.55 (0.14)	1.23 (0.68)	75.08	65.91
Summit													
partially ripe	day 0	0.18 (0.1)	0.53 (0.9)	5.72 (5.3)	8.09 (8.9)	nd	nd	nd	0.03 (0.01)	nd	0.04 (0.02)	5.90	8.67
partially ripe	1–2 °C; day 30	0.30 (0.2)	0.66 (1.0)	8.05 (7.8)	13.5 (14.8)	nd	nd	0.03 (0.01)	0.05 (0.02)	0.14 (0.05)	0.31 (0.22)	8.52	14.49
ripe	day 0	1.08 (0.6)	3.8 (3.8)	20.1 (14.5)	31.8 (30.3)	nd	nd	0.10 (0.03)	0.15 (0.10)	0.08 (0.02)	0.90 (0.55)	21.39	36.56
ripe	15 ± 5 °C; day 6	1.31 (0.6)	3.97 (3.1)	44.9 (17.8)	3.97 (44.2)	nd	nd	0.30 (0.1)	0.35 (0.17)	0.78 (0.29)	3.59 (2.67)	47.31	72.12
ripe	1–2 °C; day 30	1.03 (1.2)	2.38 (2.5)	31.5 (33.7)	2.38 (31.8)	nd	nd	0.16 (0.1)	0.16 (0.11)	0.72 (0.52)	0.96 (0.99)	33.41	35.06
Van													
partially ripe	day 0	0.09 (0.04)	0.75 (1.5)	4.86 (5.6)	13.0 (18.6)	nd	nd	nd	0.06 (0.03)	0.02 (0.01)	0.30 (0.26)	4.97	14.06
partially ripe	1–2 °C; day 30	0.65 (0.2)	0.50 (1.0)	18.6 (12.6)	12.0 (11.8)	nd	nd	nd	0.04 (0.01)	0.52 (0.26)	0.54 (0.32)	19.75	13.05
ripe	day 0	0.85 (0.4)	5.97 (6.0)	18.2 (13.4)	38.2 (38.4)	nd	0.04 (0.01)	0.10 (0.03)	0.19 (0.13)	0.18 (0.08)	2.80 (2.57)	19.37	47.14
ripe	15 ± 5 °C; day 6	2.16 (0.7)	33.4 (10.2)	60.6 (30.3)	180 (57.6)	nd	0.33 (0.1)	0.60 (0.2)	1.00 (0.38)	2.41 (0.85)	15.55 (5.29)	65.80	230.33
ripe	1–2 °C; day 30	1.46 (0.6)	13.5 (7.3)	36.0 (24.7)	88.1 (51.2)	nd	0.11 (0.04)	0.21 (0.12)	0.48 (0.20)	1.48 (0.70)	6.94 (4.02)	39.17	109.10

[a] nd: not detected. [b] Data not available. The relative amount of each compound in each type of cherry sample is shown in parentheses.

the lowest, 20 mg/100 g of fw in 2001 (**Table 4**). Higher values of anthocyanins were always determined in the ripe picking stage for all cultivars. In general, the total anthocyanins increased during storage at room and cool temperature for all of the cherry cultivars, but usually more at room temperature storage. For instance, a huge increase of 5-fold in total anthocyanins from 47 to 230 mg/100 g of fw was observed in cv. Van cherries, in 2002, after 6 days of storage at 15 ± 5 °C (**Table 4**; **Figure 3**), whereas in 2001, the increase was 3-fold (**Table 4**).

The major anthocyanin was cyanidin 3-rutinoside, which ranged from 18 to 61 mg/100 g of fw and represented 13 and 37% of the total phenolics in cherry extract of cvs. Van and Burlat, respectively. These were the two cultivars having the highest anthocyanin contents at harvest. During storage, these two cherry cultivars also became redder than other cultivars (as evaluated visually). The cv. Burlat also had higher levels of cyanidin 3-glucoside, 15 and 32 mg/100 g of fw (13 and 19% of the total phenolics), in 2001 and 2002, respectively. In the

Table 5. Flavonol and Flavan-3-ol Levels (Milligrams per 100 g of Fresh Weight) in Cherry Cultivars at Two Ripeness Stages (Partially Ripe and Ripe), after Storage at Room (15 ± 5 °C) and Cool Temperature (1–2 °C) in 2001 and 2002

cv./stage	storage conditions	rutin		catechin		epicatechin		total flavonol + flavan-3-ols	
		2001	2002	2001	2002	2001	2002	2001	2002
Burlat									
partially ripe	day 0	2.22 (2.8)	2.41 (2.7)	7.33 (9.2)	9.18 (8.9)	6.54 (8.5)	10.08 (9.4)	16.08	21.67
partially ripe	1–2 °C; day 30	2.01 (2.2)	3.22 (3.1)	5.97 (6.2)	6.15 (5.9)	7.55 (7.8)	8.29 (7.5)	15.53	17.66
ripe	day 0	3.06 (2.9)	6.46 (3.2)	5.73 (6.0)	8.68 (4.8)	4.51 (4.0)	8.98 (4.5)	13.30	24.12
ripe	15 ± 5 °C; day 6	2.13 (2.6)	8.40 (3.2)	2.41 (1.7)	8.02 (3.0)	3.18 (2.2)	8.09 (2.6)	7.72	24.51
ripe	1–2 °C; day 30	4.26 (2.8)	6.44 (3.2)	9.38 (5.8)	6.81 (3.5)	8.75 (5.2)	6.55 (3.0)	22.39	19.79
Saco									
partially ripe	day 0	6.67 (4.5)	2.78 (2.0)	9.01 (4.9)	4.79 (3.0)	5.92 (2.8)	10.23 (5.4)	21.59	17.79
partially ripe	1–2 °C; day 30	*a*	4.35 (2.5)	*a*	4.87 (2.9)	*a*	5.80 (2.6)	*a*	15.02
ripe	day 0	13.69 (4.7)	9.90 (4.3)	14.90 (5.0)	6.11 (2.7)	11.78 (4.0)	8.72 (3.7)	40.37	24.73
ripe	15 ± 5 °C; day 6	13.30 (4.2)	8.14 (2.8)	12.25 (3.8)	6.48 (2.1)	15.00 (3.9)	9.02 (2.5)	40.55	23.64
ripe	1–2 °C; day 30	13.50 (5.6)	7.22 (3.9)	9.80 (3.3)	5.25 (2.8)	27.04 (9.0)	5.89 (3.0)	50.34	18.36
Summit									
partially ripe	day 0	2.17 (2.1)	2.39 (2.9)	6.68 (7.2)	3.87 (4.4)	8.71 (9.2)	6.48 (6.5)	17.56	12.79
partially ripe	1–2 °C; day 30	4.84 (4.3)	1.86 (2.2)	5.82 (5.5)	3.99 (4.4)	5.40 (4.6)	4.06 (3.4)	16.06	9.91
ripe	day 0	2.81 (2.5)	3.39 (3.1)	7.32 (3.8)	4.36 (4.0)	11.28 (9.1)	5.18 (4.3)	21.41	12.93
ripe	15 ± 5 °C; day 6	3.39 (2.7)	3.04 (2.1)	8.13 (5.0)	4.47 (3.1)	9.89 (6.3)	5.26 (3.5)	21.41	12.77
ripe	1–2 °C; day 30	4.07 (3.8)	3.31 (3.3)	4.27 (4.5)	4.01 (4.0)	5.59 (5.6)	4.62 (4.0)	13.92	11.94
Van									
partially ripe	day 0	2.86 (3.6)	4.70 (9.3)	3.26 (4.0)	2.21 (3.5)	3.18 (3.7)	2.87 (2.1)	9.29	9.78
partially ripe	1–2 °C; day 30	3.54 (3.4)	3.89 (4.7)	4.85 (3.8)	4.13 (4.8)	4.03 (1.8)	5.02 (2.7)	12.42	13.04
ripe	day 0	4.06 (3.1)	3.92 (3.9)	4.61 (3.6)	2.42 (2.1)	4.95 (3.5)	4.09 (3.1)	13.62	10.43
ripe	15 ± 5 °C; day 6	8.28 (4.6)	9.26 (3.0)	6.56 (3.8)	3.33 (0.6)	4.97 (2.2)	6.36 (1.1)	19.80	18.96
ripe	1–2 °C; day 30	3.79 (3.1)	5.20 (3.2)	4.89 (4.1)	3.05 (2.0)	5.30 (3.1)	4.60 (2.6)	13.99	12.85

[a] Data not available. The relative amount of each compound in each type of cherry sample is shown in parentheses.

other cultivars the values were much lower, representing <6% in the total phenolics. The glucoside and rutinoside of peonidin and pelargonidin 3-rutinoside represented <5% of the total phenolics. Peonidin 3-glucoside was identified only in very low amounts in cvs. Burlat and Van (**Table 4**). Climatic conditions in 2002 clearly induced higher anthocyanin levels in both ripe and partially ripe fruits.

Flavan-3-ols and Flavonols Content. The flavan-3-ols, catechin and epicatechin, and the flavonol content (rutin) of the different cultivars are shown in **Table 5**. There were significant differences ($P < 0.001$) in the flavonol content among the cherry cultivars, but not among harvest years. Cv. Saco was the richest in this compound, containing 14 mg/100 g of fw, and cvs. Burlat and Summit had lower values, 3 mg/100 g of fw, that represented 5 and 3% of the total phenolics contents, respectively (**Table 5**). The levels of flavonol (rutin) showed a slight increase from partially ripe to ripe stage, and a higher increase was observed in cv. Saco, which varied from 3 to 10 mg/100 g of fw (3-fold), in 2002. The flavonol content of cherries remained quite constant during the storage period of ripe and partially ripe cherries at both temperatures.

The levels of flavan-3-ols, catechin, and epicatechin, differed among the cherry cultivars and the year ($P < 0.001$). At harvest, mean contents ranged from 7 mg/100 g of fw (in 2002) in cv. Van to 27 mg/100 g of fw (in 2001) in cv. Saco. In general, the flavan-3-ols content was higher in 2001, except for cv. Burlat. Epicatechin was found to be the dominant flavan-3-ol in sweet cherry cultivars. Slight variations of total flavonol and flavan-3-ols were observed during storage period (**Table 5**).

DISCUSSION

Previous studies on cherry phenolics showed that neochlorogenic acid and *p*-coumaroylquinic acid were the main hydroxycinnamic derivatives (*10*, *19*). The results of this study confirm that the two acids are the main hydroxycinamates in both partially ripe and ripe cherries, with smaller amounts of chlorogenic acid. In ripe fruits the levels of neochlorogenic acid were higher (22−190 mg/100 g of fw) and the levels of *p*-coumaroylquinic acid were lower (4−34 mg/100 g of fw) than the values obtained by Gao and Mazza (*10*), who found neochlorogenic acid in a range between 24 and 128 mg/100 g in pitted cherry and *p*-coumaroylquinic acid from 23 to 131 mg/100 g. These differences could be due to the studied cultivars and to the influence of extraction and analytical procedures.

Because these three hydroxycinnamates have been described to be involved in the copigmentation with anthocyanins (*20*, *21*), they are likely to have a major importance in the color of cherries.

Initial studies have found cyanidin 3-rutinoside and cyanidin 3-glucoside as the main anthocyanin pigments in sweet cherries (*22*−*25*). In addition, other studies (*24*, *26*) reported the presence of peonidin and two of its glycosidic derivatives in cv. Bing cherries. However, Fouassin (*27*), Harborne and Hall (*28*), and Olden and Nybom (*29*) found only cyanidin derivatives and no peonidin glycosides in cultivars of *Prunus avium*. Tanchev et al. (*30*) and Tanchev (*31*) claimed that cyanidin 3-sophoroside was present in cvs. Lambert, Helmsdorf, Somaya, Kozerskia, and Bing cherries, but this particular anthocyanin was not identified by Gao and Mazza (*10*) in any of the cherry cultivars they studied, and it was not detected in our study either. Another minor anthocyanin, peonidin 3-rutinoside, detected for the first time in sweet cherries by Gao and Mazza (*10*), was also found in the four cherry cultivars studied here. Hence, our results are generally consistent with the results reported in the literature, which identify the 3-rutinoside and 3-glucoside of cyanidin as the major anthocyanins and the same glycosides of peonidin and pelargonidin 3-rutinoside as the minor anthocyanins. In other stone fruits, such as peaches and nectarines, that belong to the same botanical family as sweet cherries, cyanidin 3-glucoside is the major anthocyanin, followed by cyanidin 3-rutinoside (*32*).

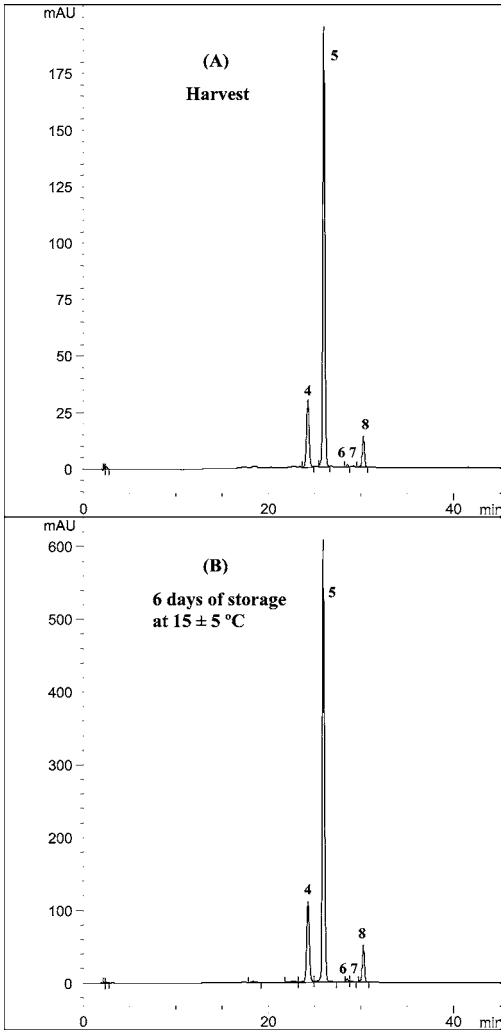

Figure 3. HPLC separation of anthocyanins in a methanolic extract of cv. Van cherries, in 2002, monitored at 520 nm. Peaks with the retention times (RT) for the two chromatograms **A** and **B**, are, respectively: (1) cyanidin 3-glucoside (RT = 24.26 and 24.28 min); (2) cyanidin 3-rutinoside (RT = 25.98 and 25.91 min); (3) pelargonidin 3-rutinoside (RT = 28.52 and 28.51 min); (4) peonidin 3-glucoside (RT = 28.93 and 28.92 min); (5) peonidin 3-rutinoside (RT = 30.25 and 30.25 min). Different scales were used to stress differences within each chromatogram.

In general, storage at 1−2 or 15 ± 5 °C resulted in an overall increase in anthocyanins in both ripe and in partially ripe cherries, which is in agreement with other findings, for other types of ripe fruits maintained at cool temperature, with high anthocyanin content, such as strawberries (*33*, *34*), blueberries (*35*), grapes (*36*), and pomegranates (*37*). We are currently examining the relationship between quality indices, notably color of cherries and pH, and anthocyanin contents.

The data in **Tables 3** and **4** suggest that the quantities of phenolic acids and anthocyanins were influenced by the climatic conditions of the two years of study. In 2001, in the last third of the fruit growth stage higher temperature (average 19 °C) and higher solar irradiation (231 h) were observed, when compared to 2002 (15 °C and 171 h, respectively), which favored the biosynthesis of phenolic acids, which tend to reach highest levels in the late stage of final maturity as referred to by Stöhr et al. (*38*) and Melin et al. (*39*) (cf. ref *40*). However, data from several more harvest years or different weather conditions are required before any firm conclusions can be drawn on these issues.

With regard to flavan-3-ols, epicatechin was found to be the major compound with smaller amounts of catechin, which is in complete agreement with other findings in cherries (*9*).

The occurrence of quercetin glucosides has been reported in sour cherries, but not in sweet cherries (*41*). However, rutin was detected in this study. In fact, in some of the samples, several other quercetin glucoside derivatives were also found (data not shown). Because quercetin glucosides—especially in onions—are known to possess significant antioxidant potency, the presence of quercetin glucoside derivatives and other phenolics, such as catechin, epicatechin, and anthocyanins in sweet cherries, may contribute to make sweet cherries a beneficial source of health protective antioxidants. Limited research on the health effects of cherries has been conducted. However, on the basis of the present data, which confirm that sweet cherry is rich in phenolics, it is likely that cherries will provide the types of health benefits associated with fruits and vegetables in general.

This study showed that the cherry cultivars have the same phenolic pattern, however, with large variations on content. Levels of phenolics are always higher in ripe than in partially ripe cherries. Both cold and room temperature storage increase phenolics levels; however, levels for cold storage cherries will never be as high as for room temperature storage cherries. The levels of the individual phenolic substances in cherries generally vary during storage, but the variation in phenolic profiles is less during a month at cool storage (where the levels may go up or down) than at storage for a few days at room temperature (15 ± 5 °C), where notably the anthocyanins and phenolic acid contents may increase. Finally, our evaluation of cherries harvested in two different years indicates that the influence of weather conditions during cherry growth may have a profound influence on the phenolics levels.

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