

Chemical Constituents and Antioxidant Activity of Sweet Cherry at Different Ripening Stages

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The development and ripening process of sweet cherry (*Prunus avium* L. cv. 4-70) on the tree was evaluated. For this purpose, 14 different stages were selected in accordance with homogeneous size and color. Some parameters related to fruit quality, such as color, texture, sugars, organic acids, total antioxidant activity, total phenolic compounds, anthocyanins, and ascorbic acid were analyzed. The results revealed that in sweet cherry, the changes in skin color, glucose and fructose accumulation, and softening process are initiated at early developmental stages, coinciding with the fast increase in fruit size. Also, the decrease in color parameter a^* was correlated with the greatest accumulation of total anthocyanins. Ascorbic acid, total antioxidant activity (TAA), and total phenolic compounds decreased during the early stages of sweet cherry development but exponentially increased from stage 8, which coincided with the anthocyanin accumulation and fruit darkening. TAA showed positive correlations ($r^2 = 0.99$) with both ascorbic acid and total phenolic compounds and also with the anthocyanin concentration from stage 8. Taking into account the reduced shelf life of sweet cherry and to ensure that these fruits reach consumers with the maximum organoleptic, nutritional, and functional properties, it is advisable to harvest sweet cherries at stage 12 of ripening.

KEYWORDS: Development; ripening; firmness; color; anthocyanins; total antioxidant activity; total phenolics; sugars; organic acids

INTRODUCTION

Sweet cherries are highly appreciated by the consumer due to their precocity and excellent quality. The main characteristics related to cherry fruit quality are color, sweetness, sourness, and firmness. In sweet cherry, as other red fruits, the ripening process is related to the change of the initial green color to red, in which accumulation of anthocyanins and degradation of chlorophyll occurred. These changes can be followed by changes in the evolution of the L^* , a^* , and b^* parameters (1). In fact, red color development in sweet cherry is used as an indicator of ripening and depends on the content and anthocyanin profile (2). Sweetness in the cherry fruit is mainly due to glucose and fructose, while sourness is primarily due to the presence of malic acid (3, 4). Fruit firmness is also very appreciated by consumers, together with a green color and freshness of the stems. However, the overall acceptance by consumers seems to be dependent on the ratio between sugar and acid concentrations (5).

There is numerous evidence supporting the hypothesis that fruits and vegetables contain several compounds that reduce the risk of several degenerative diseases, such as cancer and

cardiovascular illness. Among these compounds, special interest has been shown for anthocyanins and polyphenolics due to their antioxidant properties (6). A reduction of arthritis and gout pain has been associated to the specific consumption of cherries (7). In addition, polyphenolic compounds contribute to the sensory and organoleptic qualities of fruits, such as color, taste, and astringency (8). In the cherry, the two dominant polyphenols are caffeoyltartaric acid and 3-*p*-coumaroylquinic acid (9). However, sweet cherries are characterized to have anthocyanins as major phenolics, the aglicon cyanidin bound to the glycosides 3-rutinoside and 3-glucoside being the main compounds, and pelargonidin-3-rutinoside, peonidin-3-rutinoside, and peonidin-3-glucoside as minor contributors (1, 10).

There is no available information concerning the changes in the content of these health-promoting compounds during development and as ripening processes advance. It is necessary to ensure that cherry fruits are harvested with the quality that meets the consumer's demand, providing a good source of functional compounds. Thereafter, producers and industrials need valuable information in terms of harvesting dates for handling and commercialization purposes since harvesting is usually performed on the basis of color and size only. The aim of this work was to establish the optimum date of harvest to ensure that cherries reach consumers with the maximum functional and nutritional properties. Thus, sweet cherries were

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harvested at 14 developmental stages, in which the following parameters were analyzed: color, texture, sugars, organic acids, total antioxidant activity, total phenolic compounds, total anthocyanins, and ascorbic acid content.

MATERIALS AND METHODS

Cherry Samples. Samples of sweet cherry of cultivar 4-70 (*Prunus avium* L.) were obtained from an experimental farm in Murcia (Spain). This cultivar resulted from a private breeding program (Marvin Nies) at California and is also known as the cultivar Marvin–Niram. Since the development process is heterogeneous on the tree, cherries were harvested on May 10, 2004 and grouped in 14 different stages in accordance with homogeneous size and color. Color, weight, and texture were determined individually in 20 fruits. Then, five subsamples of four fruits were obtained for each developmental stage and ground in liquid N₂, in which total antioxidant activity, total phenolic compounds, anthocyanins, sugars, and organic acids were analyzed.

Color. External color (L*, a*, and b*) was measured on 20 cherries from each group with a Minolta chromameter (Model CR200; Minolta Camera Co., Osaka, Japan). Duplicate measurements were made at opposite sides of the fruit.

Fruit Weight. The average weight was recorded on 20 cherries from each group using a precision scale at two significant figures.

Texture. For each cherry fruit, texture was determined using a TX-XT2i Texture Analyzer (Stable Microsystems, Godalming, UK) interfaced to a personal computer. Three different texture parameters were evaluated: fruit firmness, flesh firmness, and traction force to stem removal. Fruit firmness was measured using a flat steel plate mounted on the machine. The diameter was measured, and then a force that achieved a 2% deformation of the fruit diameter was applied. Results were expressed as the ratio between the force that achieved the 2% fruit deformation and the fruit diameter (N mm⁻¹) multiplied by 100. A beveled holder prevented bruising of the opposite side. For each fruit, 1 cm² of the skin was removed, and flesh firmness was individually recorded using a 2 mm diameter probe. Penetration rate of the probe was 20 mm min⁻¹ for 10 mm after contacting the flesh, and results were expressed in N. For stem traction force, a tensile grip was used to remove the stem, and the results were expressed in N.

Antioxidant Activity and Total Phenolic Compounds. Five grams of sweet cherry tissue with pits removed was homogenized in 10 mL of 50 mM phosphate buffer, pH 7.8 and then centrifuged at 15 000 rpm for 15 min at 4 °C. The supernatant was used for total antioxidant activity (TAA) and total phenolic compound quantification. TAA was determined according to Cano et al. (11), using the enzymatic system composed of the chromophore 2,2'-azino-bis-(3-ethylthiazolyl)-6-sulfonic acid diammonium salt (ABTS), the horseradish peroxidase enzyme (HRP), and its oxidant substrate (hydrogen peroxide), in which ABTS^{•+} radicals are generated. The reaction mixture contained 1.5 mM ABTS, 15 μM hydrogen peroxide, and 0.25 μM HRP in 50 mM glycine-HCl buffer (pH 4.5) in a total volume of 2 mL. The assay temperature was 25 °C, and the reaction was monitored at 414 nm until a stable absorbance was obtained using a UNICAM Helios α spectrophotometer (Cambridge, UK). After that, a suitable amount of sweet cherry extract was added, and the observed decrease in absorbance was determined. A calibration curve was performed with L-ascorbic acid (0–20 nmol) from Sigma (Poole, Dorset, England), and results are expressed as mg of L-ascorbic acid equivalent to 100 g⁻¹. The generation of ABTS^{•+} radicals before the addition of the sweet cherry extract prevented interferences of compounds. Total phenolic compounds were quantified according to the method described by Singleton et al. (12) using the Folin–Ciocalteu reagent, and results were expressed as mg of gallic acid equivalent to 100 g⁻¹.

Organic Acids and Sugar Content. For organic acid and sugar determinations, the same extract as stated previously was used. One milliliter of the extract was filtered through a 0.45 μm Millipore filter and then injected into a Hewlett-Packard HPLC series 1100. The elution system consisted of 0.1% phosphoric acid running isocratically with a flow rate of 0.5 mL min⁻¹. The organic acids were eluted through a Supelco column (Supelcogel C-610H, 30 cm × 7.8 mm, Supelco Park, Bellefonte, PA) and detected by absorbance at 210 nm. A standard

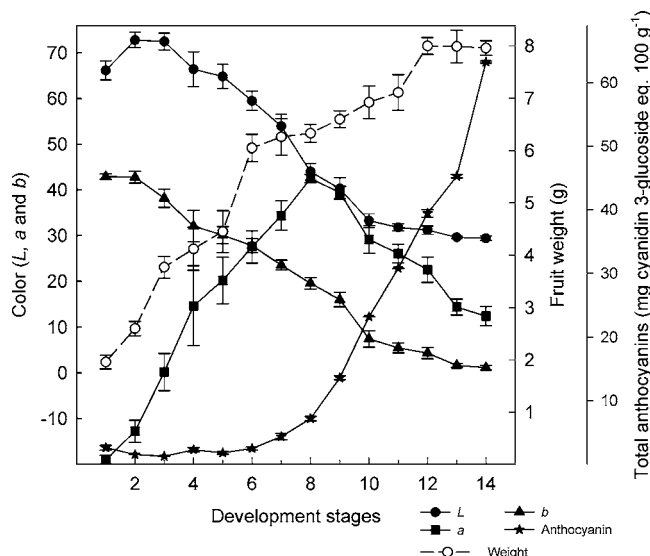


Figure 1. Evolution of fruit weight, skin color (L*, a*, and b*), and total anthocyanins over the developmental stages. Data are the mean ± SE.

curve of pure organic acids (L-ascorbic, malic, citric, and succinic acids) purchased from Sigma (Poole, Dorset, UK) was used for quantification. Results were expressed as mg of ascorbic acid 100 g⁻¹ and g 100⁻¹ (%) for the remained acids. For sugar concentrations, the same HPLC, elution system, flow rate, and column were used. The detection of sugars was obtained by a refractive index detector. A standard curve of pure sugars (glucose, fructose, sucrose and sorbitol) purchased from Sigma was used for quantification. Results were expressed as g 100⁻¹ (%).

Anthocyanin Determination. The method described by Lee et al. (13) was adapted to cherry tissue. Two grams of fruit tissue was homogenized in 4 mL of methanol and left for 1 h at -18 °C. Extracts were centrifuged at 15 000 rpm for 15 min at 4 °C. The supernatant was loaded onto a C18 Sep-Pak cartridge, previously conditioned with 5 mL of methanol, 5 mL of pure water, and then with 5 mL of 0.01 N HCl. The cartridge was washed with 5 mL of pure water and then eluted with acidified MeOH (0.01% HCl). Absorbance of the collected fraction was measured at 530 nm. Total anthocyanin contents (14) were calculated using cyanidin-3-glucoside (molar absorption coefficient of 23 900 l cm⁻¹ mol⁻¹ and molecular weight of 449.2 g mol⁻¹), and results were expressed as mg 100 g⁻¹.

Statistical Analysis. The overall least significant differences (Fisher's LSD procedure, $p < 0.05$) were calculated and used to detect significant differences. All analyses were performed with SPSS software package v. 11.0 for Windows (15). Linear regressions were performed between measurement variables.

RESULTS AND DISCUSSION

Fruit Weight and Color Evolution. The cherry fruit weight increased along the selected developmental stages (Figure 1); the maximum size was reached at stage 12. With respect to external color parameters, the luminosity (L*) and b* (indicator of yellow–blue changes) showed the highest values at early stages of development (stages 1–3) and a linear decrease until stage 10 of development, from which the values remained significantly unchanged until the end of the ripening. Contrarily, parameter a* increased sharply from stage 1 to 8, at which the maximum a* value was detected, and then a continuous diminution was observed (Figure 1). These results indicate that in the cherry fruit, skin color changes are initiated at early development phases coinciding with the fast increase in fruit size. When total anthocyanins were determined, an exponential increase was observed (correlation coefficient $r^2 = 0.99$), with very low concentrations between stages 1 and 6 (≈ 2 mg of cyanidin-3-glucoside equivalent to 100 g⁻¹), and a sharp increase

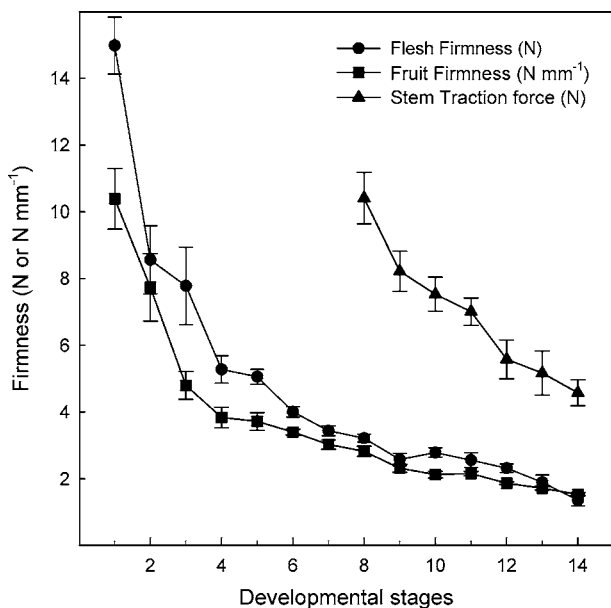


Figure 2. Evolution of texture: fruit and flesh firmness and stem traction force over the developmental stages. Data are the mean \pm SE.

from stage 8 until 14, for which the highest anthocyanin concentration was reached (63.26 ± 0.16 mg cyanidin-3-glucoside equivalent to 100 g^{-1}). It is interesting to point out that the beginning of the anthocyanin accumulation was correlated ($r^2 = 0.985$) to the decrease in color parameter a^* from stage 8. The 4-70 cultivar could be considered as medium-colored cherry fruit since cherries with anthocyanin concentrations below 40 and over $80 \text{ mg } 100 \text{ g}^{-1}$ are considered to be light- and dark-colored, respectively (1). It has been observed that the predominant anthocyanins in cherry are cyanidin-3-rutinoside and cyanidin-3-glucoside, the peonidin-(3-glucoside and 3-rutinoside) and pelargonidin-3-rutinoside being considered as minor (2, 16).

Texture. Whole fruit and flesh firmness in the cherry showed a sharp decrease in the first stages of development and was less pronounced from stage 4 to 14 (Figure 2). Both texture parameters showed a negative exponential decay correlation ($r^2 = 0.986$ and 0.978 for fruit and flesh firmness, respectively) with the increase in fruit size. The traction force to remove the stems could be only determined from stage 8 of development since at earlier stages the required force was too high, and stem removal could not be achieved. Nevertheless, the same behavior for firmness was observed for this parameter, that is, a continuous decrease in the traction force during development (Figure 2). Unlike most of fruits, cherry softening does not seem to be dependent on pectin depolymerization since negligible activity of polygalacturonase was reported during fruit ripening (17) but rather on increases in β -galactosidase activity (18).

Sugars and Organic Acid Contents. The main sugars found in cherry over development were glucose and fructose, for which a linear accumulation was observed over developmental stages ($r^2 = 0.998$ for both sugars) with final concentrations of 6.57 ± 0.01 and $8.43 \pm 0.13\%$, for glucose and fructose, respectively. Contrarily, sucrose and sorbitol concentrations were very low (between 0.1 and 0.20%) and did not experience significant changes over development (Figure 3). The final sugar concentrations found for cultivar 4-70 were in agreement with those reported for other sweet cherry cultivars harvested at the commercial ripening stage (4, 19). With respect to organic acid contents (Figure 3), the predominant one was malic acid, which was found to increase over the developmental stages (from 0.59

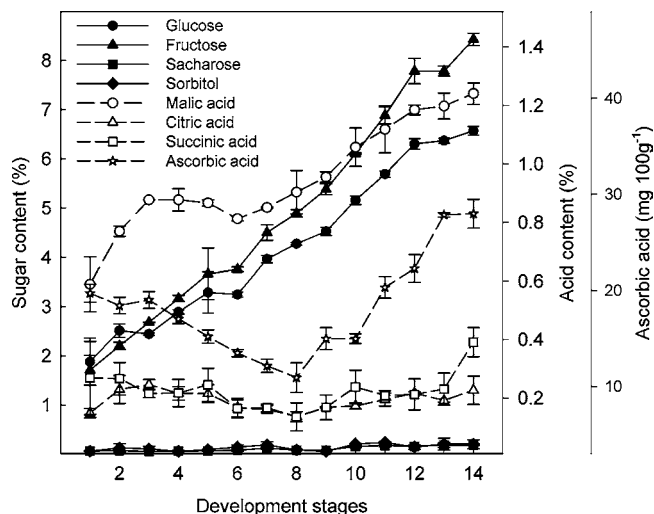


Figure 3. Evolution of sugars (glucose, fructose, sucrose, and sorbitol) and organic acids (malic, citric, succinic, and ascorbic acids) over the developmental stages. Data are the mean \pm SE.

± 0.09 to $1.24 \pm 0.04\%$). For citric and succinic acids, no significant changes were observed during fruit development. Malic acid has been found to be the major organic acid contributing to the acidity in the species of *Prunus*, such as plum, peach, apricot, and nectarine (20). In these fruits, the acidity decreases over the development and ripening, while an accumulation of malic acid was observed in the sweet cherry. This was in agreement with the increase in total acidity found as the harvesting date was delayed in "Lapins" cherries (21).

It is interesting to point out that in the sweet cherry color changes, increase in sugar content and decrease in texture parameter started at the early phases of cherry development, at which the fruits had only reached 20% of their final weight. However, in other fruits, such as plums, peaches, dates, and loquats, these changes related to ripening occurred when the fruits had almost finished their growth (22–26).

Ascorbic Acid, TAA, and Total Phenolic Compounds. Ascorbic acid levels halved during the early phases of development (from 1 to 8) and then progressively increased until the end of the ripening process, reaching final concentrations of $27.96 \pm 1.50 \text{ mg } 100 \text{ g}^{-1}$ (Figure 3), which were similar to those found in other cherry cultivars at harvest (27, 28). A similar behavior was found for both total antioxidant activity (TAA) and the total phenolic compound, for which decreases at early stages of development followed by increases from stage 7 were observed (Figure 4). TAA reached the maximum activity at stage 14 ($50.03 \pm 1.32 \text{ mg}$ of ascorbic acid equivalent to 100 g^{-1}), which coincided with the maximum concentration of total polyphenolic compounds ($99.88 \pm 3.40 \text{ mg}$ of gallic acid equivalent to 100 g^{-1}). The levels of total phenolics were found to be at the same concentration in other cherry cultivars, the neochlorogenic acid and 3'-*p*-coumaroylquinic acid being the main components (29). When linear regressions were performed, highly positive correlations ($r^2 = 0.99$) were found between TAA and both ascorbic acid and total phenolic compounds (Figure 5). Thereafter, from stage 6 to 14 and coinciding with the greatest accumulation of anthocyanins, a highly positive correlation ($r^2 = 0.99$) was also found between TAA and anthocyanin concentration. This indicates that when the sweet cherry is developing intensity in its red color, the anthocyanins could also account for the TAA in the sweet cherry. The correlation between TAA and phenolic compounds has been found in several fruits (30–32). Specifically, in analyzing

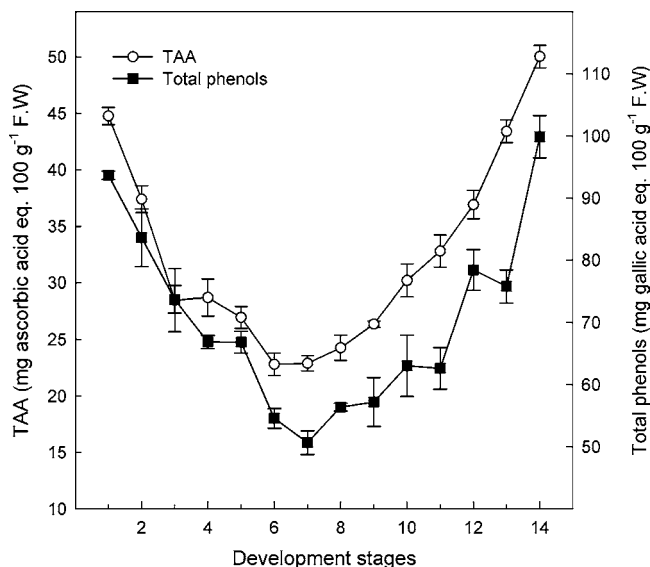


Figure 4. Total antioxidant activity (TAA) and total phenolic compound evolution over the developmental stages of sweet cherry. Data are the mean \pm SE.

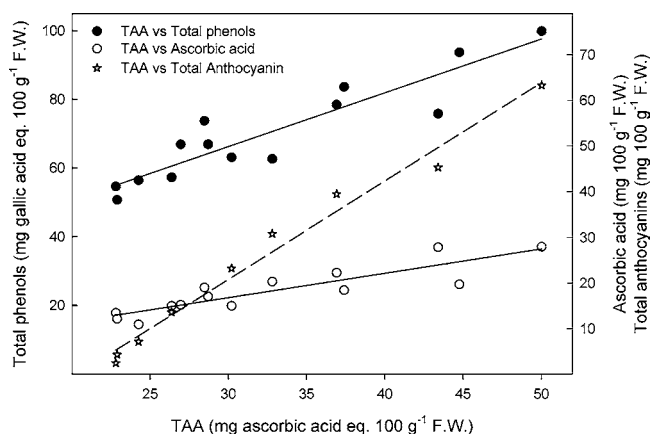


Figure 5. Correlations between total antioxidant activity (TAA) and total phenols or ascorbic acid concentration or total anthocyanins taking into account all data from the 14 developmental stages.

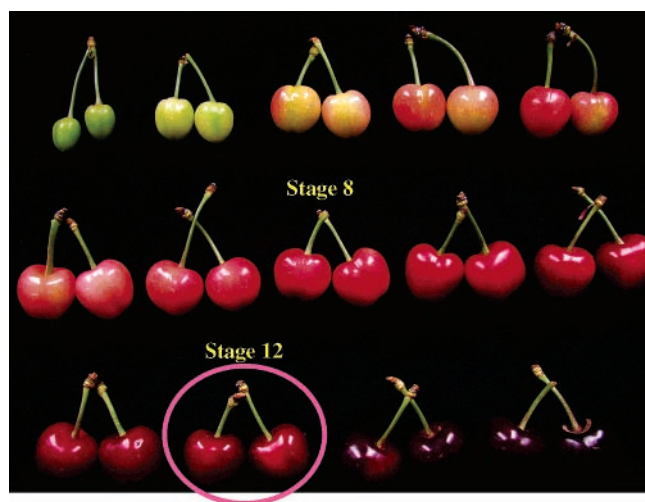


Figure 6. Photography displaying the fruits at the 14 stages of development (from top to bottom and from left to right, stages 1–14).

different fruit portions of four sweet cherry cultivars, a good correlation has been found between total phenol and TAA,

although a poor correlation was obtained with anthocyanins (10). It is well-known that phenolic compounds contribute to fruit quality and nutritional value in terms of modifying color, taste, aroma, and flavor and also in providing health-beneficial effects (33).

Conclusion. It is well-known that the sweet cherry is a highly perishable fruit, and in some cases, does not reach the consumer at optimal quality after transport and marketing (34–36). Then, from the point of view of the reduced shelf life addressed for the sweet cherry, and to ensure that these fruits reach consumers with the maximum nutritional and functional properties, it is advisable to harvest the sweet cherry at stage 12 of ripening (Figure 6). At this stage, the fruit has reached its maximum size and in a short period of time would develop the maxima organoleptic, nutritional, and functional quality attributes.

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