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Maturity Stage at Harvest Determines the Fruit Quality and Antioxidant Potential after Storage of Sweet Cherry Cultivars

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Eleven sweet cherry cultivars were harvested at three maturity stages (S1 to S3) based on skin color and stored at 2 °C for 16 days and a further period of 2 days at 20 °C (shelf life, SL) to analyze quality (color, total soluble solids, and total acidity) and bioactive compounds (total phenolics and anthocyanins) and their relationship to total antioxidant activity (TAA), determined in hydrophilic (H-TAA) or lipophilic (L-TAA) fraction. For all cultivars and maturity stages, the ripening process advanced during postharvest storage with increases in color intensity and decreases in acidity, as well as enhancements in phenolics, anthocyanins, and TAA in both H-TAA and L-TAA, although important differences existed among cultivars. The results showed that sweet cherry should be harvested at stage S3 (4 days later than the commercial harvest date) since after 16 days of cold storage + SL, the highest antioxidant capacity was achieved for both H-TAA and L-TAA.

KEYWORDS: Phenolics; anthocyanins; hydrophilic and lipophilic total antioxidant activity; bioactive compounds; postharvest

INTRODUCTION

Sweet cherry is an important fruit with high commercial importance in Spain, although given its perishable nature, the application of cold storage is a necessary postharvest tool to maintain fruit quality till consumption. Among the factors determining the consumer's acceptability, total soluble solids (TSS), acidity, and color are the most important (*1, 2*). For this reason, producers use a number of parameters to establish the optimum time for harvesting, the most reliable being skin color (*3*). Red color development in sweet cherry is used as an indicator of quality and ripening, and is due to the accumulation and profile of anthocyanins (*4*−*6*).

Nowadays, increased intake of fruit and vegetables has been associated with reduced incidence of degenerative diseases due to their antioxidant potential (*7*−*9*). Among these compounds, special interest has been focused on anthocyanins and polyphenolics due to their antioxidant properties (*10*). In cherries, the two dominant polyphenols are caffeoyltartaric acid and 3-*p*-coumaroylquinic acid (*11*). However, sweet cherries are characterized by having anthocyanins as major phenolics, the aglicone cyanidin bound to the saccahride moieties 3-rutinoside and 3-glucoside being the main compounds, and pelargonidin-3-rutinoside, peonidin-3-rutinoside, and peonidin-3-glucoside being the minor phenolics (*4, 12*).

Organoleptic, nutritive, and bioactive compounds of sweet cherry at the time of harvest differ among cultivars (*13*−*16*), but apart from an early paper by Gonçalves et al. (*17*) with four sweet cherry cultivars, there is no additional information about the changes occurring in the above bioactive compounds during the postharvest life of sweet cherries. Therefore, the aim of this article was to determine the changes in quality and bioactive compounds during storage of a wide range of sweet cherry cultivars. In addition, the behavior of phenolic and anthocyanin compounds and their relationship to total antioxidant activity (TAA), analyzed for the first time in two separate fractions (hydrophilic and lipophilic, H-TAA and L-TAA), during storage as affected by maturity stage at harvest will be discussed.

MATERIAL AND METHODS

Plant Material and Experimental Design. The experiment was carried out on a commercial plot (Finca Los Frutales, Villena Alicante, Spain) using 10 years-old sweet cherry trees of 11 cultivars (Brooks, Cristalina, Newstar, No 57, NY-6479, Prime Giant, Santina, Somerset, Sonata, Sunburst, and Sweetheart) on Santa Lucía rootstock. All cultivars were in the same plot, and the trees for each cultivar were distributed in paired rows of about 50 trees in each. Fruits were harvested totally at random from multiple trees at 3 maturity stages (S1, S2, and S3) based on fruit color. For all

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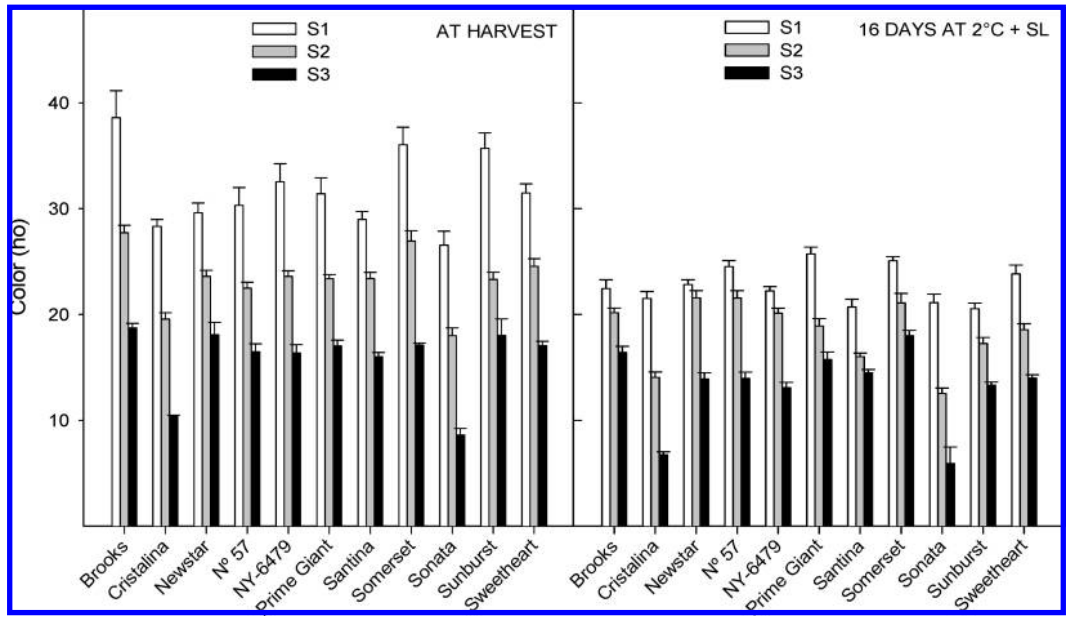

Figure 1. Color hue angle (ho) at harvest and after 16 days at 2 °C + 2 days at 20 °C (SL) of 11 sweet cherry cultivars harvested at three maturity stages (S1, S2, and S3). Data are the mean ± SE.

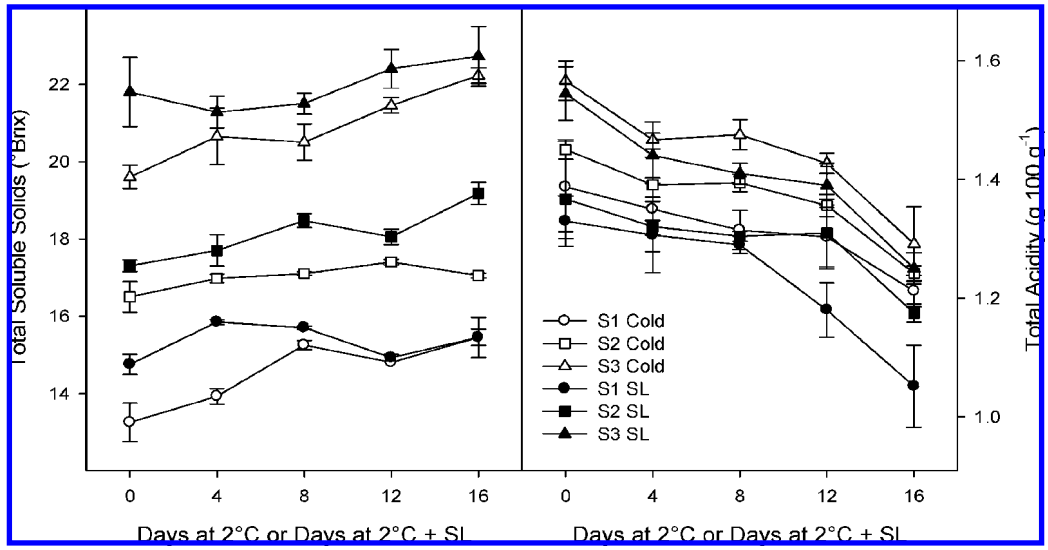

Figure 2. Evolution of total soluble solids and total acidity during storage at 2 °C or at 2 °C + 2 days at 20 °C (SL) of NY-6479 sweet cherry cultivar harvested at three maturity stages (S1, S2, and S3). Data are the mean ± SE.

cultivars, S2 corresponded with commercial harvesting with the following scores according to the color chart from Centre Technique Interprofessionel de Fruits et Légumes (CTIFL, Paris): 3 for Brooks and Somerset, 5 for Cristalina and Sonata, and 4 for the remaining cultivars. S1 and S3 were fruits harvested 4 days earlier or later than the commercial harvest date, respectively. For each cultivar and maturity stage, about 500 cherries were picked and immediately transferred to the laboratory. Then, 300 homogeneous fruits in color, size, and without visual defects were selected for each cultivar and maturity stage, and randomly grouped in 30 lots of 10 fruits for cold storage at 2 °C with a RH of 85% in darkness during 16 days. After, 0, 4, 8, 12, and 16 days, 6 lots were sampled at random from cold chambers, from which 3 were analyzed immediately and 3 after a shelf life period of 2 days at 20 °C (SL) to simulate commercial practices. In these fruits, color was individually measured, and then the edible portion of each lot was cut in small pieces to determine in duplicate total soluble solids (TSS), total acidity, total anthocyanins, total phenolics, and antioxidant activity.

Ripening Parameters. Color was determined in a Minolta colorimeter (CRC200, Minolta Camera Co., Japan) and expressed as hue angle (ho). TSS was determined from the juice obtained for each subsample with a digital refractometer Atago PR-101 (Atago Co. Ltd., Tokyo, Japan) at 20 °C and expressed as % (°Brix). Total acidity (TA) was determined by automatic titration (785 DMP Titrino, Metrohm) with 0.1 N NaOH up to pH 8.1, using 1 mL of diluted juice in 25 mL of distilled H_2O, and results expressed as g malic acid equivalent per 100 g^{-1} fresh weight.

Total Antioxidant Activity, Total Phenolic, and Total Anthocyanin Determination. Total antioxidant activity (TAA) was quantified according to Arnao et al. (*18*), which enables one to determine TAA due to both hydrophilic and lipophilic compounds in the same extraction. Briefly, for each subsample, 5 g of tissue was homogenized in 5 mL of 50 mM Na-phosphate buffer at pH 7.8 and 3 mL of ethyl acetate, then centrifuged at 10,000*g* for 15 min at 4 °C. The upper fraction was used for total antioxidant activity due to lipophilic compounds (L-TAA) and the lower for total antioxidant activity due to hydrophilic compounds (H-TAA). In both cases, TAA was determined using the enzymatic system composed of the chromophore 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), the horse radish peroxidase enzyme (HRP), and its oxidant substrate (hydrogen peroxide, H_2O_2), in which $ABTS^{\bullet+}$ radicals are generated and monitored at 730 nm. The reaction mixture contained 2

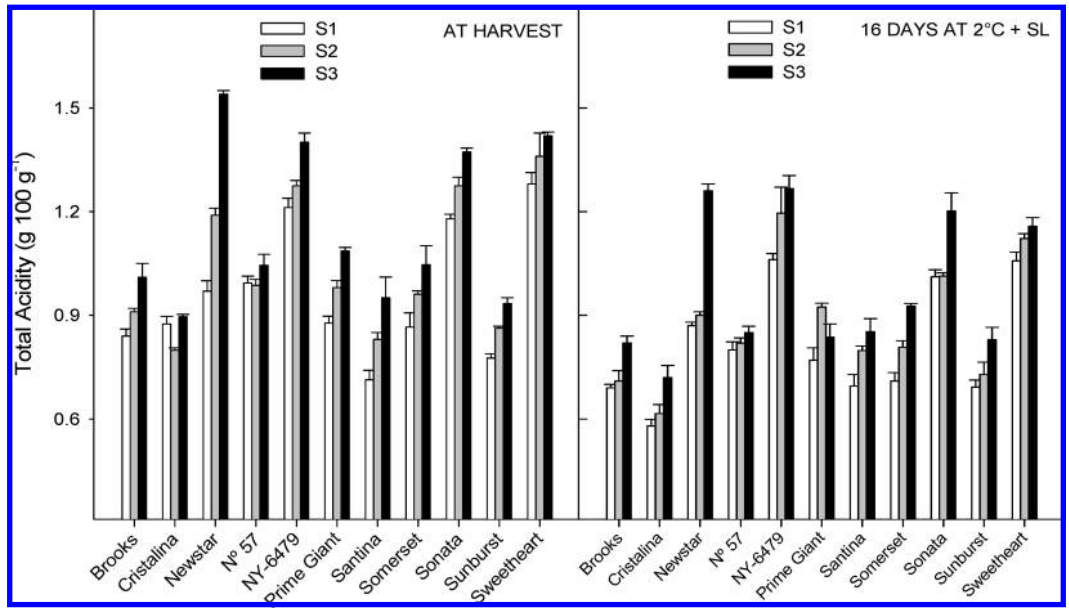

Figure 3. Total acidity at harvest and after 16 days at 2 °C (cold) + 2 days at 20 °C (SL) of 11 sweet cherry cultivars harvested at three maturity stages (S1, S2, and S3). Data are the mean ± SE.

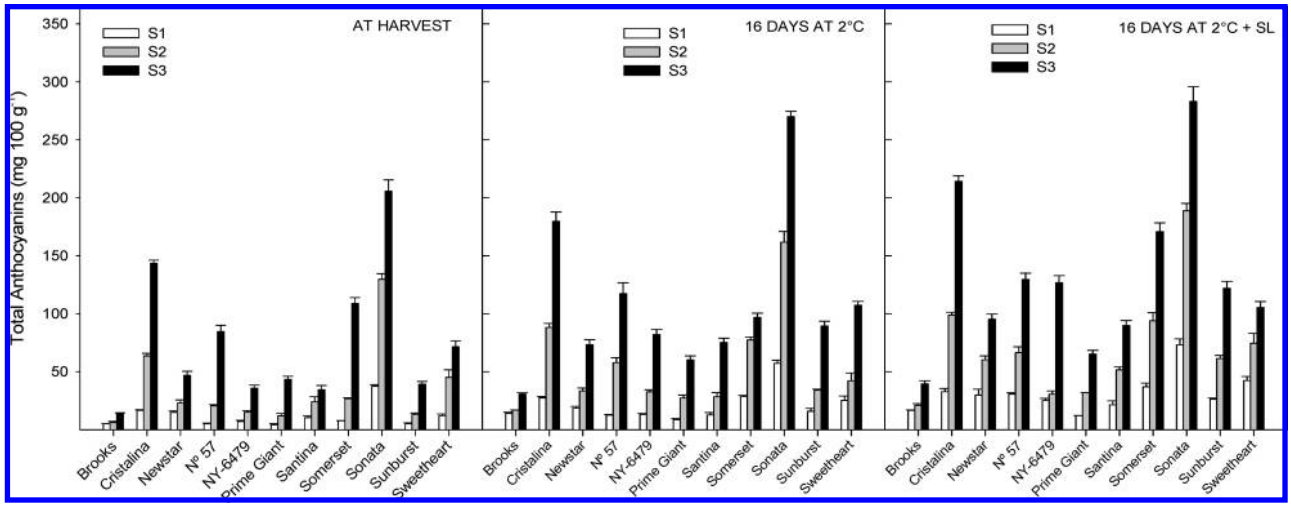

Figure 4. Total anthocyanins at harvest, after 16 days of storage at 2 °C and after 16 days at 2 °C + 2 days at 20 °C (SL) of 11 sweet cherry cultivars harvested at three maturity stages (S1, S2, and S3). Data are the mean ± SE.

mM ABTS, 15 μM H_2O_2, and 25 μM HRP in 50 mM Na-phosphate buffer (pH 7.8) in a total volume of 1 mL. The decrease in absorbance after adding the extract was proportional to TAA of the sample. A calibration curve was performed with Trolox ((*R*)-(+)-6-hydroxy-2,5,7,8-tetramethyl-croman-2-carboxylic acid) (0−20 nmol) from Sigma (Madrid, Spain), and the results are expressed as mg of Trolox equivalent 100 g^{-1}.

Total phenolics were extracted according to the Tomás-Barberán et al. protocol (*19*) using water/methanol (2:8) containing 2 mM NaF (to inactivate polyphenol oxidase activity and prevent phenolic degradation) and quantified using the Folin−Ciocalteu reagent (*20*), and the results (mean ± SE) were expressed as mg gallic acid equivalent 100 g^{-1} fresh weight.

Total anthocyanins were determined according to García-Viguera et al. (*21*) adapted as previously reported (*6*) and calculated as cyanidin 3-glucoside equivalent (molar absorption coefficient of 23900 L cm^{-1} mol^{-1} and molecular weight of 449.2 g mol^{-1}) and the results expressed as mg 100 g^{-1} fresh weight. The results were the mean ± SE.

HPLC-DAD Anthocyanin and Phenolic Compounds Analysis. Anthocyanin and phenolics were assayed by high performance liquid chromatography coupled with a diode array detector (HPLC-DAD) as previously described (*19*). One milliliter from the extracts obtained for total anthocyanin and phenolic quantification was filtered through a 0.45 μm Millipore filter and then injected into a Hewlett-Packard HPLC series 1100 equipped with a C18 Supelco column (Supelcogel C-610H, 30 cm × 7.8 mm, Supelco Park, Bellefonte, USA) and detected by absorbance at 510 or 340 nm. The peaks were eluted by a gradient using the following mobile phases: 95% water + 5% methanol (A); 88% water + 12% MeOH (B); 20% water + 80% MeOH (C); and MeOH (D) at a rate of 1 mL min^{-1}. Peaks were identified using authentic standards by comparing the retention times and peak spectral analysis. The anthocyanin standards (cyanidin 3-glucoside, cyanidin 3-rutinoside and pelargonidin 3-rutinoside) were provided by Dr. García-Viguera, while the hydroxycinnamic acids were purchased from Sigma (Sigma, Madrid, Spain).

Statistical Analysis. Experimental data were subjected to ANOVA. Sources of variation were cultivar and storage. The overall least significant differences (Fisher's LSD procedure, $P < 0.05$) were calculated and used to detect significant differences among cultivars and storage time. All analyses were performed with SPSS software package v. 11.0 for Windows (SPSS, 2001) (*22*). Correlations were performed between total anthocyanins and ho and between H-TAA and total phenolics taking into account all sampling data.

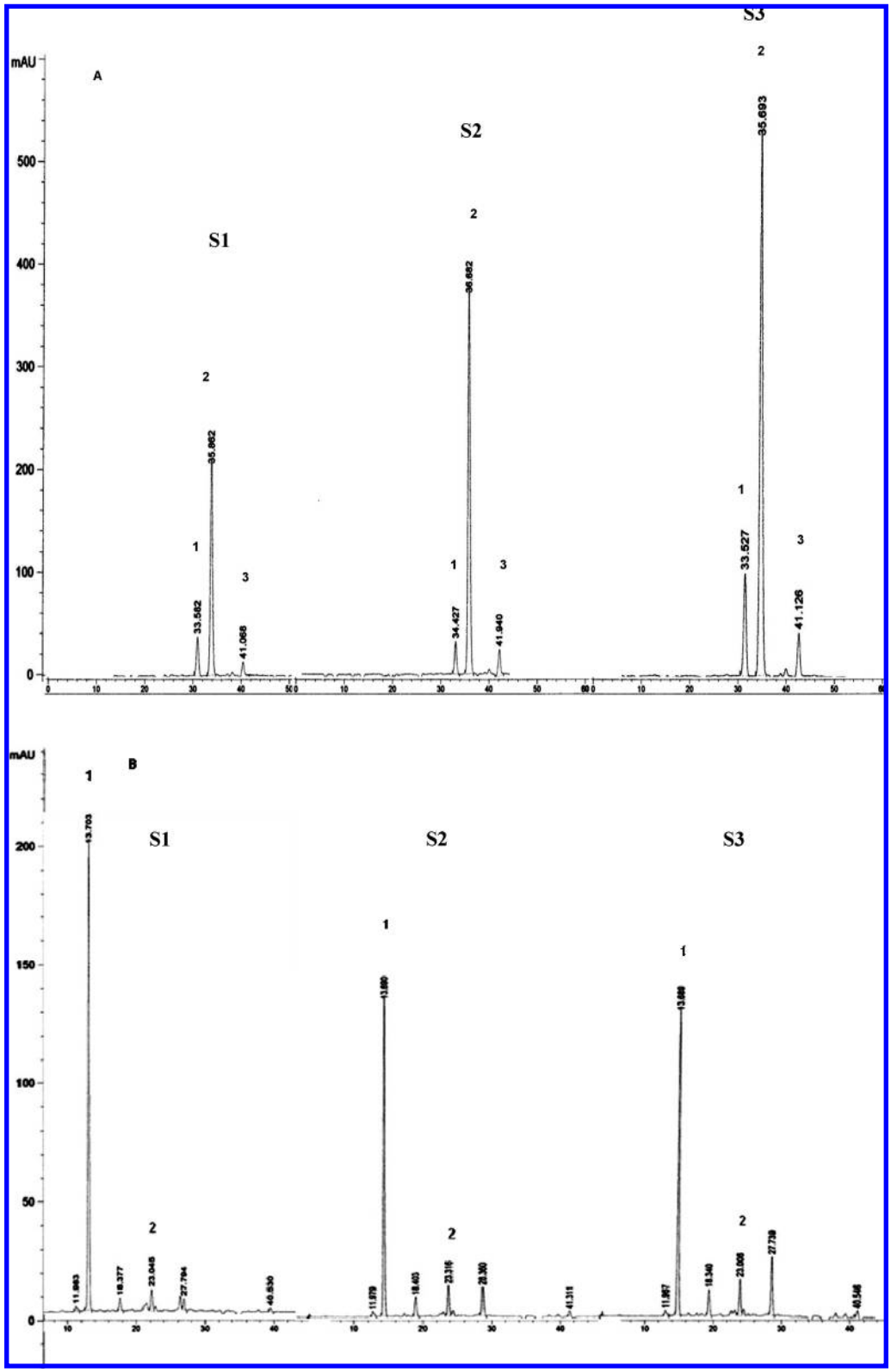

Figure 5. HPLC-DAD chromatograms for anthocyanins (**A**) and hydroxycinnamic acids (**B**) in Sonata sweet cherry harvested at 3 maturity stages (S1, S2, and S3). For anthocyanin peaks: (1) cyanidin 3-glucoside, (2) cyanidin 3-rutinoside, and (3) pelargonidin 3-rutinoside. For hidroxycinnamic acids: (1) neochlorogenic acid and (2) 3′*p*-coumaroylquinic acid.

RESULTS AND DISCUSSION

Sweet Cherry Ripening Parameters. It is widely accepted that the most important parameters determining sweet cherry acceptability by consumers are bright red color and flavor, which is mainly due to the ratio between TSS and TA (*1*, *2*), although important differences exist among cultivars and maturity stages. In fact, during maturity on the tree (from S1 to S3), reductions in color ho were observed for all cultivars, which reflect the changes from bright red to dark-red color (**Figure 1**). However, at commercial harvest (S2) Brooks and Somerset showed the highest ho (≈27), which corresponded with a bright red color, while the lowest ho values were obtained for Cristalina and

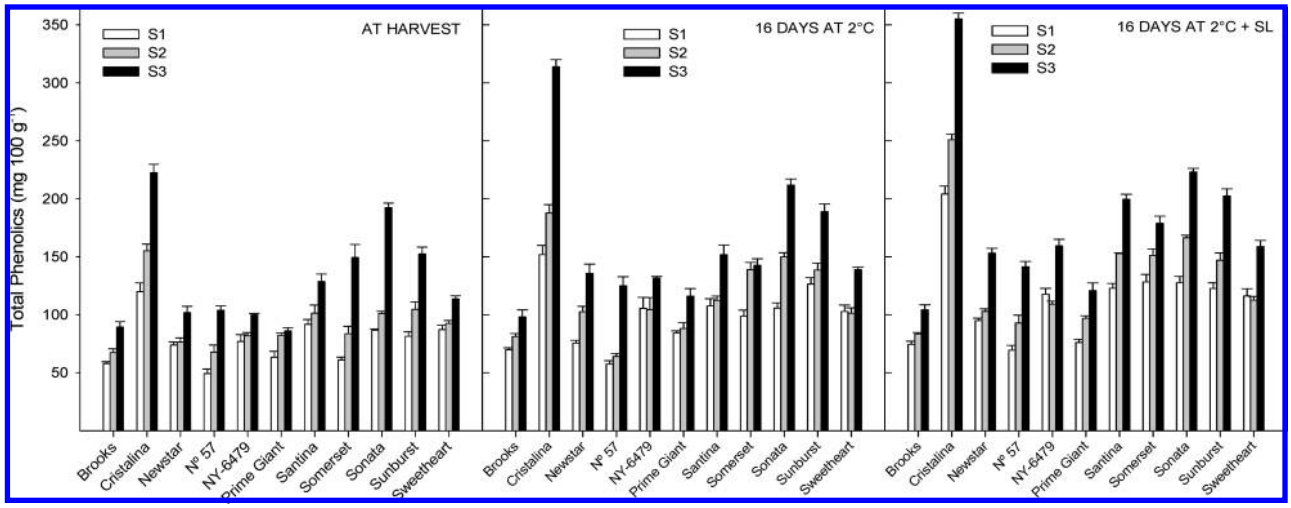

Figure 6. Total phenolics at harvest, after 16 days of storage at 2 °C and after 16 days at 2 °C + 2 days at 20 °C (SL) of 11 sweet cherry cultivars harvested at three maturity stages (S1, S2, and S3). Data are the mean ± SE.

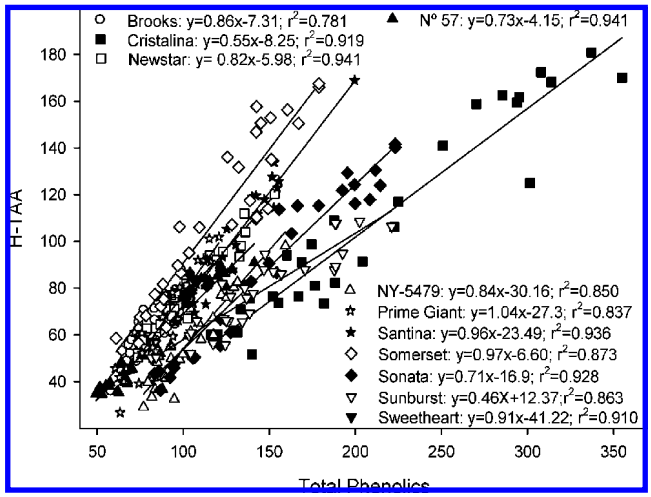

Figure 7. Correlation between hydrophilic total antioxidant activity (H-TAA) and total phenolics in 11 sweet cherry cultivars, taking into account data for all maturity stages and sampling data during storage at 2 °C and subsequent shelf life.

Sonata (≈14), which had a dark-red color. During storage, color evolved in all cultivars leading to significant ($P < 0.05$) decreases in ho after 16 days at 2 °C + SL (end of the experiment), especially for those fruits picked at stage S1. It is interesting to point out that those fruits harvested at S1 stage reached an ho color value at the end of the experiment close to those shown for S2 at harvest. On the contrary, the fruits picked at the S2 stage did not get the dark-red color of S3 at harvest. From these results, it can be inferred that during postharvest storage the color changes occurred at much slow rates than during ripening on the tree (*23*). Moreover, when fruits were harvested at S3 stage (ho below 19), only slight changes in color were observed, as has been shown in Burlat cherries (*24*).

With respect to TSS and TA, significant ($P < 0.05$) increases occurred among the 3 maturity stages (from S1 to S3) as shown in **Figure 2** for NY-6479 as an example. However, during storage the evolution of these parameters was quite different since significant decreases occurred in TA during cold storage and subsequent SL, at the 3 maturity stages, while SST generally increased when fruits were transferred at 20 °C after cold storage. Among cultivars and maturity stages, differences existed in relationship to TSS and TA at harvest, with values of 13−21 °Brix (data not shown) and 0.7−1.5 g 100 g^{-1}, respectively, with Newstar, NY-6479, Sonata and Sweetheart being those with high acidity levels. During storage, the most significant differences were observed in TA for which a reduction was observed with a 15−17% on average (**Figure 3**). Accordingly, in other cherry cultivars a general decrease in TA during postharvest storage was reported (*24*−*27*), while TSS slightly diminished in Ambrunes (*27*), Sciazza, and Ferrovia (*25*), remained unchanged in Van (*26*), and increased in Burlat, the increase being attributed to dehydration (*24*).

Antioxidant Compounds and Total Antioxidant Activity. At harvest, the concentration of anthocyanins was clearly affected by cultivar and maturity stage (**Figure 4**), with the highest levels being found for Sonata and Cristalina picked at S3 and the lowest for Brooks. For all cultivars and maturity stages, significant increases ($P < 0.05$) in anthocyanin content were found during cold storage and subsequent SL, as can be seen in **Figure 4** after 16 days at 2 °C and after subsequent SL. The concentration of anthocyanins was negatively correlated (exponential decay) with color ho ($y = 605 \times e^{-0.13x}$, $R^2 = 0.879$) taking into account data for all cultivars, maturity stages, and sampling data during storage. These results were in agreement with those previously reported (*28*, *29*), in which total anthocyanins increased during storage and were correlated negatively with color parameters (L*, a*, b*, Chroma, and ho) in other cherry cultivars. The accumulation of anthocyanins during storage is attributed to normal sweet cherry ripening, as has been found in other commodities such as berries and plum (*30*−*32*). The HPLC-DAD chromatograms revealed that in all cultivars the main anthocyanins were cyanidin 3-rutinoside, followed by cyanidin 3-glucoside and pelargonidin 3-rutinoside, which increased with ripening from S1 to S3, as can be seen in **Figure 5A** for Sonata cultivar as an example. The anthocyanin profile in our cultivars agreed with those found in Burlat, Saco, Summit, Van, and Lambert Compact cultivars (*28*, *29*).

With respect to total phenolics, an increase in total phenolics as maturity advanced was observed (from S1 to S3) for all cultivars, although significant differences ($P < 0.05$) existed among them (**Figure 6**). The levels of total phenolics at S2 stage (70 to 150 mg 100 g^{-1}) were within the same concentration range to those found in other cherry cultivars at commercial harvesting (*15*, *33*). During cold storage and subsequent SL, a general increase (over 40−60% on average) in phenolics was observed for all cultivars and maturity stages, in accordance with results from Burlat and Saco, although for Summit and

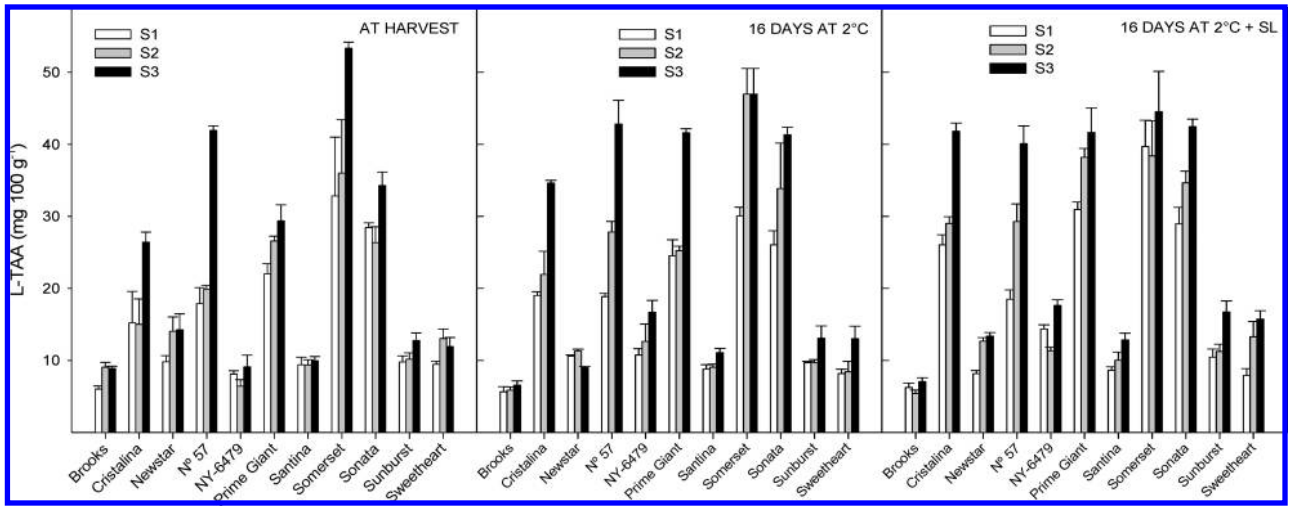

Figure 8. Lipophilic total antioxidant activity (L-TAA) at harvest, after 16 days of storage at 2 °C and after 16 days at 2 °C + 2 days at 20 °C of 11 sweet cherry cultivars harvested at three maturity stages (S1, S2, and S3). Data are the mean ± SE.

Van, decreases were observed (*17*). In addition, the increase in total anthocyanins was positively correlated with the enhancement in total phenolics, which would indicate that anthocyanins are the main phenolic compounds in sweet cherry according to previous reports (*4, 12*), followed by the hydroxycinnamic acid derivatives neochlorogenic acid and 3′-*p*-coumaroylquinic acid (*12, 33*). In fact, we have found in these sweet cherry cultivars that nechlorogenic acid was the predominant hydroxycinnamic acid followed by 3-*p*-coumaroylquinic acid, as shown in **Figure 5B** for Sonata, in agreement with previous reports (*28, 29*). Since phenolic compounds contribute to fruit quality in terms of modifying color, taste, aroma, and flavor (*34*), those cultivars with higher phenolics content will have higher quality.

The total antioxidant activity was determined due to both hydrophilic and lipophilic compounds. In most papers, only antioxidant activity due to hydrophilic compounds has been addressed for sweet cherry (*6, 16, 17*), while this is the first time that antioxidant activity in both hydrophilic and lipophilic extracts has been measured during sweet cherry postharvest storage, although evidence exists in our previous work during sweet cherry on-tree ripening (*23*). The only paper in which L-TAA and H-TAA have been quantified separately is that of Wu et al. (*35*), in a wide range of fruits and vegetables at commercial harvest (including 4 cherry cultivars, although no names or maturity stages were reported). In this study, H-TAA increased in a way similar to that of total phenolics during cold storage and subsequent SL, regardless of cultivar (data not shown). In fact, a positive relationship was found between H-TAA and total phenolics for all cultivars taking into account data from all sampling dates and maturity stages with correlation coefficients ranging in between 0.78−0.94 (**Figure 7**). Although not determined in this study, it is well known that ascorbic acid can also contribute to H-TAA (*6*). Recently, it has been reported that the ingestion of certain foods with high amounts of phenolics has antioxidant activity in vivo by increasing the plasmatic antioxidants (*36*). In this sense, sweet cherry extracts showed dose-dependent antioxidant effect in the low-density lipoprotein assay (*17, 37*). The L-TAA was significantly lower than H-TAA since L-TAA accounted for 20−30% of the total antioxidant capacity. As above, significant differences ($P < 0.05$) in the L-TAA were observed among cultivars (**Figure 8**), although only for some cultivars a significant increase was observed from S1 to S3 maturity stages and during storage and subsequent SL (Cristalina, N° 57, Prime Giant, and Sonata). No literature is available to contrast the L-TAA results in sweet cherry, but the presence of tocopherol in a group of fruits, including sweet cherry, was correlated to the lipophilic activity (Cho et al. (*38*)), although the correlation coefficient was weak ($r^2 = 0.584$). In this sense, more studies are needed to get a better knowledge about L-TAA and the lipophilic constituents with antioxidant activity in sweet cherry.

In conclusion, during postharvest storage of sweet cherry, the ripening process advanced at a much lower rate than ripening on the tree (*23*), which is manifested by increases in both color and TSS and decreases in TA. Although there are great differences in the content of bioactive compounds (total phenolics and anthocyanins) at harvest among cultivars and maturity stages, their behavior during postharvest was essentially the same, which was a general increase after cold storage and subsequent SL. Moreover, these hydrophilic compounds were positively correlated with the H-TAA, although the presence of lipophilic compounds contributing to L-TAA should not be disregarded since this fraction accounted for 20−30% of the total antioxidant capacity. Given the relationship between the intake of fruits and the reduction of human diseases due to the occurrence of antioxidant phytochemicals (*8−10*), the sweet cherry should be harvested at stage S3 (4 days later than commercial harvest date) since after 16 days of cold storage + SL, the highest antioxidant capacity was achieved for both H-TAA and L-TAA.

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