PhD Thesis

Bioactive Compounds, Antioxidant Activity and Quality of Plum and Sweet Cherry Cultivars as Affected by Ripening On-Tree, Cold Storage and Postharvest Treatments

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Que la Tesis Doctoral titulada "**Bioactive Compounds, Antioxidant Activity and Quality of Plum and Sweet Cherry Cultivars as Affected by Ripening On-Tree, Cold Storage and Postharvest Treatments**", ha sido realizada por Da. Huertas María Díaz Mula, bajo su dirección y supervisión, y autorizan que sea presentada para optar a la obtención del Título de Doctor Europeo por la Universidad Miguel Hernández.

En Orihuela a 12 de Mayo de dos mil once.

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Abstract

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ABSTRACT

Plum and sweet cherry are one of the most important stone fruits grown commercially in Spain and are of the most important commodities consumed worldwide due to their excellent quality attributes and high degree of acceptation by consumers. However, these fruits are highly perishable and the application of cold storage and other postharvest technologies are therefore necessary to maintain fruit quality from harvest to consumption.

In this Thesis a comparative study on the evolution of physical, chemical and nutritive parameters and bioactive compounds during development and on-tree ripening and postharvest cold storage was performed in a wide range of plum ad sweet cultivars. Eight plum cultivars: 4 purple ('Angeleno', 'Blackamber', 'Black Diamond' and 'Larry Ann') and 4 yellow ('Golden Japan', 'Golden Globe', 'Songold' and 'TC Sun') skin coloured, and 11 sweet cherry cultivars: 'Brooks', 'Cristalina', Newstar', 'Nº 57', 'NY-6479', 'Prime Giant', 'Santina', Somerset', 'Sonata', 'Sunburst' and 'Sweetheart' were chosen.

During on-tree ripening fruit weight evolution demonstrated the double-sigmoid growth pattern for all plum and sweet cherry cultivars and the main changes related to ripening, such as colour, total soluble solids (TSS), total acidity (TA), firmness started at early stages of fruit development, with significant differences among cultivars. The decrease in colour Hue angle was highly correlated with the increase in carotenoids in yellow plums and with total anthocyanins in purple plums and sweet cherries, showing that carotenoids are the main chemical compounds responsible for colour changes from green to orange/yellow occurring during ripening of the yellow plum cultivars, while anthocyanins were the main pigments of sweet cherry and purple plum cultivars. This correlation was found either during on-tree ripening or during cold storage.

In sweet cherry the major anthocyanin was cyanidin-3-rutinoside followed by cyanidin-3-glucoside and pelargonidin 3-rutinoside, while the hydroxycinnamic acids derivatives neochlorogenic acid and 3'-*p*-coumaroylquinic acid were the main phenolic compounds. The cold storage of plums and sweet cherries induced a delay in fruit metabolism although the ripening process went on measured by the decrease in acidity, the increase in TSS and softening as well as the ethylene production in those plum cultivars that showed a climacteric-ripening pattern ('Blackamber', 'Larry Ann', 'Golden Globe' and 'Songold').

In addition, the cold storage experiment in sweet cherry was performed with fruits harvested at 3 ripening stages (S1, S2, and S3), showing that at the end of storage time (16 days of cold storage + 2 days at 20 ºC) sweet cherries harvested at S1 reached the

ripening stage of S2 at harvest, but the fruits picked at S2 did not get the ripening stage of S3 at harvest.

Total antioxidant activity (TAA) due to hydrophilic (H-TAA) and lipophilic (L-TAA) compounds increased during on-tree ripening and during cold storage in all the plum and sweet cherry cultivars assayed, and were correlated to total phenolics and total carotenoids, respectively, demonstrating that phenolics are the main hydrophilic compounds contributing to H-TAA while the lipophilic nature of carotenoids contributed to L-TAA. It was found that at the usual commercial harvest dates for these cultivars do not assure the highest content of bioactive compounds and related antioxidant activity, since a delay of 7 or 4 days led to increases in these phytochemicals and TAA, for plums and cherries, respectively.

The effect of modified atmosphere packaging (MAP) on maintaining plum quality was assayed on 4 plum cultivars (2 with yellow skin, 'Golden Globe' and 'Songold', and 2 with purple skin, 'Blackamber' and 'Larry Ann') thermo-sealed in baskets with 2 distinct films with medium (film M) and high (film H) gas permeability. Fruit stored with macro-perforated film served as a control and lost their quality attributes very rapidly, manifested by accelerated colour changes, softening, decrease in acidity and increase in TSS. The use of MAP retarded these changes, the efficacy being higher in the fruit packed with film M compared with film H as a result of the delay in postharvest ripening, which could be attributed to the effect of MAP on reducing ethylene production rates. With the use of these packages, the storage time with fruit having high quality attributes could be increased 3–4 weeks more as compared with control plums.

The changes in bioactive compounds (total phenolics and total carotenoids, and individual anthocyanins) as well as H-TAA and L-TAA in the peel and the flesh of these plum cultivars during storage under MAP conditions were studied. Results revealed that in all cultivars, total phenolics and H-TAA increased in the peel and flesh during storage, as well as the two identified anthocyanins: cyanidin-3-glucoside and cyanidin-3-rutinoside in the purple cultivars. These changes were significantly delayed in fruit stored under MAP conditions. Total carotenoids and L-TAA increased in the yellow cultivars (in both peel and flesh) while decreases were observed in the purple cultivars, these changes were also delayed by the use of MAP. Positive correlations were found between H-TAA and total phenolics and between L-TAA and total carotenoids.

Results suggest that MAP does not impart any negative effects on TAA or phytochemicals and just reflects the delay of the ripening process occurring in the plums stored under MAP conditions, which led to an increase of the storage time with fruit having high quality attributes of 3-4 weeks more as compared with control plums.

Postharvest treatment with salicylic acid (SA), acetylsalicylic acid (ASA) or oxalic acid (OA) at 1 mM were performed on 'Cristalina' and 'Prime Giant' sweet cherry cultivars harvested at commercial ripening stage and then fruits were stored for 20 days under cold temperature. These treatments delayed the postharvest ripening process, manifested by lower losses in acidity and firmness, lower color changes, and higher quality attributes maintenance in treated cherries as compared with controls.

In addition, total phenolics, anthocyanins and antioxidant activity increased in untreated fruit during the first 10 days of storage and then decreased, while in fruits of all treatments, these parameters increased continuously during storage without significant differences among treatments. Thus, postharvest treatments with natural compounds, such as SA, ASA or OA, could be innovative tools to extend the storability of sweet cherry with higher content of bioactive compounds and antioxidant activity as compared with control fruits.

Finally, another postharvest treatment was an edible coating based on sodium alginate applied at several concentrations (1, 3 or 5% w/v) in 'Sweetheart' cherry cultivar harvested at commercial maturity stage. A significant delay in the evolution of the parameters related to postharvest ripening, such as colour, softening and loss of acidity, and reducing respiration rate, was obtained in alginate-coated fruits. In addition, the edible coatings showed a positive effect on maintaining higher concentration of total phenolics and TAA, which decreased in control fruits associated with the over-ripening and senescence processes. Overall, the results obtained from quality parameters and antioxidant activity suggested that the maximum storability period for alginate-coated cherries with optimal quality and enhanced antioxidant activity could be extended up to 16 days at 2°C plus 2 days at 20°C, while this period for control fruits was only 8 days at 2°C plus 2 days at 20°C.

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Introduction

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1. FRUIT RIPENING

Fruit is considered as a commercially important and nutritionally essential food commodity due to the provision of nutrients such as sugars, organic acids, vitamins and minerals, as well as other non-nutrient constituents including dietary fiber and secondary metabolites with health-beneficial effects. From the botanical point of view, fruits are highly diverse ranging from dry seed capsules (both dehiscent and non-dehiscent) to relatively large complex fleshy fruits which have developed bright colour and complex aromas to attract animals for seed dispersion. In most cases, fruits are formed from a fertilized ovary although other parts of the flower or inflorescence may also contribute to fruit formation, such as receptacle tissues and sepals for strawberry and pineapple, respectively. However, in a few species fruits are set and mature without fertilization and without seed development. Such fruits are called parthenocarpic fruits and are known in some figs, pears, apples, peaches, cherries, table grapes, bananas and citrus cultivars

1.1. Fruit Growth

The time required for fruit growth varies widely among species and genotypes, ranging from anthesis to ripening from 3 weeks in strawberry to 60 weeks in 'Valencia' orange, although, in fruits of many species this interval is about 15 weeks. However, it should be taking into account that fruit growth rate varies greatly among seasons, environmental conditions, cultural practices an even among different fruit in the same crop.

Fruit growth involves various degrees of cell division and cell expansion. During fruit set, when a flower has been successfully pollinated (and exceptionality in parthenocarpic), fruit becomes an active carbohydrate sink, and many of its tissues become meristematic. In some fruits, such as currants and blackberries, cell division is completed by the time of pollination, although in most of them cell division occurs for a short time after pollination and in still others, as avocado, cell division continues throughout the life of the fruit. Thus, in most species, increase in cell size makes the greatest contribution to total fruit expansion. For example, in grape, the increase in cell number accounts for a doubling of fruit size, whereas the increase in cell volume accounts for a 300-fold size increase (Monselise, 1986).

Fruit growth on plant can be followed by physical measurements such as weight, length, width and volume. The evolution of these parameters shows a simple or double-sigmoid curve depending of fruit type. In general, the double sigmoid type is characteristic of stone fruits, as cherry, peach, apricot, plum and olive, as well as of some non-stone fruits as grape and currant. In this double sigmoid growth curve, four distinct stages (S1-S4) could be established, as can be seen in **Figure 1** for 'Larry Ann' plum and

Figure 2 for 'Sweetheart' sweet cherry as examples. S1 is the first exponential growth phase and characterized by cell division and elongation. S2 shows little or null fruit growth but the endocarp hardens to form a solid stone. S3 is the second exponential growth phase due to cell enlargement, while in S4 the fruit growth rate decreases and fruit ripening occurs.

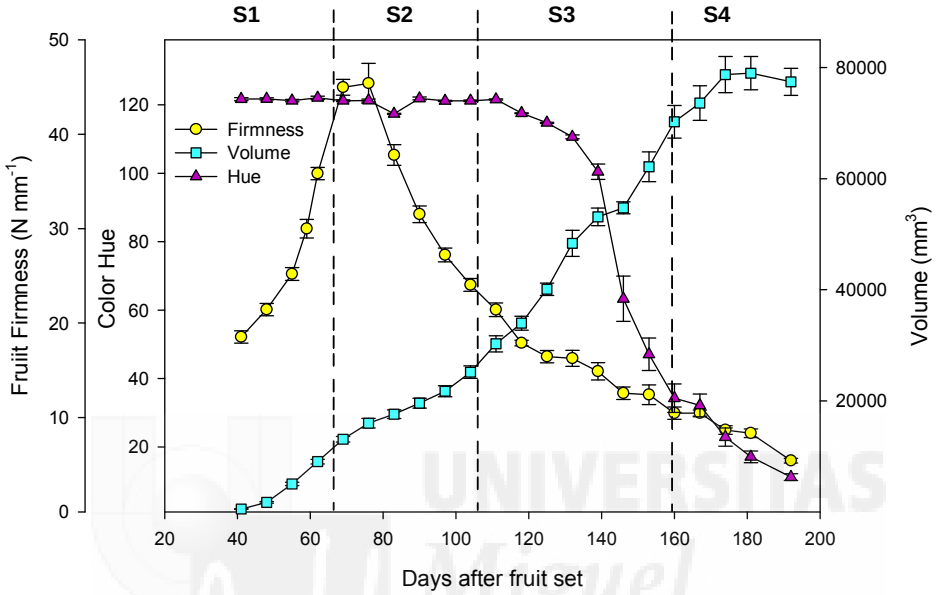


Figure 1: Evolution of fruit volume, firmness and skin colour (expressed as Hue angle) during 'Larry Ann' plum development on tree. S1-S4 phases represent the four phases of the double sigmoid growth of this stone fruit (Díaz-Mula, Valero and Serrano, unpublished data).

Contrarily, fruits containing a large number of seeds (such as apple, pear, orange, pepper, banana, avocado, strawberry, mango and lemon) show a single sigmoid growth curve, with a first phase of cell division and slow growth followed by a second phase of fast growth due to cell expansion, and finally the third phase with reduced growth till reaching the maximum fruit size (Valero and Serrano, 2010).

1.2. Fruit Ripening

Fruit ripening is a highly coordinated, genetically programmed process occurring at the later stages of maturation and involving a series of physiological, biochemical and sensory changes leading to the development of an edible ripe fruit with desirable quality parameters to attract seed dispersion agents (Brady, 1987; Lelièvre at al., 1997; Giovannoni, 2001). The main changes associated with ripening include colour (loss of green colour and development of yellow, orange, red and other colour characteristics

depending on species and cultivar), firmness (softening by cell-wall degrading activities), taste (increase in sugars and decline in organic acids), and flavour (production of volatile compounds providing the characteristic aroma).

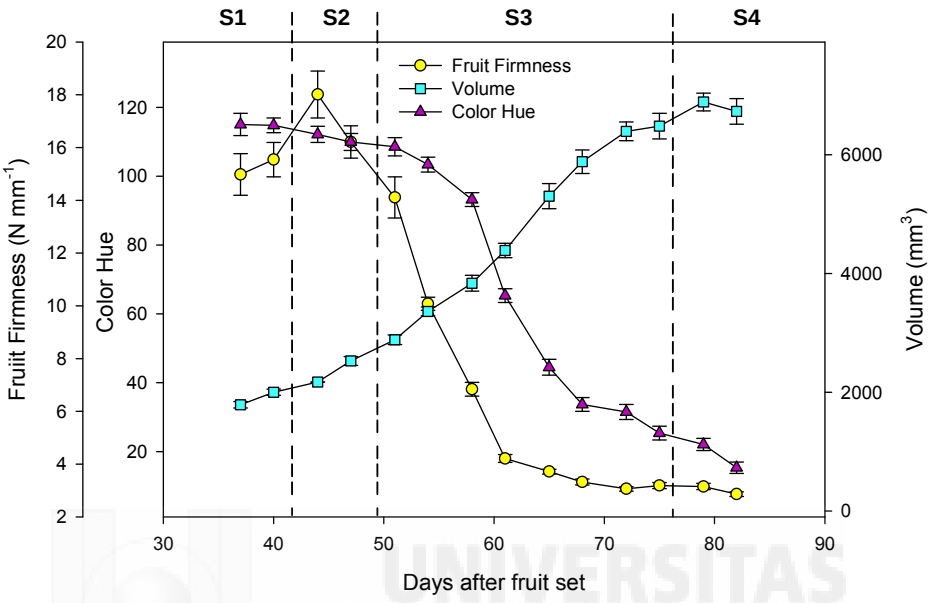
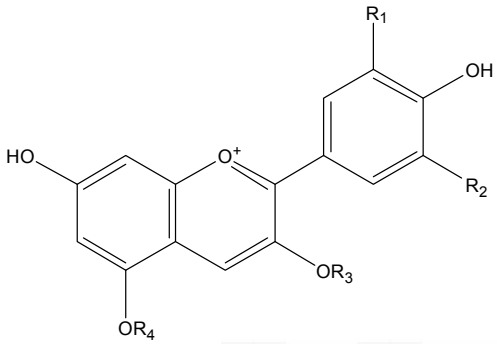


Figure 2: Evolution of fruit volume, firmness and skin colour (expressed as Hue angle) during 'Sweetheart' sweet cherry development on tree. S1-S4 phases represent the four phases of the double sigmoid growth of this stone fruit (Díaz-Mula, Valero and Serrano, unpublished data).

However, from the point of view of consumers, ripening could be defined as the composite of processes leading to changes in colour, texture, flavour, aroma, sugars, organic acids, and other nutritive components, that occur from the last stages of growth through the earliest stages of senescence, rendering a fruit attractive for consumption (Tucker and Grierson, 1987). Specific biochemical and physiological changes varying among species although generally include altered sugar metabolism, softening, colour changes, synthesis of aroma volatiles and increased susceptibility to pathogen infection, suggesting that the underlying genetic mechanisms which regulate fruit ripening are well conserved between fruits of different species (Adams-Philips et al., 2004; Giovannoni, 2004). The time of ripening varies with the developmental stage of fruits. In fruits with a single sigmoid pattern of growth, ripening usually occurs during the final phase of slow growth, while in fruits with a double sigmoid growth curve, ripening begins during the phase S3, along the second phase of fast growth. **Figure 1** and **2** show that Hue angle in both, 'Larry Ann' plum and 'Sweetheart' sweet cherry starts to decrease at this phase indicating the change of skin colour from green to the characteristic red-purple of this fruits.

1.2.1. Colour changes

The colour changes are due to loss of chlorophyll, and concomitant synthesis of the characteristic pigment for each fruit, that is anthocyanins or carotenoids. Anthocyanins are hydro-soluble pigments located in the vacuole that are responsible for the blue, red and purple colour of the fruits and classified as flavonoids with glycosilated derivatives of the 3,5,7,3'-tetrahydroxyflavylium cation (**Figure 3**).



R_1	R_2	Aglycone	Abbreviation	Color
H	H	Pelargonidin	Pg	Orange
OH	H	Cyanidin	Cy	Orange-Red
OMe	H	Peonidin	Pn	Orange-Red
OH	OH	Delphinidin	Dp	Bluish-Red
OMe	OH	Petunidin	Pt	Bluish-Red
OMe	OMe	Malvidin	Mv	Purple

R_3 or R_4 could be H or sugar

Figure 3: General structure of anthocyanidin (tetrahydroxiflavylium cation) and the different substituent for R_1 and R_2 to form the free aglycones, which are later glycosilated to form anthocyanins.

The free aglycones (anthocyanidins) are highly reactive with sugars to form the glycosides and all anthocyanins are *O*-glycosilated. The main aglycones found in fruits are pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin while the most relevant sugars are D-glucose, L-rhamnose, D-galactose, D-xylose and arabinose (Francis, 1989; Welch et al., 2008).

In general, anthocyanin concentration increases during ripening in a range of pink, red and purple coloured fruits, both climacteric and non-climacteric ones, although great variations exists in the total anthocyanin content at commercial harvest among fruit species and cultivars as well as in the predominant anthocyanin. Thus, strawberry has comparatively low total anthocyanin concentration, ranging from 20 to 60 mg 100 g^{-1}, depending on cultivar, the major anthocyanin being pelargonidin 3-glucoside (Lopes da Silva et al., 2007). In blackberry higher concentration has been reported, from 70 to 200 mg 100 g^{-1}, depending on cultivar too, in which cyanidin 3-glucoside contributed in more than 70% (Fan-Chiang and Wrolstad, 2005). However, the greatest variation among cultivars has been found on sweet cherry, with variations between 2 and 300 mg 100 g^{-1}, from light-coloured to dark cherry cultivars, in all of them the major being cyanidin 3-

rutinoside and cyanidin 3- glucoside (Gao and Mazza, 1995; Mozetiĉ et al., 2002; Chaovanalikit and Wrolstad, 2004). Great variations have been also found among table grape cultivars (6-200 mg 100 g^{-1}) and even the major anthocyanin (cyanidin 3-glucoside, peonidin 3-glucoside or malvidin 3-glucoside) was different depending on cultivar (Carreño et al., 1997; Orak, 2007). For red purple plum cultivars total anthocyanin concentration is higher in the skin (100-800 mg 100 g^{-1}) than in the flesh (2-100 mg 100 g^{-1}) and in both tissues cyanidin 3- glucoside has been reported as the major anthocyanin (Tomás-Barberán et al., 2001; Vizzotto et al., 2007). Anthocyanins are also present in peaches at total concentration ranging from 1.5-260 mg 100 g^{-1} from white to red skin genotypes (Vizzotto et al., 2007). However, these anthocyanin levels could be considered as illustrative, since environmental conditions and cultural practices have a great effect in the anthocyanin content for a particular fruit. For instance, the total anthocyanin content of 'Cabernet Suavignon' was determined as 108 and 194 mg 100 g^{-1} in 2001-2002 consecutive years (González-Neves et al., 2004).

On the other hand, carotenoids are the most widespread group of pigments in nature which are present in all photosynthetic organisms responsible for most of yellow to red colour of fruits and flowers. In fruits, carotenoids are C_{40} tetraterpenoids formed from eight C5 isoprenoid units joined head to tail resulting in a symmetrical molecule located in the chromoplasts. The transition from chloroplast to chromoplast can be visualized during the ripening process by exploiting the autofluorescence of chlorophyll and carotenoids of purified plastid fractions (Egea et al., 2010).The hydrocarbon carotenoids are known as carotenes (ß-carotene, lycopene, etc.) while xanthophylls are oxygenated derivatives containing at least one hydroxyl group and then being more polar than carotenes. On the other hand, carotenoids can be acyclic (eg. lycopene), monocyclic (γ-carotene) or dicyclic (α- and ß-carotene). **Figure 4** shows chemical structures of the main carotenoids found in fruits, which exist generally under the all-*trans* form (the most stable) although occurrence at much lower concentrations has been also found of *cis* isomers (Rodriguez-Amaya and Kimura, 2004).

Fruit and vegetables vary qualitative and quantitatively in their carotenoid composition, with green vegetables having a defined qualitative pattern, with lutein, ß-carotene, violaxanthin and neoxanthin being the main carotenoids, while fruits exhibit a carotenoid composition much more complex and variable. During fruit ripening, large variations have been found not only in the pigment profile but also in the concentration, with a general increase in the ripening process for all fruits and higher contents in the skin than in the flesh. However, important differences in the pigment concentration at commercial ripening stage have been found among species and cultivars, and are also affected by several factors such as environmental conditions and cultural practices. For carotenoids, the reported levels (mg 100 g^{-1}) are in the range of 0.1-4 for apricot (Kurz et al., 2008), 2-7 for tomato (Kaur et al., 2006), 0.02-5 for *Citrus* sp. (Fanciullino et al.,

2008), 1.5-3 for papaya (De Souza et al., 2008), 0.1-2 for loquat (Zhou et al., 2007), 0.2-20 for pepper (Topuz and Ozdemir, 2007), and 3-7 for watermelon (Perkins-Veazie et al., 2001).

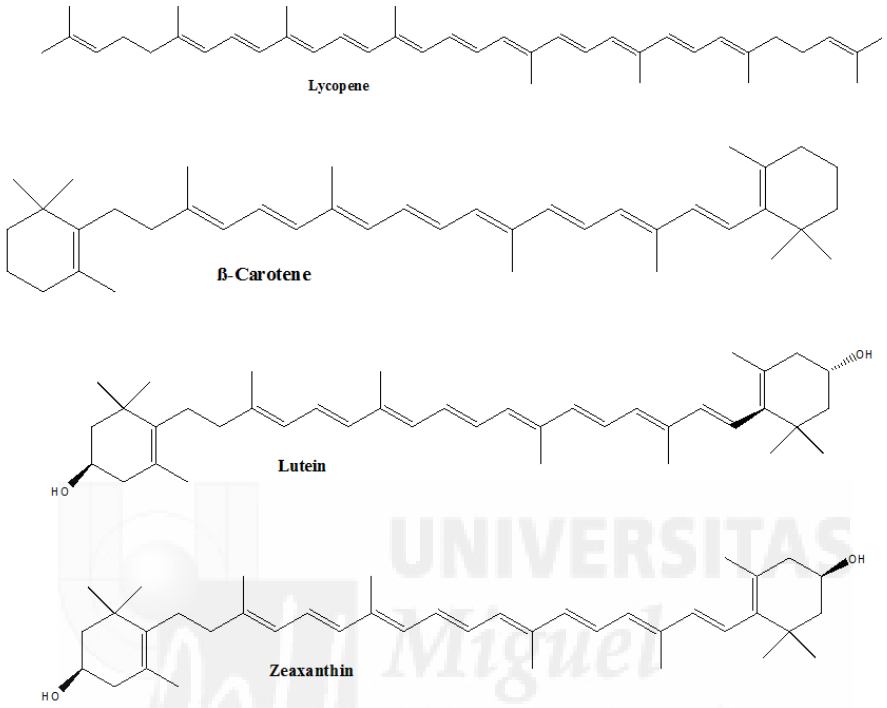


Figure 4: Chemical structures of the main carotenoids found in fruits.

1.2.2 Sugar and organic acids

The vacuole is the most important organelle for fruit quality due to the compounds responsible for the taste and flavour (sugars and organic acids) are accumulated within the vacuole at high concentrations (Shiratake and Martinola, 2007). Thus, the levels of sugars and organic acids are important factors in determining the taste of ripe fleshy fruit, and the relative content of these constituents depends on the activity and the interaction of sugar and acid metabolism (Rhodes, 1980). Sugar accumulation during fruit growth and ripening is mainly a matter of carbon import in the form of sucrose (and sorbitol in Rosaceae' species) from photosynthetic leaves, leading to increase in total soluble solids (TSS). However, important differences in sugar content at commercial harvest exist among species and cultivars. For example, in tomato cultivars sugar content ranges from 4 to 7.5% (Guillén et al., 2006; Zapata et al., 2008) and in sweet cherry cultivars from 13 to 20% (Usenik et al., 2008). The translocated assimilates enter in the metabolism to form fructose, glucose and sucrose, which accumulate in the growing fruit as occur in sweet cherry and plum. However, other fruits, such as mango, kiwifruit and banana, among

others use the translocated sugars to form starch, the main reserve carbohydrate, which is hydrolysed into sugars during ripening (Berüter, 2004). In addition, the hydrolysis of sucrose into fructose and glucose is also intensified during fruit ripening and the main sugar at fruit ripening stage depends on plant species as it is shown in **Table 1.**

Table 1: Major sugar and organic acid in some fruits.

Fruit	Sugar	Organic acid	Reference
Table grape	Glucose	Tartaric	Valero et al., 2006.
Strawberry, blueberry, date plum persimmon, mango, tomato (domesticated), *Citrus* spp. (lime, orange, lemon)	Fructose	Citric	Glew et al., 2005; Albertini et al., 2006. Kafkas et al., 2007; Wang et al., 2008; Zapata et al., 2008; Thanaraj et al., 2009.
Apricot, plum, nectarine, peach, loquat	Sucrose	Malic	Amorós et al., 2003; Aubert et al., 2003; Cascales et al., 2005; Aubert and Chanforan, 2007; García-Mariño et al., 2008; Morandi et al., 2008.
Tomato (wildtype)	Sucrose	Citric	Kortstee et al., 2007.
Pomegranate, sweet cherry	Fructose	Malic	Mirdehgham et al., 2006; Serrano et al., 2005a.

Thus, glucose is the major sugar in table grape, while fructose is the predominant in berries, mango and citrus species. Those fruits that accumulate fructose or glucose show very low concentrations of sucrose. However, apricot, plum, nectarine and peach have sucrose as main sugar, which is accumulated during the stage S3 as a result of a rise in the activity of sucrose synthase [EC 2.4.1.13] (Morandi et al., 2008). Interestingly, wild type tomatoes have sucrose as main sugar while domesticated fruits accumulate fructose followed by glucose (Kortstee et al., 2007; Zapata et al., 2008). The proportions of fructose, glucose, and sucrose are important in the perception of taste since fructose is 1.8 times sweeter than sucrose, while glucose is only 60% sweetness than sucrose (Yamaguchi et al., 1970).

Developing fruits are extremely acidic due to accumulation of many organic acids, although mature fruits do not taste acidic because the large amounts of accumulated sugars and the decrease of total acidity (TA) than usually occur during ripening. Nevertheless, as in sugar concentration, important differences are observed among fruit species and cultivars in total acidity at harvest, i.e. from 0.4 to 1.7 % in tomato cultivars

(Guillén et al., 2006; Zapata et al., 2008), from 1.0 to 1.5 % in sweet cherry cultivars (Usenik et al., 2008), while in plums these values ranged between 0.7 and 1.6 % (Crisosto et al., 2007). Another factor determining fruit acidity is the type of organic acids, the most important being malic, citric, tartaric, quinic, oxalic, fumaric, and succinic acid, since each of them has a unique taste that contributes in different way to the overall flavour of fruits (**Table 1**).

Malic acid is the main organic acid of fruits belonging to Rosaceae family for both *Prunus* (plum, apricot, peach, nectarine, sweet cherry) and *Malus* (apple, pear) genera. For these fruits, TA is very high at the beginning of development with accumulation of malic acid during the first rapid growth phase that takes place at the end of the cell division phase, but diminishes over the maturation and ripening processes, with the exception of sweet cherry for which a continuous increase in acidity occurs during on-tree ripening (Serrano et al., 2005a). The decline in TA in plum cultivars appears to be related to the dilution process caused by the intense water uptake that occurs during the second fast growth phase (García-Mariño et al., 2008). The enzymes involved in malic acid synthesis are phospho*enol*pyruvate carboxylase [EC 4.1.1.31] and NAD-malate dehydrogenase [EC 1.1.1.37], while NADP-malic enzyme [EC 1.1.1.37] is the responsible for malic acid degradation, all of them located at the cytosol (Etienne et al., 2002). On the contrary, citric acid is the characteristic organic acid of *Citrus* sp. (orange, lemon, mandarin, lime and grapefruit), which accounts between 50-80% of the total organic acids, although other fruits such as tomato, mango and small berries have also this organic acid as predominant. During maturation of citrus fruits, citric acid generally decreases with the exception of lemon, in which remains constant. *Citrus* sp. shows a single sigmoid growth pattern (Monselise, 1986) and citric acid accumulates during the second stage of development. The enzymes responsible for citric acid synthesis are citrate synthase [EC 4.1.3.7] and aconitase [EC 4.2.1.3], located at the mitochondria, while the cytosolic aconitase and NADP-isocitrate dehydrogenase [EC 1.1.1.42] are involved in citric acid catabolism (Etienne et al., 2002). Tartaric is the predominant organic acid in grapes and its diminution has been a useful parameter for checking the maturation process of this fruit (Mato et al., 2005).

For practical purposes and as an index of fruit ripening, the ratio between soluble solids and acidity (TSS/TA) is used rather than the soluble sugars content alone since it is related to the overall consumer appreciation. The proportions of individual acids are also important, since citric acid masks the perception of sucrose and fructose, while malic acid enhances sucrose perception (Lobit et al., 2003).

1.2.3 Fruit softening

The metabolic events responsible for the textural changes leading to fruit softening during maturation and ripening involve loss in turgor pressure (due to an accumulation of osmotic solutes in the apoplast), degradation and other physiological changes in the composition of membranes, modifications in the symplast/apoplast relations, degradation of starch, and modifications in the cell wall structure and dynamics. The relative contribution of each event in fruit softening is not clear, and probably depends on the species, although changes in cell wall composition, especially cell wall mechanical strength and cell-to-cell adhesion, have been considered to be the most important factors (Lasbrook, 2005; Brummell, 2006; Goulao and Oliveira, 2008). Flesh of freshly fruit is composed mainly by parenchymatic cells, having a thin primary cell wall composed by cellulose microfibrils embebed in a matrix of glycan polysaccharides (formerly known as hemicelluloses), pectic substances, enzymatic and structural proteins, mineral ions and some phenolics. The external rind of primary cell wall is known as middle lamella being common to two adjacent cells and providing intercellular connections, in which the major components are pectins accompanied by proteins and with absence of cellulose microfibrils (Rose and Bennett, 1999; Carpita and McCann, 2000; Jarvis et al., 2003).

According to their softening behaviour, fruits can be divided into two categories: those that soften greatly to a melting texture, due to swelling of the cell, as they ripen (e.g. tomato, peach, strawberry or kiwifruit) and those that soften moderately, without cell swelling, and characterized by a crisp fracturable texture (apple or cranberry). In addition, the time of softening is different in each fruit, since softening may start after finishing fruit growth (e.g. in pome fruits) or before the fruits stop growing (e.g. avocado and strawberry). Then, considering the wide range of fruit types, softening may proceed via different mechanism among fruit species and even among cultivars that belong to the same species. Nevertheless, changes in the structure of the cell wall by dissolution of the middle lamella and disruption of the primary cell wall, which will be commented bellow, are considered to be common to all freshly fruit species.

When examined by electron microscopy, the first change observed in a ripening fruit is dissolution of the middle lamella, leading to diminution of intercellular adhesion, followed by disruption of the ordered structure of the primary cell wall and certainly fruit cell wall becomes noticeable thinner during ripening. However, cell wall component disassemble and hydrolysis varies considerably among species and cultivars. In general, it is considered that pectin degrading enzymes are mostly implicated in fruit softening, although other enzymes acting on the glycan polysaccharides and cellulose microfibrils also contribute to the softening process as will be commented bellow (Brownleader et al., 1999; Rose and Bennett, 1999; Brummell and Harpster, 2001; Lasbrook, 2005; Brummell, 2006; Prasanna et al., 2007; Goulao and Oliveira, 2008).

Enzymes that act on pectins are mainly polygalacturonase [EC 3.2.1.15, PG], pectin methylesterase [EC 3.1.1.11, PME], ß-galactosidase [EC 3.2.1.23, ß-GAL] and pectate lyase [EC 4.2.2.2, PL], all of them existing in multi-gene families, with a subset of one or more gene family members regulating the cell wall modification processes associated to fruit ripening.

PME de-esterifies polyuronides by removing methyl groups from de C6 position of galacturonic acid residues of high-molecular-weight pectins, releasing methanol and protons and leading to changes in pH and charge in the cell wall, since negatively carboxyl groups are created. This allows the aggregation of polyuronides into a calcium-linked gel structure, and makes the polyuronides susceptible to degradation by PG. PME action begins in the middle lamella and spreading throughout the cell wall during ripening which may be a prerequisite for PG activity during fruit ripening. PGs catalyze the hydrolytic cleavage of α-(1→4) galacturonide linkages and can be exo- or endo- acting types. The exo-PG removes single galacturonic acid units from the non-reducing end of polygalacturonic acid, whereas the endo-PG cleaves such polymers at random. Both exo and endo-PG types are found in fruit, although the fruit ripening specific enzyme usually referred as PG is of the endo-acting type. In addition, it is generally accepted that PG is primarily responsible for dissolution of the middle lamella during fruit ripening, although PG-independent solubilisation also exist.

PL also preferentially acts on de-esterified homogalacturonic acid by cleaving the α-1,4-linkages between its galacturonic acid residues. Exo-PL acts from the non-reducing end, whereas endo-PL acts randomly on de-esterified galacturonans. Either endo-PG, PL or both are detected in most ripening fruit, and their activities lead to degradation of homogalacturonan, the main component of the middle lamella and in turn to reduce intercellular adhesion and firmness. ß-GAL cleaves the galactan or arabinogalactan side chains of ramnogalacturonan I by hydrolyzing terminal ß –D-galactosyl residues, acting as an exo-enzyme. This enzyme also acts on short chain oligomers of galactose units present as glycoproteins or glycolipid and the terminal galactosyl residues of xyloglucan side chains. Then, ß-GAL causes a decrease in polymeric galactose and an increase in free galactose.

Structural modifications in hemicellulose-cellulose domains are due to xyloglycan endotransglycosylase [EC 2.4.1.207] (namely XTH, XET or EXGT), endo-ß-1,4-glucanases [EC 3.2.1.152, EGases], ß-D-xylosidase [EC 3.2.1.37], endo- ß-mannan transglycosylases [EC 3.2.1.25, manase) and expansins. XET**s** cleave internal 1,4 linkages within the ß-D-glucan backbone of xyloglucans and transfer the newly formed potentially reducing end to the C-4 position of the glucose unit at the non-reducing end of another xyloglucan polymer or oligosaccharide. EGase**s** (also referred as cellulases) hydrolyze internal linkages of (1→4) ß-D-glucan chains adjacent to un-substituted residues of xyloglucan, integral and peripheral regions of non-crystalline cellulose and possibly glucomanan, where sufficient

consecutive (1→4) ß-D-linked glucan residues occur for substrate binding, resulting in loosening of the cellulose-xyloglucan network. Mannase catalyzes the hydrolysis of mannan polymers and expansins act by causing a reversible disruption of hydrogen bonding between cellulose microfibrils and matrix polysaccharides, particularly xyloglucan, resulting in a disassembling of the hemicellulose network and increasing the accessibility of other cell wall degrading enzymes to their substrates.

It is probably that fruit of all species have the same range of enzymatic activities, acting all of them together in a cooperative interdependent way to achieve controlled changes in softening. However, it must be remembered that important differences have been reported among species and even among cultivars, since the expression of cell wall degrading enzymes is regulated both in time and amount in each particular fruit (Brummell, 2006; Bennett and Labavitch, 2008; Goulao and Oliveira, 2008). In general, depolymerization of matrix glycans begins during early ripening, and it is followed by loss of galactan and arabinan side chains of ramnogalacturonan I and dimethylesterification of polyuronides, and finally pectin depolymerization, by endo- or exo-PG, starts at mid or late ripening depending on fruit. In addition, fruits with a crisp fracturable texture at ripeness (apple, watermelon, bell pepper) have a low grade of pectin solubilisation and depolymerisation compared to the melting-flesh fruit, suggesting that the integrity of intercellular connections is an important component of crispness.

1.2.4. Aroma compounds

Fruit aromas are perceived by human nasal olfactory epithelium, a relatively small area of the mucous-covered inner surface of the nasal cavity. Most fruits produce a significant number of volatile compounds and their qualitative and quantitative composition determines fruit aromatic characteristic. Many of these volatile compounds are produced in trace amounts, which are below the thresholds of most analytical instruments, but can be detected by human olfaction (Zhu et al., 2005; Goff and Klee, 2006; Song and Forney, 2008; Defilippi et al., 2009).

Fruit volatile composition includes array of chemicals from various classes, such as alcohols, aldehydes, esters, ketones and terpenes, and it plays a principal role in the market success of any fruit. In addition, the recognized flavour of a particular type of fruit is usually absent in the early stage of its development and, instead, is acquired during the ripening process as a consequence of volatile accumulation. Volatiles responsible for fruit aroma can be classified as “primary” or “secondary”, indicating whether they are present in intact fruit tissue or produced as a result of tissue disruption. Volatiles collected from intact fruit reflect the consumer smelling and perceiving ripening signals of the fruit, while volatiles generated after tissue disruption may better represent the flavour perception during eating.

To identify and quantify fruit aroma compounds, the most useful technique is gas chromatography-mass spectrometry (GC-MS). However, aroma extraction methods affect the profile and concentration of the extracted volatile compounds, and important differences also occur between aroma released by intact fruit and those determined after fruit ground and extraction (Guillot et al., 2006; Aubert and Chanforan, 2007; Song and Forney, 2008; Defilippi et al., 2009). The electronic nose (e-nose) has also been utilized to study changes in aroma compounds in many fruits such as apples, peach, and apricots. This technique is based on electrochemical sensors that allow for the analysis of aroma intensity and has the advantage of being a non-destructive technique, but it cannot, however, identify particular aroma compounds (Benedetti et al., 2008). Finally, to relate the contribution of volatile compounds to fruit aroma and flavour, human olfactory analysis is required, since humans can smell volatile at ppb levels or lower (Goff and Klee, 2006). Thus, the combination of sensory analysis of fruit flavour with instrumental analysis provides greater insights into the impact of volatile compounds on flavour than either alone (Baldwin et al., 2007; Song and Forney, 2008).

An overwhelming number of chemical compounds have been identified as volatile compounds in fresh fruit, based on their quantitative abundance and olfactory thresholds, although only a fraction of these compounds have been identified as fruit flavour impact compounds. In **Table 2** some examples about aroma substances and description of their odour are showed.

Table 2: Aroma substances in fruits and a description of their odour.

Aroma substance	Odour description
Acetaldehyde	Pungent, penetrating
Acetone	Sweet, pungent
Ethyl acetate	Ether-like, pineapple, anise
Methyl butyrate	Apple
Dimethyl disulfide	Onion, cabbage
Ethyl butyrate	Fruity, pineapple
Butyl acetate	Fruity
1-Methyl-ethyl butyrate	Apple
Hexanal	Cut grass
Hexenal	Sweet, almond, green
2-Hexenal	Green leaf
Heptanone	Banana
Methyl hexanoate	Ether-like, pineapple
Butyl butyrate	Fruity, pear
Ethyl hexanoate	Fruity
Hexyl acetate	Fruity, apricot
Linalool	Fruity, floral, citrus
B-lanone-trans	Warm, woody, balsamic, rose

Citrus fruits possess unique aromas rarely found in other fruit species, mainly due to mono and sesquiterpenes (the major components of citrus essential oils), which accumulate in specialized oil glands in the flavedo and oil bodies into the juice sacs. The monoterpene limonene normally accounts for over 90 % of essential oils of the citrus fruit, although several unique sesquiterpenes compounds present in small quantities have a profound effect on flavour and aroma of each particular citrus fruit species and cultivars (Sharon-Asa et al., 2003). In mango cultivars mono and sesquiterpene hydrocarbons also dominated the compound profiles, while aldehydes occurred at lower concentration, although they are responsible for the flesh, grassy and fatty-green in some mango cultivars and they impart the characteristic smell in these fruits, even at their extremely low concentrations (Pino et al., 2005; Pandit et al., 2009). In tomato fruits over 400 volatile compounds have been detected, the most important contributor to tomato fruit aroma being hexanal, hexenal, hexenol, 3-methylbutanal, 3-methylbutanol, methylnitrobutane and isobutyl thiazole and a general increase in these and other volatiles occur during ripening (Zhu et al., 2005; Birtic et al., 2009). Accordingly, volatiles in apple also increase during ripening and the aroma profile changes from an abundance of aldehyde volatiles to a profile dominated by esters (Dixon and Hewett, 2000; Villatoro et al., 2008).

Esters have been reported as the main components of aroma of Charentais cantaloupe melons, and among them, 2-methylpropyl acetate, ethyl butyrate, ethyl 2-methyl butyrate, butyl acetate, 2-methylbutyl acetate, benzyl acetate and hexyl acetate were the most abundant, followed by some thioesters, all of them being at low concentration in long shelf life cultivars as compared to mid and wild type ones (Aubert and Bourgen, 2004; Pech et al., 2008). Hexyl acetate and butyl acetate have been also identified as the main contributors to apricot fruit aroma, together with ethyl acetate, linalool, α-terpineol, γ-hexalactone and γ- and δ-decalactone (Aubert and Chanforan, 2007; Defilippi et al., 2009). Finally, in plum cultivars, low quantities of aroma compounds have been identified. For instance, Lozano et al. (2009) identified 40 volatile compounds in six plum cultivars, with guanidine, 3-hexen-1-ol and the esters 4-hexen-1-ol acetate and hexyl acetate being present in all cultivars at the greatest proportions.

The aroma components in sweet cherry are a wide range of organic compounds, such as aldehydes, alcohols, esters acids and terpenes. Among them, the most important compounds contributing to aroma are hexanal, *(E)*-2-hexenal, *(E)*-2-hexenol, 1-penten-3-ol, nonanal, *(E,Z)*-2,6-nonadienal, benzylalcohol, linalool and bezylaldehide, although differences in aroma profile and concentration of each particular compound have been found depending on cultivar and ripening stage (Matteis et al., 1992; Girard and Kopp, 1998; Bernalte et al., 1999; Serradilla et al., 2010; Sun et al., 2010).

1.2.5. Bioactive compounds and antioxidant activity

Foods from plant origin contain hundreds of non-nutrient constituents with significant biological activity, generally called "bioactive compounds" or phytochemicals with antioxidant activity. The word antioxidant is increasingly popular in modern society, as it gains publicity through mass media coverage concerning to its health benefits. According to the dictionary, the term antioxidant refers to a substance that opposes oxidation or inhibits reactions promoted by oxygen or peroxides, although a more biologically relevant definition is synthetic or natural substances that prevent or delay oxidation reaction in biological systems.

Free radicals are molecules with one or more unpaired electrons, such as superoxide anion ($O\bullet^{2-}$) hydroxyl radical (OH•) and peroxyl radical (ROO•) namely as reactive oxygen species (ROS), among others. These free radicals are generated during normal aerobic metabolism, inflammatory processes and macrophages action. They are short-lived and highly reactive, constantly seeking for another electron to be paired causing oxidation in other molecules such as lipids, proteins and nucleic acids and spreading oxidation chains. Thus, free radicals are implicated as mediators in the ageing process, in degenerative and chronic deteriorative, inflammatory, and auto-immune diseases, diabetes, hypertension, cancer, arthritis, brain dysfunction, etc. In living cells there are defence systems against these free radicals. The primary defence system directly interacts with harmful free radicals by preventing their formation or by removing them as soon as they are formed and avoiding the damage of the body's cellular components. Enzymes such as catalase [EC 1.11.1.6, CAT] and superoxide dismutase [EC 1.15.1.1, SOD], and small molecules like vitamin C, E and other food minor components, generally known as antioxidants, are involved in this primary defence system. The secondary defence system consists on other enzymes and antioxidants which repair the already damaged biomolecules. Thus, problems leading to cellular ageing and the above commented diseases only arise when the balance between free radicals and antioxidant defence systems tilts to the side of free radicals (Yeum et al., 2004; Willcox et al., 2004; Tsao and Akhtar, 2005).

In this sense, fruit and vegetable consumption has shown protective effects against several chronic diseases associated to ageing including atherosclerosis, cardiovascular diseases, cancer, cataracts, blood pressure increase, ulcerous, neurodegenerative diseases, brain and immune dysfunction and even against bacterial and viral diseases. The impact of this scientific inquiry has resulted in the development of many epidemiological studies to correlate the presence of these bioactive compounds on alleviating one or more of the above diseases. The protection that fruit and vegetables provide against these degenerative diseases has been attributed to several antioxidant compounds, which vary widely in chemical structure and function in plant tissues and are grouped in vitamins (C and E), carotenoids, phenolic and thiol (SH) compounds (Kris-Etherton et al., 2002;

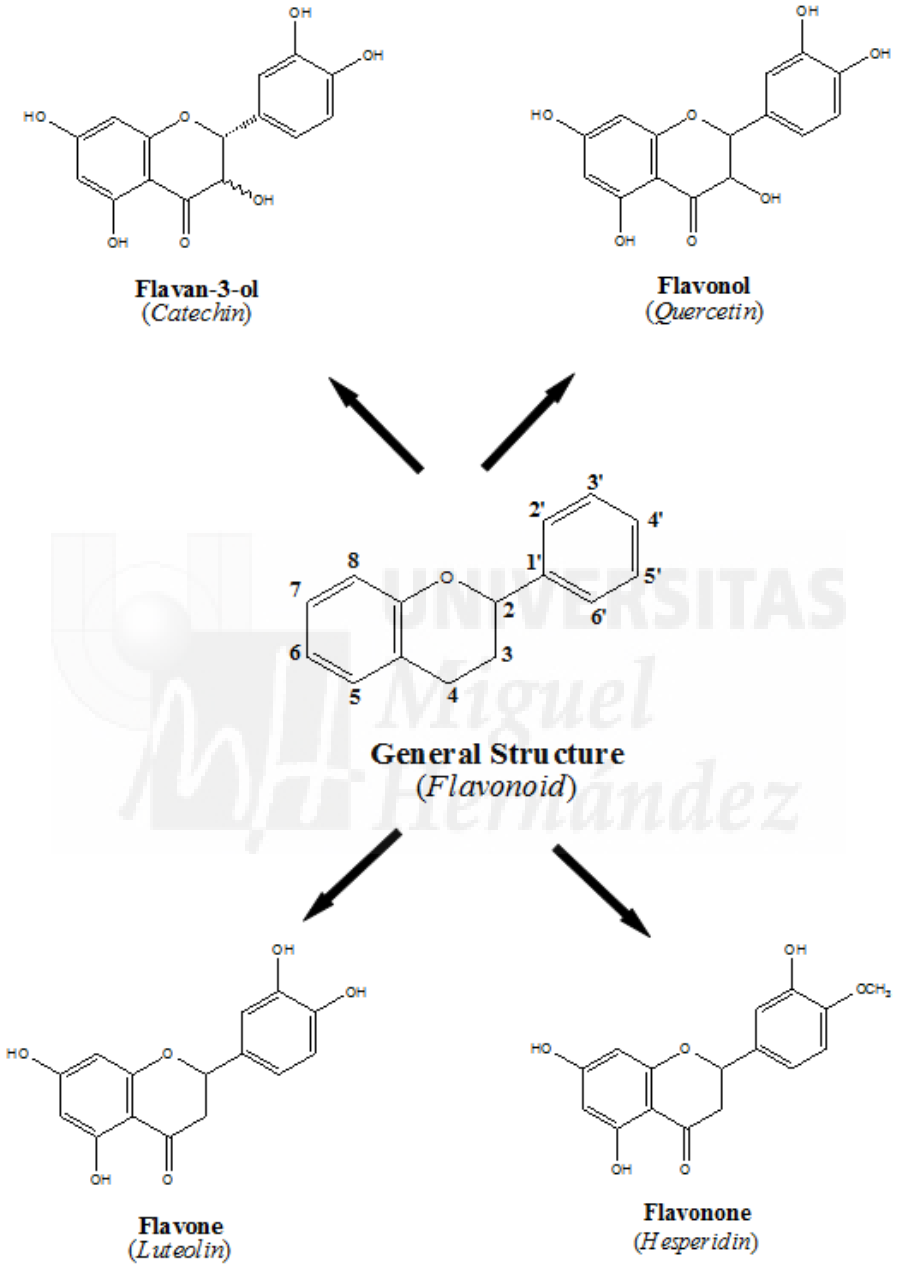
Pennington, 2002; Scalbert et al., 2005; Nichenametla et al., 2006; Lister et al., 2007; Saura-Calixto and Goñi, 2009). In this sense, there is growing scientific evidence that dietary antioxidants may be a critical mediator of the beneficial effects of the Mediterranean diet.

These bioactive compounds experience variations along fruit ripening on-tree as well as during postharvest storage and processing. These changes as well as the impact of several postharvest technologies on the content of fruit phytochemicals will be commented in **Section 4** of this Introduction.

Table 3 and **Figure 5** show the phenolic groups and subgroups and the chemical structures of the most commonly found in fruits. From all of them, flavonoids are the most important group with ca. 8,000 different compounds already identified. A direct relationship has been found between the total phenolic compounds and the total antioxidant activity (TAA) during the ripening of plums, peaches, peppers, nectarines and sweet cherries (Gil et al., 2002; Cevallos-Casals et al., 2006; Deepa et al., 2007).

Table 3: Main phenolic groups and subgroups found in fruits.

Phenolic groups	Subgroups	Compounds
Phenolic acids (C_6-C_1)		Gallic acid, Ellagic Acid
Hydroxycinnamic acids (C_6-C_3)		*p*-Coumaric acid, Caffeic acid, Ferulic acid
Stilbenes (C_6-C_2-C_6)		*Cis*-Resveratrol, *Trans*-Resveratrol
Flavonoids (C_6-C_2-C_6)	Flavonols	Quercetin, Kaempherol, Myrcetin, Isorhamnetin
	Flavan-3-ols	Catechin, Epicathechin, Gallo-catechin, Proanthocyanidins
	Flavones	Apigenin, Luteolin, Chryoeriol
	Flavanones	Naringenin, Herperidin, Eriodyctol
	Anthocyanindins	Cyanidin, Delphinidin, Malvidin, Petunidin, Peonidin, Pelargonidin


Flavan-3-ol
(Catechin)
Flavonol
(Quercetin)
General Structure
(Flavonoid)
Flavone
(Luteolin)
Flavonone
(Hesperidin)

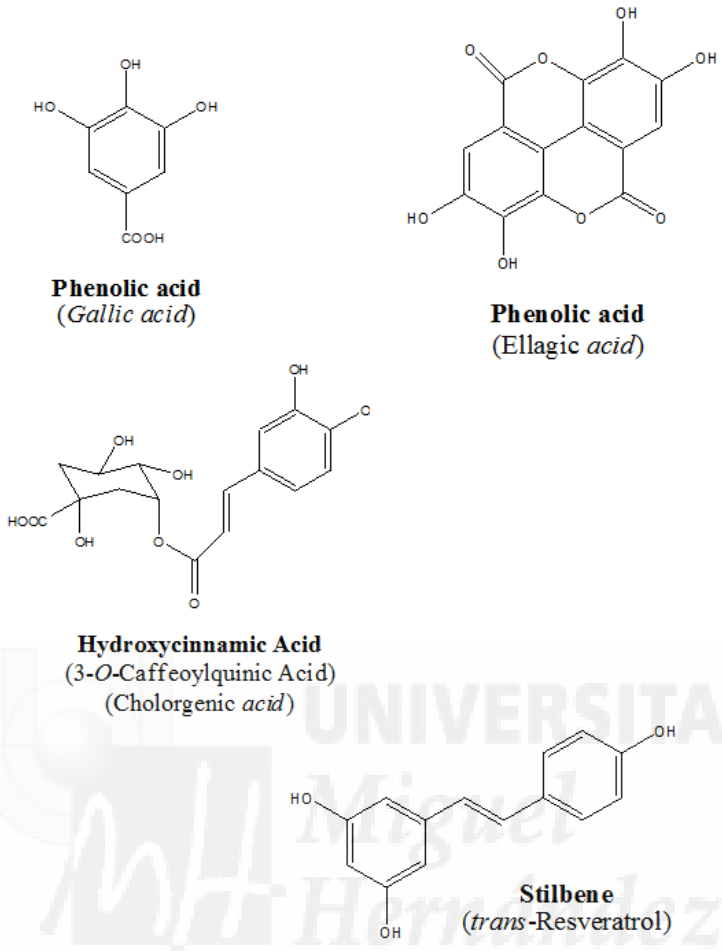


Figure 5: General structures of some flavonoid subgroups and other phenolic compounds.

1.2.6. Physiological changes

Fruit ripening physiology has been classically defined as either climacteric or non-climacteric, which differs in their pattern in both ethylene production ad respiration rates. In this sense, climacteric fruits, such as tomato, avocado, banana, peach and plum, among others (**Table 4**), show a sharp increase in respiration rate and ethylene production at the onset of ripening. For these fruits, ethylene is considered as the plant hormone responsible for their ripening process. On the contrary, non-climacteric fruits, such as sweet cherry, pomegranate, pepper, grape and citrus fruits, among others (**Table 4**), show comparatively low profile and a gradual decline in their respiration pattern and ethylene production through the ripening process (Lelièvre et al., 1997; Giovannoni, 2001; 2004; 2007; Adams-Phillips et al., 2004; Barry and Giovannoni, 2007). This physiological

behaviour of the fruits has a great importance in the postharvest biology and technology of these commodities (Martínez-Romero et al., 2007a).

Table 4: Examples of fruit having a climacteric or non-climacteric ripening pattern.

Climacteric Fruit	**Non-Climacteric Fruit**
Tomato	Pomegranate
Peach	Grape
Nectarine	Pepper
Plum	Blackberry
Apricot	Sweet cherry
Apple	Strawberry
Pear	Orange
Banana	Lemon
Watermelon	Cucumber
Kiwifruit	Cranberry
Mango	Loquat
Cherimoya	Blueberry

Ethylene is a very simple molecule with two carbon atoms linked with a double bound and naturally occurring as gaseous form. The first indications of a gaseous compound affecting plant tissues were reported in the nineteenth century, with the observation that illuminating gas streetlights caused senescence and defoliation in neighbouring trees. In the early twentieth century (1901), Neljubov identified ethylene as the causative agent of this effect, and he is recognized as the discoverer of this plant hormone. Later, Gane (1934) proved that plants produce ethylene, although only after the establishment of gas chromatography (1959) this compound could be quantified. Ever since the discovery of ethylene, continuous efforts have been made to clarify its biosynthesis pathway (Yang and Hoffmam, 1984; Pech et al., 1992). Ethylene biosynthesis, perception, signal transduction and its regulation at biochemical, genetic and biotechnological levels is well documented and covered by a number of excellent reviews (Sisler and Serek, 1997; Bleecker and Kende, 2000; Ecker, 2002; Wang et al., 2002; Stearns and Glick, 2003; De Paepe and Van der Straeten, 2005; Yoo et al., 2009).

In higher vascular plants, ethylene is synthesized from the amino acid methionine, which is converted to S-adenosyl-methionine (SAM) by the addition of adenine and consumption of ATP. SAM is then transformed to 1-aminocyclopropane-1-carboxylic acid (ACC) by the enzyme ACC-synthase (ACS) with the generation of the by-product 5'-methylthioadenosine, which is recycled to methonine (Yang's cycle). Thus, ethylene can be produced at high rates even with a small pool of free methionine. Finally, ACC is oxidized to ethylene by ACC-oxidase (ACO, formerly named as EFE, ethylene-forming enzyme). The main step controlling ethylene biosynthesis is ACS and the subsequent pool of ACC. Early studies reported the up-regulation of this pathway (**Figure 6**) by observations of ACC accumulation and increases in the activities of both ACS and ACO enzymes (Yang and Hoffman, 1984; Abeles et al., 1992; Kende, 1993). More recently, it has been reported that ACS and ACO are encoded by multigene families. In climacteric fruit, once the ethylene is being synthesized at low amounts, the internal production of ethylene rapidly increase, this is the autocatalytic ethylene production and the onset of the ripening process begin at this stage (Yang and Hoffman, 1984). As a hormone, ethylene binds to a receptor and the signal is transduced through a complex mechanism to trigger specific biological responses.

Ethylene biosynthesis and action can be diminished by chemical compounds which differ in their structure and act at different levels, such as modifying ACS and ACO activities, blocking receptor sites, diversion of SAM through polyamine biosynthesis or through the removal of ethylene (Martínez-Romero et al., 2007a).

Climacteric fruits synthesize small amounts of ethylene during the growing period (0.1-0.2 μL kg^{-1} h^{-1}), but can increase markedly (up to 1,000-fold) associated with the ripening process. In general, climacteric fruits have high rates of ethylene production and are also highly sensitive to this plant hormone (at concentrations of 0.03-0.1 μL L^{-1}). Conversely, non-climacteric fruits produce very low amounts of ethylene and exhibit low sensitivity to this plant hormone (over 0.2 μL L^{-1}). However, some non-climacteric fruits such as *Citrus* show a good response to exogenous ethylene, the phenomenon being used for the de-greening agricultural practice (Goldschmidt et al., 1993). The distinction between climacteric and non-climacteric fruits is not absolute, since there are also a number of species in which different varieties and cultivars exhibit both climacteric and non-climacteric behaviour. Thus, plum cultivars have generally been categorized as climacteric fruit, although 'Shiro', 'Golden Japan' and 'Ruby Red' behave as suppressed-climacteric fruits (Abdi et al., 1997; Zuzunaga et al., 2001). Accordingly, melon fruits comprise both climacteric and non-climacteric genotypes. Thus, *Cucumis melo* var. *cantalupensis* has a fast ripening rate and a short shelf life with high ethylene production rate, while *C. melo* var. *inodorous* is unable to produce autocatalytic ethylene and has a slow ripening rate associated with a long shelf life. In addition, the inheritance of the climacteric character seems to be dominant, since crossing climacteric with non-

climacteric melons generates climacteric melons, although the genetic control involved is still unknown (Pech et al., 2008).

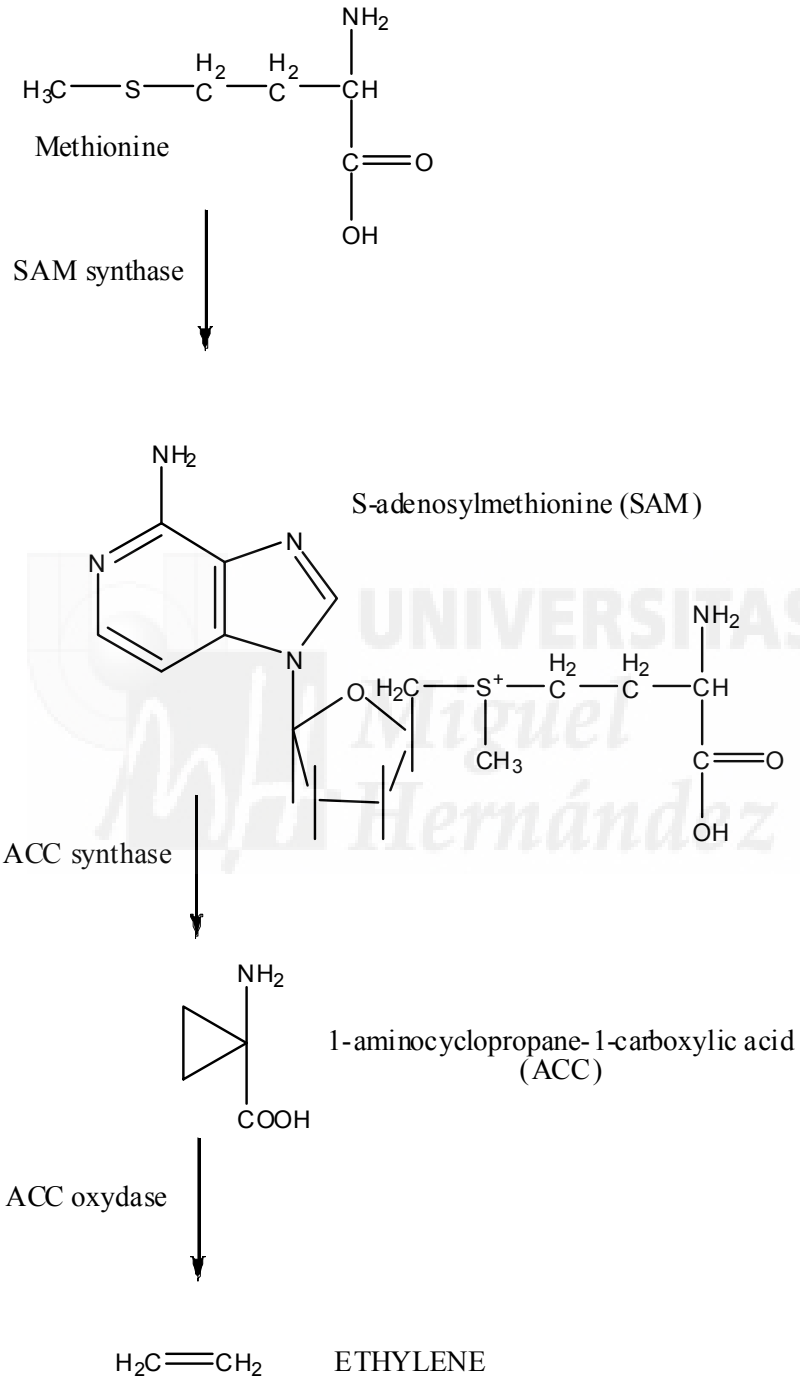


Figure 6: Ethylene biosynthesis pathway.

As commented above, ripening of climacteric fruits is regulated by ethylene, although there are ethylene-dependent and independent ripening processes (Barry and Giovannoni, 2007; Pech et al., 2008; Pirrello et al., 2009). Thus, in many fruits such as tomato, apple, banana and melon pulp colouration, sugar accumulation and loss of acidity are ethylene-independent processes, whereas de-greening and yellowing of the rind, softening of the flesh, development of peduncle abscission zone, aroma formation and climacteric respiration are totally or partially ethylene-dependent. Thus, antisense ACO melons and apples from plants silenced for ACS or ACO exhibited strong reduction of softening, but significant residual softening still persisted, indicating the presence of an ethylene-independent component of softening. Moreover, in antisense ACO tomatoes ethylene production was inhibited at 97 %, but not softening, which could also show that the 3 % of residual ethylene was sufficient to active cell wall degradation events. These examples point out that the relevance of ethylene-dependent or independent component of softening are different among fruit species and probably among cultivars. In this sense, it has been showed that depolymerisation of pectins and xyloglucans are strongly ethylene-dependent, although specific genes of each family of the cell-wall degrading enzymes have been categorized as ethylene-dependent, totally ethylene-independent or partially ethylene-dependent.

By other hand, chilling injury (CI) caused by storage of many tropical and subtropical fruit at low but non-chilling temperatures has been shown to be ethylene-dependent in melon, avocado, pineapples and 'Shamouti' oranges, while in 'Fortuna' mandarins ethylene treatments enhanced resistance to CI, showing that the role of ethylene in CI may vary from one fruit to another (Pech et al., 2008). However, chlorophyll degradation and synthesis of pigments such as anthocyanins and carotenoids are also ethylene-dependent processes, even in non-climacteric fruits as grape berries, pepper and citrus fruits. These results support the hypothesis that although ethylene synthesis does not increase during the ripening of these fruits, alteration in ethylene responsiveness might be able to mediate physiological changes associated with ripening in them (Adams-Phillips et al., 2004; Barry and Giovannoni, 2007).

Clearly, ethylene is required for the normal ripening of many fruits and in its absence the ripening process fails to process, rendering the product unacceptable. However, once initiated, ripening is a one-way process and ethylene leads to stimulate over-ripening and decay, leading to rapid loss of fruit quality. Then, in post-harvest storage conditions it is important to control ethylene effects, not only in fruits but also in vegetables and ornamental crops in order to maintain their quality and enlarger storage possibilities.

In general, both climacteric and non-climacteric commodities have higher respiration rates in the early stages of development that steadily decline during maturation, but in climacteric fruits a rise occurs that coincides with ripening or

senescence (Yang and Hoffman, 1984). The respiration rate is usually very high during the early stages of fruit development and decreases as plant organs mature on tree, as can be observed in plums (Zuzunaga et al., 2001), and tomato (Serrano et al., 2008b), for which respiration rate peaked at pink stage of ripening and deceased in pink, light red and red fruits. The respiration rate of citrus fruits continuously declines during fruit ontogenity and remains nearly constant during natural maturation or in detached mature fruits (Rodrigo and Zacarías, 2006). In addition, the respiratory intensity of sweet cherry depended on the cultivar and agroclimatic conditions during the development of the fruit, in which a relationship has been established between respiratory intensity and the date of harvesting, with late harvested cultivars having reduced respiration rate than early fruits (Jaime et al., 2001).

It is well known that respiration rate of fruits strongly determines their transit and postharvest life, since at the senescent stage of climacteric fruit development there is a rise in respiration, presumably in order to obtain more energy for metabolic processes. Then, this situation must be avoided when the produce is being submitted to prolonged storage.

2. CHANGES IN FRUIT QUALITY DURING POSTHARVEST STORAGE

The agricultural products can be classified as: (a) durables, comprising cereal grains, oilseeds, and grain legumes, and (b) perishables, comprising succulent storage organs such as fleshy fruits, vegetables, rhizomes, and tubers. The harvested commodities are essentially plant organs with physiological functions quite different from tissues of the mother plant. In freshly fruits, as the ripening-senescence process sets in, the produce quality deterioration and the susceptibility of the stored products to decay caused by microbial spoilage and pathogens increases progressively. In this sense, the required fruit quality will depend on the final use and it is well accepted that the postharvest quality is determined by pre-harvest factors and an appropriate handling, processing, packaging and retailing. In today's modern society, fruits are exposed to a wide range of events commencing with the separation from the mother plant and finishing up on plates, all of them affecting the produce freshness and quality.

2.1. Concept of Quality

There is an increasing consciousness of quality, particularly in the fruit and health sector, which strongly demands research activities regarding the production of defined quality, the preservation of quality during marketing, as well as the possibilities to evaluate quality parameters and to integrate this into production processes. During the postharvest chain (from harvesting to retailing) the concept of fruit quality is frequently used, but its significance is different depending on the level at which is used: growers, producers, handlers, packers, distributors, retailers, markets, and finally and the most important being consumers. However, for all of them the term of quality is related to the degree of excellence and absence of defects of a fresh produce, which implies either sensory attributes (appearance, colour, texture, flavour, and aroma), nutritive (chemical components used to obtain energy) and functional properties (vitamins and other non-nutrients phytochemicals). Shewfelt (1999) suggested that the own produce characteristics determine the quality, but the consumer's acceptability is determined by its perception and satisfaction. Thus, quality can be oriented to the product or to the consumer's point of view. Product-oriented quality is generally measured with the aid of analytical determinations, while consumer-oriented quality is based on acceptability or its willingness to purchase the fresh produce. Products offered to consumers in the market should fulfill certain external requirements that are perceived by the senses of sight and touch because they are important for the purchase decision. In this sense, dietary guidelines recommending the consumption of fresh fruits (5-a-Day Campaign) will not be succeeded if consumers show certain dissatisfaction with the product quality, which limits the fruit consumption due to the development of undesirable characteristics.

For the fresh produce market, specific minimum quality standards exist in many countries; however, owing to the international nature of the fresh produce market, there is a trend towards international standardization of quality grades. The European Commission was one of the first organizations to develop international standards for fresh fruits (MAFF, 1996). Many of these standards have been adopted by the Organization for Economic Cooperation and Development (OECD). Usually, standards required for multiple retail outlets are considerably more stringent than these minimum standards, and will be defined for the supplier by the retailer. From the point of view of fruit quality, the factors which limit storage and shelf-life fall into the following categories: weight loss, appearance, texture, flavour/aroma and decay. In addition, occurrence of mechanical damage along the handling process will accelerate the changes in the above factors with a faster reduction in fruit quality.

With regard to overall quality, it is much clearer that quality of fresh fruit or vegetable changes from harvest to consumption. These changes are due to physiological or technological processes related to fruit ripening and ending with senescence, and thus determining the shelf life of a particular fruit. The increase in the storage life of fruits is carried out through the development of new postharvest technologies aimed to reduce the rate of deterioration and to maintain the desirable characteristics of the fruits leading to a great expansion of the opportunities for the industry to supply high quality fruits to local and export markets. However, the developing of most effective handling procedures and innovative postharvest technologies to assure the quality without compromising the safety and nutritional value of fruits depends on a better understanding of fruit biology and physiology. In this sense, it is necessary to get a better knowledge about the main changes occurring during the normal postharvest life of fruits which lead to quality deterioration: weight loss, appearance, softening, flavour and aroma, and decay occurrence. All these quality traits will be enhanced if mechanical damage is occurring along the commercialization process (from harvest to consumers).

2.2. Weight Loss

Pre-harvest conditions largely affect fruit quality, its chemical composition, texture and postharvest moisture loss (Gómez-Galindo et al., 2004). At time of harvest, the water status of the produce is usually high but after harvesting there are two factors that lead to fruit weight loss: a) water can no longer be taken up from soil due to the interruption of the plant's natural life cycle, and b) water transpiration, which is a physical process by which water vapour can permeate the stomas and epidermis. Water is also lost through lenticels, which are gaps in the periderm formed to enable gas exchange for respiration. If the epidermis or periderm is damaged, water loss can be massively exacerbated. The rate of postharvest water loss is dependent primarily on the external

vapour pressure deficit, although other factors will influence the situation. Products with a large surface to volume ratio, such as leaf crops, will lose greater percentages of their water far quicker than large spherical fruits. The specific structure of the cuticle and the extent of suberisation in the periderm appear to be more important than thickness in improving resistance to the movement of water vapour. Produce varies in the percentage of water which can be lost before quality is markedly reduced (Acked, 2002). Fruits with thick peels can lose a considerable amount of moisture from the skin without compromising edible quality, for example citrus species and bananas. Fruit species is a key factor that determines the rate of weight loss. Thus, for example, lemon, pepino and tomato had significantly lower percentages of weight loss (below 5%) than stone fruits (apricot, peach, nectarine, and plum). In addition, there are also differences among cultivar, since between the 'Black Diamond' (BD) and 'Golden Japan' (GJ) plums, the latter experienced much lower losses of weight (Ben-Yehoshua, 1987; Valero and Serrano, 2010). When fruits exhibit considerable weight loss, the quality of the product is then regarded as poor due to loss of turgidity, and consumers do not accept a fruit which is soft, dull and wrinkled. During postharvest, turgidity is a necessary condition for the fresh appearance of the fruit since affects negatively to texture properties and colour attributes, and thus temperature and relative humidity are among the main factors affecting the water status of the fresh fruits.

2.3. Colour

Appearance is the vital factor for consumers in deciding the purchase of fresh produce. Essential components of visual quality include colour and colour uniformity, glossiness, and absence of defects in shape or peel and freedom from diseases. From these components, colour contributes more to the assessment of quality than any other single appearance factor. Colour mainly defines the aesthetic value of food, predetermines consumers' expectation of flavour and taste and modulates appetite. Therefore, the manufacturer will try best to retain the natural appearance of the raw material. However, during storage of fruits colour may be altered through the action of light, temperature, oxygen, metal ions and endogenous enzymes (Stintzing & Carle, 2004). In addition, the colour of fruits and vegetables will vary during seasons depending on their intra- and infraspecific variabilities, the edaphic factors at the site of cultivation and postharvest treatments.

To investigate colour quality in a systematic way it is necessary to objectively measure colour, as well as pigment concentration. In this context, colour denotes the visual appearance of the product whereas pigments or colorants are the chemical compounds that impart the observed colour. The CIEL*a*b* system (International Commission on Illumination, Vienna) has been adopted by the food industry for measuring

colour of food products. While this system does not necessarily give an accurate definition of colour, it is very effective for measuring colour differences and tracking colour changes during storage (Wrolstad et al., 2005).

Most fruits experience colour changes as part of the ripening process. Unripe fruit is usually green (the so-called 'ground colour') and in many types of fruit, the green colour becomes lighter during ripening and maturation owing to breakdown of chlorophyll, for example in apples, pears, grapes and papaya. These colour changes affects both peel and pulp tissues, and in many cases the colour of the fruit is a strong indicator of the eating quality and shelf life, for example, tomatoes, plums, sweet cherries and bananas. The increase in a* parameter reflects the change from greenish to orange-red while the decrease in Chroma is related to peel darkening. Accordingly, four sweet cherry cultivars picked at two ripening stages (partially-ripe and ripe) showed reductions in colour parameters after 6 days of storage at room temperature (Hue angle from ≈40 to 16, on average) which were correlated with an increase in anthocyanin levels (Gonçalves et al., 2007).

2.4. Firmness

Fruit texture is not a term easily to define since it is composed of a wide range of attributes including type of tissue, water content, and cell wall composition, among others. Bourne (1980) defined: "The textural properties of a food are that group of physical characteristics that are sensed by the feeling of touch, are related to the deformation, disintegration and flow of the food under the application of a force, and are measured objectively by the functions of force, time and distance". It is generally accepted that changes in texture occur normally during growth and development on tree but go on during postharvest storage of fruits, with changes in texture being due to changes in the chemistry of the middle lamella and primary cell wall components: pectins, cellulose, and hemicelluloses that accelerate fruit softening as has been commented in **Section 1**.

The breakdown of the cell wall and dissolution of the middle lamella that accompanies fruit ripening is at least partially caused by the degradation of pectic polysaccharides by enzymes capable of altering cell wall texture such as PME, endo- and exo-PG, α- and ß-GAL, cellulase, α-L-arabinosidase, ß-glucosidase, ß-xylosidase, α-L-fucosidase, α-L-rhamnosidase, arabinoxylanase, feruloyl esterase and XET, among others (Brownleader et al., 1999; Brummell, 2006; Goulao and& Oliveira, 2008). However, differences exist in the type and extent of the modification of the polysaccharides of the cell wall and in the expression and regulation of cell wall-modifying enzymes depending on the fruit type and even among cultivars of the same fruit species. Thus, for example, after 7 days of storage at 20°C, lemon and pepino showed significantly lower percentages

of firmness loss (26-40%) than tomato (55%) or stone fruits (apricot, peach, nectarine, and plum), with 70-90% of loses in fruit firmness. In this sense, fruit species is an important factor determining the rate of softening. Interestingly, different cultivars from the same species could also show a different behaviour, since 'Black Diamond' plum exhibited much higher firmness loss (85%) than 'Golden Japan' plums (28%) (Valero and Serrano, 2010).

Excessive fruit softening is one of the main factors responsible for the limitations of shelf life, storage and marketability, and thus a poor relationship between firmness at harvest and after storage occurs in those fruits with accelerated softening pattern (stone fruits), while in those commodities with moderate softening rate there is a positive correlation (Goulao and Oliveira, 2008).

The accelerated softening also contributes to the increased occurrence of physical damages during handling, and for higher susceptibility to pests and diseases. In fact, it has been reported very recently that the cell wall disassembly during tomato ripening is mediated cooperatively by PGs and expansins (EXPs), and then the tomato susceptibility to the necrotropic pathogen *Botrytis cinerea* increased, while the simultaneous suppression of these degrading enzymes in transgenic fruits led to a dramatic reduction in the susceptibility to this fungus (Cantu et al., 2009). Interestingly, the suppression of just one enzyme (either PG or EXP) did not reduce the decay occurrence.

2.5. Flavour, Taste and Aroma

In addition to their external appearance and texture changes in fruits, aroma and flavour are becoming key factors that determine the choice or not to purchase a fruit. Apart from sugars and organic acids, aroma volatiles contribute also to fruit flavour. When a fruit is consumed, the interaction of taste, odour and textural feeling provides an overall sensation which is best defined by "flavour". Flavour results from compounds that are divided into categories: those responsible for taste and those contributing to odours, the latter often designated as aroma substances. However, there are compounds which provide both sensations. It is generally accepted that "flavour life" of the product has either not been reached or has been exceeded before consumption, the main consequence being a dissatisfaction and lower demand for a particular fruit by consumers but also commercial consequences.

The compounds responsible for taste are generally nonvolatile at room temperature. Therefore, they interact only with taste receptors located in the taste buds of the tongue. The four important basic taste perceptions are provided by: sour, sweet, bitter and salty compounds. Taste is a fruit attribute considered as a quality indicator too and cannot be separated from other characteristics of the produce. In most cases, the taste of fresh fruits is usually disregarded, since it is an internal attribute that cannot be determined by non-destructive taste measurement, as well as the large variation among

the fruits harvested at the same time. It is important to point out that taste is being used currently to differentiate one cultivar from other, with the economical repercussion. Most growers and marketers use the °Brix determination to measure the TSS, which primarily estimates the sugars content in a particular fruit and thus it provides the degree of sweetness. In this sense, consumers are quite familiar with the attribute sweetness as a preferable attribute. However, in recent years the perception of taste by consumers is not only related to the content of sugars, and TA is becoming an important factor. For that reason, the ratio between TSS and TA (TSS/TA) is being used as a criterion for ripening index and the degree of a fruit acceptance (Crisosto et al., 2002; 2003).

During postharvest there is a general increase in the content of soluble solids, as has been reported for nectarines, apricots, kiwifruits and strawberries (Aubert et al., 2003, Aubert & Chanforan, 2007; Park et al., 2006; Hernández-Muñoz et al., 2006). This increase in soluble solids is much higher in those fruits that accumulate larger amounts of starch during development on plant, such as mango or bananas. However, different behaviour has been reported for tomato cultivars with either maintenance, increases and even decreases (Kaur et al., 2006; Serrano et al., 2008b).

It is clearer the behaviour of fruit acidity during postharvest ripening, since a net decrease in TA has been reported during postharvest storage in kiwifruit, strawberry, tomato, sweet cherry, plum, nectarine and several apricot cultivars, although the magnitude of the diminution is greatly dependent on the fruit species. (Aubert et al., 2003, Aubert and Chanforan, 2007; Park et al., 2006; Hernández-Muñoz et al., 2006; Valero and Serrano, 2010). The reduction of the acidity associated to postharvest ripening has been attributed to the fact that organic acids are substrates for the respiratory metabolism in detached products.

Aroma substances are volatile compounds which are perceived by the odour receptor sites of the olfactory system at the nasal cavity. The concept of aroma substances, like the concept of taste substances, should be used loosely, since a compound might contribute to the typical odour or taste of one food, while in another food it might cause a faulty odour or taste, or both, resulting in an off-flavour. In this sense, one of the major problems in postharvest storage and handling of fruits is the development of off-flavours and loss of authenticity. The appearance and sensation of off-flavours are highly associated with over-maturation and the accumulation of the ethanol fermentation products acetaldehyde and ethanol, which are also volatiles. In general, the higher the storage temperature, the greater is the production of acetaldehyde and ethanol.

In the case of tomato, hexanal, *(E)*-2-heptenal, *(E,E)*-2,4-decadienal, 6-methyl-5-hepten-2-one, geranylacetone, 2-isobutylthiazole, 1-nitro-2-phenylethane and geranial increased during postharvest storage at 20 °C and only methyl salicylate decreased

(Krumbein et al., 2004). These authors proved that the intensity of the attribute "tomato-like" aroma increased during storage but also the undesirable attribute "mouldy", and suggested that 2-isobutylthiazole could responsible for the "off-flavour" occurrence detected in stored tomatoes, which would affect to the sensory quality, and on the contrary, the increase in gernaylacetone would contribute to the "tomato-like" flavour.

Similarly, the storage at 20 °C of 28 apricot cultivars induced a general increase in volatile compounds (1.4-8 fold), the most discriminating compounds being lactones (γ-decalactone, γ-nonalactone, γ-octalactone, γ-hexalactone, and γ-jasmolactone), which increased 1-45 fold and were responsible for the fruity aroma of apricot (Aubert & Chanforan, 2007). In the case of nectarines, the level of volatiles could be equal to or higher in the postharvest ripened nectarines compared with the tree-ripening fruits. Nevertheless, although the volatile level, in particular lactones and C13-norisoprenoids were notably higher in samples ripened at 20 °C than in those attached to the tree, no differences by sensory evaluation were detected among them with respect to the intensities of "peach odour" or "peach aroma" (Aubert et al., 2003). In sweet cherry, relevant changes in aroma constituents associated with long storage time have been reported, including a decrease in *(E)*-2-hexenal and 1-hexanol and an increase of 2-methyl-propanal, and 2-methyl-butanal, with a negative impact on flavour (Serradilla et al., 2010).

2.6. Decay

As stated above, fruit and vegetables contain a wide range of organic acids and high water activity, and then they are good substrates for microbial spoilage. However, the low pH of fruits leads to their spoilage being predominantly by fungi, while vegetables, in contrast, have pH values closer to neutrality and thus both fungi and bacteria cause spoilage (Moss, 2008). In general, mould spoilage of fruits does not lead to health hazard, since the commodities are usually rejected, but the economical loses are considerably important due to decay occurrence.

Fungal pathogens exploit through main routes to penetrate the host tissue: 1) through wounds caused by biotic and/or abiotic agents during growth or storage, 2) through natural openings such as lenticels, stem-ends and the pedicel-fruit interfaces, and 3) by directly breaching the host cuticle. The incidence of fruit decay during postharvest is the result of pre-harvest latent infection or contamination at harvest which is manifested during storage, transport or marketing of the fleshy fruits leading to a net reduction of the postharvest shelf life. An active pathogen can start its attack process immediately after spores land on the host tissue or can remain inactive for months until the harvested fruits ripen, this period being designated as "quiescent stage" (Prusky & Lichter, 2008). Thus, the agricultural industries aimed to offer fresh produce that are with

high quality standards and less prone to postharvest decay. There are a wide range of factors that influence the occurrence of decay and its severity that can be grouped at three levels: at pre-harvest (type and amount of inoculums, cultivar, climatic and environmental conditions and ripening stage at harvest), at harvest (manual or mechanical methods) and at postharvest (handling procedures, storage conditions and postharvest treatments). It is estimated that in the developed countries, about 25% of all perishable fruits harvested are lost between harvest and consumption, while this percentage increases up to 50-70% in underdeveloped countries. Then, appropriate postharvest management is of special importance to provide commodities with high quality standards and reduced incidence of decay.

The germination of the spores and mycelium growth is strictly dependent on the temperature, which is considered the limiting factor for the development of the disease. On a general basis, the optimal temperature for growth of most storage fungi is 20-25°C, though some species prefer higher or, more rarely, lower temperatures. However, the optimum for growth is not necessarily identical to the optimum for germination. The deviations of the optimal temperature will prolong the required time for initiation of the germination and the mycelium growth and the duration of the incubation period of the disease, that is the time until the appearance of decay symptoms (Barkai-Golan, 2001). The high RH required for the protection of fruits from dehydration and weight loss can stimulate the pathogen development during storage since the severity of decay is enhanced by the condensation of mist over the fresh fruit or vegetable surface. In this sense, the susceptibility of many fruits to fungal decay is enhanced when the pathogen encounter tissues with elevated levels of turgidity when, as occurs under high RH. In many cases, the increased decay rate should be attributed to moisture held within the wounds, lenticels or stomata under these conditions. Fungal spores use this moisture for germination previously to their penetration into the tissues. This is the main reason by some fruit commodities, such as grapes and strawberries cannot be washed during the handling process, since the excess of humidity will favour the decay occurrence.

On the other hand, the ripening stage at harvest influences the fruit susceptibility to fungal decay since TA, nutrient availability and tissue turgidity change during the ripening process, and especially when senescence is initiated, these changes being related to the enhancement of fungal decay. **Table 5** summarizes the main fungi responsible for diseases in specific fruits and can be observed that stone fruits (peach, nectarine, plum, sweet cherry and apricot), pome fruits (pear and apple), tomato and pepper are the main hosts for fungal development and incidence of decay (Valero and Serrano, 2010).

Table 5: The main fungal pathogens of the fruits and the correspondent diseases

Fungal species	Fruit target	Diseases
Alternaria citri	Citrus fruits	Stem-End Rot
Alternaria alternata	Tomato, Apple, Grapes, Pepper, Melon, Plum, Peach, Nectarine, Apricot, Pear, Sweet Cherry,	Fruit Rot, Dark Spot Sooty Mould
	Avocado, Mango, Papaya, Persimmon	Stem-End Rot, Black Rot
Penicillium expansum	Apple, Pear	Blue Mould
Penicillium digitatum	Citrus Fruits	Green Mould
Penicillium italicum	Citrus Fruits	Green Mould
Aspergillus niger	Tomato, Grape, Date, Melon,	Black Rot, Brown Rot
Fusariumm verticilloides	Banana, Pineapple	Black Heart
Botrytis cinerea	Strawberry, Raspberry, Grape, Persimmon, Tomato, Pepper, Melon, Stone Fruits, Pome Fruits	Gray Mould
Cladosporium herbarum	Date, Grape, Pome and Stone Fruits, Papaya, Fig, Tomato, Pepper, Melon	Olive-Green Mould Sooty Mould
Colletotrichum gloesporoides	Avocado, Mango, Papaya, Guava, Citrus, Pome and Stone Fruits	Anthracnose
Monilinia fructicola	Stone Fruits	Brown Rot
Monilinia fructigena	Pome Fruits	Brown Rot
Mucor piriformis	Tomato, Strawberry, Raspberry, Melon	Watery Soft Rot
Rhizopus stolonifer	Strawberry, Raspberry, Sweet Cherry, Grape, Avocado, Papaya, Tomato, Pepper, Melon	Watery Soft Rot

3. POSTHARVEST TREATMENTS TO MAINTAIN FRUIT QUALITY

Fruits and vegetables are highly perishable, the rate of deterioration being dependent on the respiration rate, which varies primarily with the type of produce, the temperature of storage and the level of physiological stress caused by harvesting or postharvest processing. Thus, it is necessary store the fruit under cold conditions and cool it as quickly as possible after harvest in order to arrest the deteriorative and senescence processes and to maintain a high level of quality that ensures customer satisfaction. In fact, temperature control is the single most important factor to maintain quality and reduce the deterioration rate of harvested commodities, since it is widely accepted that the rate of deterioration after harvest is closely related to the respiration rate of the harvested product and this is dependent on temperature (Kader, 2002).

3.1. Low Temperature Storage

The main effect of the low temperature application during postharvest storage is a reduction of the fruit metabolism and consequently a delay of the evolution of the parameters related to fruit ripening and quality loss. Respiration rate of a produce is dependent on a wide range of variables, from which the temperature is considered as the most important in modulation this physiological parameter. As can be seen in **Figure 7**, the respiration rate of some fruits increased as the assay temperature was enhanced, although differences exist among fruit types. Thus, at 30°C oranges showed a respiration rate of ≈ 40 mg kg^{-1} h^{-1} while in banana was ≈ 170 mg kg^{-1} h^{-1}. Moreover, differences were also found in the temperature at which the maximum respiration rate was achieved, this temperature being 50°C for apple and orange, 45°C for lemon and 40°C for banana. The diminution of the temperature led to a reduction in the respiration rate for all fruits, and taking into account the concept of Q_{10}, that is the reduction of the reactions between two temperatures that differ in 10°C, the calculated Q_{10} was 1.63 for apple and 1.9 for the remained fruits. Interestingly, at the lowest temperature (1°C) there were still differences among fruits and followed the same sequence, with banana having the highest and orange the lowest respiration rate (≈ 40 and 5 mg kg^{-1} h^{-1}, respectively).

The reduction in storage temperature has the added advantage of reducing the production and sensitivity of ethylene, with special interest in the climacteric fruits since this hormone accelerates the ripening process and senescence. In fact, the maximum ethylene production in these climacteric fruits (banana and apple) was obtained at 40°C, and the reduction of the temperature significantly decreased the ethylene production (**Figure 7**), although differences existed between two fruits for whichever temperature.

Thus, at 40°C banana produced the highest rate (≈ 230 nL g^{-1} h^{-1}) compared with the apple (≈ 9 nL g^{-1} h^{-1}).

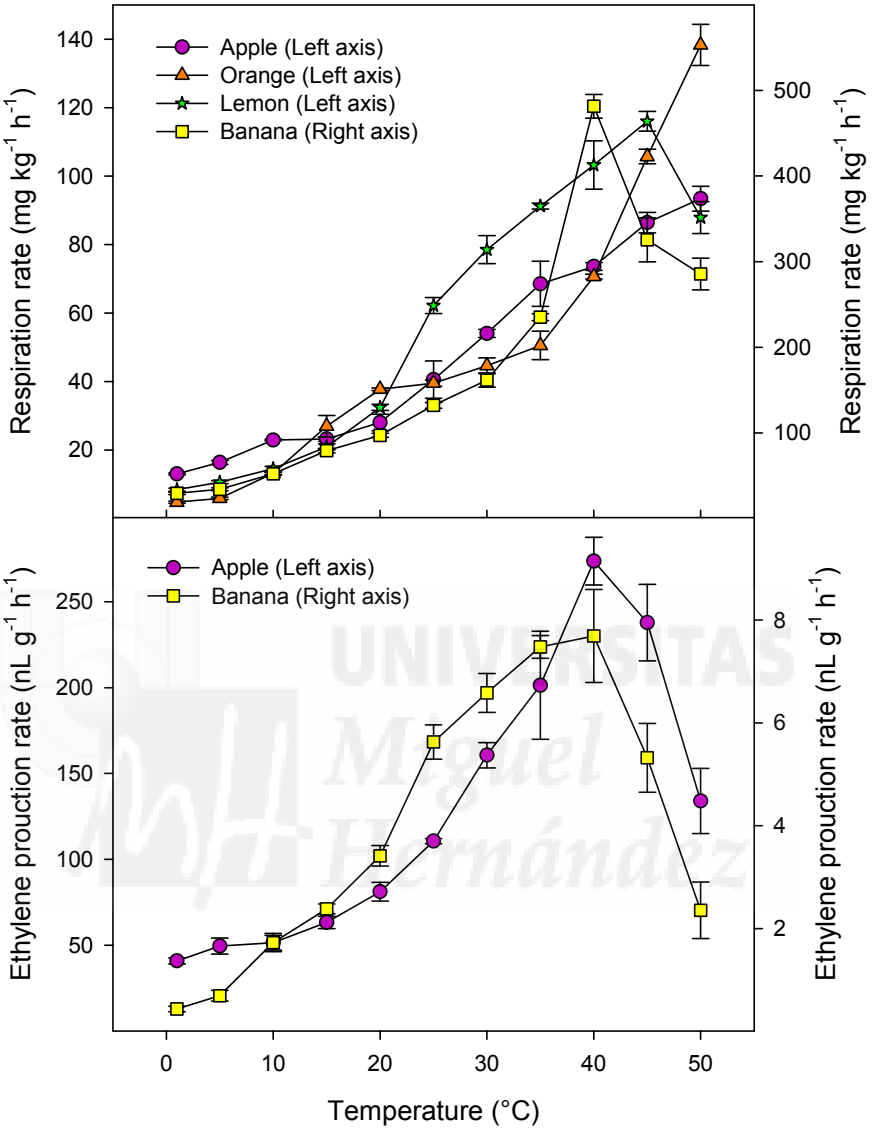


Figure 7: Respiration and ethylene production rates in some fruits as affected by temperature. Data are the mean ± SE of determinations made in five fruits (Valero and Serrano, 2010).

These effects in slowing down the rates of respiration and ethylene production by low temperature affect to the parameters related to fruit ripening and quality, such as colour, acidity or texture. According to these evidences it is clear that vegetable products need to be stored at low temperatures as soon as possible after harvesting in order to reduce the metabolic activity of fruit tissues and the evolution of the postharvest ripening process leading to maintenance of fruit quality (Valero and Serrano, 2010).

3.2. Application of Heat

The application of heat treatments to fruit commodities and its effect on physiological, biochemical, nutritive and quality parameters has been widely studied in recent years. Heat treatments can also be used to inhibit ripening processes or to induce resistance to chilling injury (CI) and external skin damage during storage, thus extending storability and marketing (Lurie, 1998; Paull and Chen, 2000).

There is no a general rule for the combination of heat treatment/exposure time to gain a specific effect on a particular fruit type. Thus, temperature in the range of 39-53°C and duration 10 min-12 hours is needed to achieve an effective decay control in tomato, orange, mango and pear, and to reduce mechanical damage before handling in plum, while higher temperatures act as quarantine insect method (46-58°C). In addition, is it interesting to point out that in very recent years, the hot treatments have shown a positive effect on increasing nutritive and functional properties of some fruits such as pomegranate, tomato, mango and kumquat (Valero and Serrano, 2010).

On a general basis, the effect of heat treatments on fruit ripening is a delay of some parameters related to fruit physiology (ethylene production and respiration rate) and quality deterioration (softening, colour evolution, increase in soluble sugars, and reduction in acidity), the overall quality of fresh produce exposed to an appropriate temperature and time being significantly better than in non-heated commodities. However, sometimes there is a more advanced evolution in some ripening characteristics, since heat acts as a stress-environmental condition inducing tissue injury, with negative effects on quality (Paull and Chen, 2000). In general, mild heat treatments (38-55°C) are preferred to reduce the ripening process and/or maintain fruit organoleptic quality, although the duration is wide variable, from seconds (for apple) to several minutes (4-60 min), for pomegranate, tomato, sweet cherry, pitaya and mango) and even to 12 hours for peach (Valero et al., 2002b; Serrano et al., 2004a; Valero and Serrano, 2010).

In climacteric fruits, the inhibition of ripening by heat may be mediated by its effect on the ripening hormone ethylene and the enzymes responsible for its biosynthesis ACS and ACO (Lurie 1998; Serrano et al., 2004c). The mechanism by which heat delays and/or inhibits ethylene production is variable. Thus, temperatures in the range of 35-38°C tend to accumulate ACC and to decrease ethylene production, due to inhibition of ACC oxidase activity. However, higher temperatures induce lower concentration of ACC, thorough inhibition of ACS activity. This means that ACS is less sensitive to heat treatments than ACO. In addition, the differences in response to heat between ACS and ACO may be related to difference in their turnover rates (Paull and Chen, 2000). Any way, it is clear that the effect of heat temperature on ethylene biosynthesis is reversible, since fruits exposed to high temperature during long period can recover their ability to ethylene synthesis when are removed from heat, and even the rates of ethylene are higher in

heated than in non-heated produce (Lurie, 1998). Thus, in a wide range of fruits (papaya, apple, melon, and mango) a full recovery of ACO activity occurred within 3 days after removal from heat, for which new protein synthesis was required as well as reactivation of previously synthesized mRNA, which were attenuated during the heating process.

However, there are some exceptions to these general effects of heat treatments, such as the enhancement of colour found in tomato (McDonald et al., 1999), the increase in total acidity in pomegranate (Mirdehghan et al., 2007a; 2007b), and the absence of effect on fruit firmness recorded in tomato, sweet cherry and peach (McDonald et al,. 1999; Drake et al., 2005; Jin et al., 2009).

3.3. Calcium

Calcium is an essential plant nutrient, since the divalent Ca^{2+} is required for structural roles in the cell wall and membranes, as a counter-cation for inorganic and organic anions in the vacuole and as an intracellular messenger in the cytosol. There are two distinct areas in the cell wall with high Ca^{2+} concentrations, the middle lamella and the extension surface of the plasma membrane. In both sites, Ca^{2+} has essential structural functions, namely, the regulation of the membrane permeability and related processes and the strengthening of the cell walls.

Calcium treatments represent a safe and potentially effective method for increasing the quality and storage life of a wide range of fruit species, since they reduce postharvest spoilage, softening, ethylene production and senescence rate. Calcium applications may be performed by pre-harvest treatments in the irrigation system or by spraying the tree canopy with calcium solutions, although they are more effective when calcium is applied directly to the fruit surface, as little or no subsequent translocation of calcium occurs from leaf to fruit (Kadir, 2004). By other hand, calcium may be applied as post-harvest treatments, by dipping fruits in solutions of calcium salts o by vacuum infiltration. However, some damage can occur if calcium is applied at high concentration (Valero et al., 1998c; Ferguson and Boyd, 2001; Serrano et al., 2004c; Bakshi et al., 2005).

Most of the reports on calcium treatments have been focused on their effect on fruit firmness. Calcium, as a constituent of the cell wall, plays an important role in forming cross-bridges among pectic substances, leading to stabilization of the plant cell wall and protection from the cell wall degrading enzymes, specifically from the pectolitic enzymes (White and Broadly, 2003; Serrano et al., 2004c). The most important, PG breaks the glycosidic links between units of non-esterificated galacturonic acids. If calcium interacts with these carboxylic groups without esterification reduces their number and then, the PG action decreases. In fact, calcium content in the nutrient solutions affected positively whole fruit firmness of cantaloupe and honeydew melons (Serrano et al., 2002; Lester and Grusak, 2004; Madrid et al., 2004). The softening process started two days

early on melon treated with low calcium concentration and coincided with increases in ß-GAL and PG activities, while in melon irrigated with high calcium concentration the softening process was lower and delayed on time and no PG activity was detected (Serrano et al., 2002). However, in other experiments performed in California, calcium fertirrigation had no effect on firmness losses during honeydew or muskmelon postharvest storage and was not correlated with calcium concentration in fruit tissue (Johnstone et al., 2008). Thus, these contradictory results could be attributed to different soil characteristics or to other cultural or environmental factors.

Postharvest calcium treatments have been also effective in retaining firmness during storage in a wide range of fruits, such as apple (Conway et al., 1994; Chardonnet et al., 2003), mango (Suntharalingam, 1996), kiwifruit (Hopkirk et al., 1990), peach (Manganaris et al., 2007), plum (Valero et al., 2002b), blueberry (Hanson et al., 1993), strawberry (García et al., 1996; Lara et al., 2004), nectarine (Manganaris et al., 2005), lemon (Valero et al., 1998c; Martínez-Romero et al., 1999; Safizadeh et al., 2007), tomato slices (Artés et al., 1999; Pinheiro and Almeida, 2008), cantaloupe and honeydew melon cylinders (Luna-Guzman et al., 1999; Saftner et al., 2003) and zucchini slices (Izumi & Watada, 1995), among others. In other experiments, calcium lactate has been more effective on increasing texture and crispness of fruits and vegetables than calcium chloride (Martín-Diana et al., 2007). In addition, calcium treatment makes the fruit less susceptible to mechanical damage during processing, handling and packaging, as has been shown in lemon (Martínez-Romero et al., 1999) and plum fruits (Serrano et al., 2004a). Accordingly, in papaya dipping or vacuum calcium treatments increased storage life and diminished the softening process, the effect being greater as increased calcium chloride concentration up to 2.5% and with the vacuum treatment (Mahmud et al., 2008).

In addition, the increase in TSS was delayed by postharvest infiltration calcium treatments on papaya, due to slower changes from carbohydrates to sugars, as well as the decrease in TA in papaya and strawberry, by reducing the enzymatic reactions of respiration (Lara et al., 2004; Mahmud et al., 2008), showing a delay on the ripening process in these treated fruits.

The relationship between calcium treatments and aroma volatile production is of interest, although only a few works on this subject have been published. For example, pre-storage apple treatments with $CaCl_2$ caused enhanced emission of some impact compounds and improved the aroma quality after middle-term storage. In addition, sensory analysis by means of a consumer panel indicated higher acceptance scores for calcium-treated fruits (Ortiz et al., 2009). After middle-term storage the effect of calcium treatment on increasing production of volatiles probably arises from enhanced supply of precursors for ester production as a consequence of increased pyruvate decarboxylase and alcohol dehydrogenase activities, while after long-term storage, the enhancement of

alcohol-acyltransferase activity might also contribute. Thus, postharvest calcium treatments have the potential to improve aroma quality of cold stored apple.

Other beneficial effects of calcium infiltration on postharvest quality of fruit are probably mediated through the stabilizing influence of Ca^{2+} on cell membranes leading to a delay in membrane protein and phospholipid catabolic processes and to a reduction of ion leakage during postharvest storage of fruit (Picchioni et al., 1998). These effects of calcium ions on increasing membrane integrity have as consequence the maintenance or enhancement of cell turgor pressure which contributes to delay fruit softening and weight loss during postharvest storage as have been shown in papaya fruits (Eryani-Raqeeb et al., 2009), which could be also attributed to the effect of calcium on decreasing water vapour diffusivity through the cell wall structure.

In addition, a role of calcium on maintaining membrane stability under stress conditions, such as low temperature storage, has been also addressed. This calcium effect could explain the fact that pre- and postharvest calcium treatments have a positive effect on reducing CI as has been observed in mandarins (D'Aquino et al., 2005), lemons (Safizadeh et al., 2007) and peaches (Manganaris et al., 2007). In addition, an effect of calcium on reducing browning, which occurs as a result of oxidation of membrane phospholipids and polymerization of polyphenols, has been observed, with especial interest in fresh-cut fruits such as apples, pears and melons (Martín-Diana et al., 2007; Alandes et al., 2009).

Calcium treatment is also effective in controlling postharvest decay caused by *Penicillum expansum*, *Botrytis cinerea*, *Glomerella cingulata* and *Gloeosporium* in apple and strawberry, as well as on papaya fruit, the effect being higher when calcium treatment was performed by vacuum infiltration compared to the dipping one (García et al., 1996; Lara et al., 2004; Mahmud et al., 2008). Additional reduction of decay has been reported by combined heat and calcium treatments in 'Golden Delicious'(Conway et al., 1994), and 'Gala' apples (Conway et al., 1999), in cactus pear (Schirra et al., 1997) and in fresh-cut melon (Aguayo et al., 2008), showing that these combined treatments may be a useful alternative to the chemical fungicides in controlling postharvest decay. Calcium chloride infiltration was also effective on reducing anthracnose (*Colletotrichum gloeosporioides*) disease incidence on papaya fruits, in a dose-dependent manner from 1.5 to 3.5%, this effect being increased by the combined treatment chitosan-calcium (Eryani-Raqeeb et al., 2009).

The mechanisms by which exogenous calcium reduces fruit decay and increases fruit firmness are closely related, and are attributed to the increased calcium bound to the cell wall, that is to an interaction between the cell wall pectins and Ca ions maintaining the structural integrity of the cell wall. Thus, calcium enhances tissue resistance to fungal attack by stabilizing or strengthening cell walls, thereby making them

more resistant to harmful enzymes produced by fungi and also delays softening of fruits (Conway et al., 1994; 1999; Lara et al., 2004; Eryani-Raqeeb et al., 2009). Although prolongation of storage life as a result of calcium application is thought to be due mainly to the role of calcium in ameliorating physiological disorders and thus indirectly reducing pathogen activity, direct effects of calcium on the pathogen have also been recognized (Barkai-Golan, 2001), such as interfering with spore germination and germ tube elongation of *P. expansum* and *B. cinerea*.

Calcium treatments in fruits have been related to longer postharvest life as a result of reduced rates of respiration and ethylene production. Thus, pre-harvest treatments with calcium solution have shown an effect on delaying ethylene production and ripening process on climacteric fruits, such as tomato (Wills et al., 1997), persimmon (Agustí et al., 2004), pears (Gerasopoulos & Richardson, 1999), peach and nectarines (Serrano et al., 2004b). In addition, apple tree treatments with calcium chloride by foliar sprays decreased ethylene production and respiration rate in apple fruits at harvest and after postharvest storage under controlled ultralow oxygen atmosphere conditions, both physiological parameters being correlated with fruit calcium content (Recasens et al., 2004). In addition, postharvest calcium treatment can inhibit ethylene production and fruit ripening of climacteric fruits, such as apple and plum, ethylene production being more reduced with increasing calcium concentrations. This effect has been attributed to a decrease in the activity of ACO (Serrano et al., 2004c).

Calcium treatment has also effect on reducing the ethylene production induced by mechanical damage. This ethylene production is so-called wound-inducible ethylene and it seems to be a general response of plant tissues to injury through an activation of ACS and ACO (Kato et al., 2000). However, in calcium infiltrated plums before the application of mechanical damage, the increase in wound ethylene was significantly lower. Similarly, respiration rate increase as a consequence of the mechanical damage is also reduced in calcium-treated plums. In addition, during prolonged storage, the climacteric peak of both, ethylene production and respiration rate occurred earlier and reached higher values in damaged control fruits than in calcium treated ones, showing a net effect of calcium treatment on protecting fruit tissues against mechanical damage (Serrano et al., 2004a).

3.4. Polyamines

Polyamines (PAs) are organic cations containing amino groups which are present in all eukaryotic cells (both animal and plant). In plant organs, PAs are positively implicated in plant growth and differentiation as well as in stress responses, the main PAs being putrescine (Put, 1,4-diaminobutane), spermidine (Spd, 1,8-diamino-4-azaoctane), and spermine (Spm, 1,12-diamino-4,9-diazadodecane).

In plants, the PA biosynthetic pathway is shown in **Figure 8**, in which connection with the ethylene biosynthesis is also provided (Kumar et al., 1997; Valero et al., 2002). Two pathways lead to Put formation from arginine. One involves the transformation from arginine to ornithine, which is catalysed by the enzyme arginase. Then, ornithine is transformed to Put catalysed by ornithine decarboxylase (ODC). The other route involves decarboxylation of arginine by arginine decarboxylase (ADC) to form agmatine and then agmatine is first converted by agmatine iminohydrolase to N-carbamoylputrescine which is finally converted to Put by N-carbamoylputrescine amidohydrolase. The existence of two alternative routes (ADC/ODC) for the synthesis of Put could be explained by the differential compartmentalization of the two enzymes (ADC is chloroplast-localized and ODC is generally considered to be cytoplasmic), resulting in the specific regulation of different plant processes. The enzymes ADC and ODC can be inhibited by the reversible inhibitors DL-α-difluoromethylarginine (DFMA) and DL- α -difluoromethylornithine (DFMO), respectively. Decarboxylate S-adenosylmethionine (DCSAM), which is formed from S-adenosylmethionine (SAM) by the enzyme SAM decarboxylase (SAM-DC), can be used as an aminopropyl donor in the conversion of Put to Spd and from Spd to Spm by two reactions catalysed by two separate and distinct enzymes, Spd synthase and Spm synthase, respectively. It has been demonstrated that Put and Spd levels inhibit ODC mRNA translation. Methylglyoxal-bis-guanylhydrazone (MGBG) and cyclohexylamine are irreversible inhibitors of SAM-DC and Spd synthase activities, respectively. SAM is an important metabolic crossroad in the regulation of nitrogen metabolism, since it is also a precursor of ethylene via ACC, in a reaction catalysed by ACC synthase and finally, ACC is converted to ethylene by ACC oxidase. Furthermore, in some plants, the methyl moiety of SAM can be transferred to Put via Put-N-methyl-transferase, to form N-methyl-Put, which serves as a precursor of nicotine and other alkaloids.

Ethylene and PAs (Spd and Spm) biosynthesis share the common precursor SAM and are known to exert opposite effects in fruit ripening and senescence, since reduced levels of PAs have been correlated with increased ethylene production, fruit ripening and senescence, while high endogenous concentrations of PAs are associated with a delay in these processes. Thus, a balance between these two opposite growth regulators is crucial to retard or accelerate ripening and senescence (Serrano et al., 1996; Pandey et al., 2000; Valero et al., 2002a; Valero and Serrano, 2010). In this sense, several experiments have shown that exogenous application of PAs during the growing season (pre-harvest) can decrease ethylene production and delay the ripening process in apricot (Paksasorn et al., 1995), peach (Bregoli et al., 2002), mango (Malik et al., 2003; Malik and Singh, 2006), nectarine (Torrigiani et al., 2004) and plum (Khan et al., 2008).

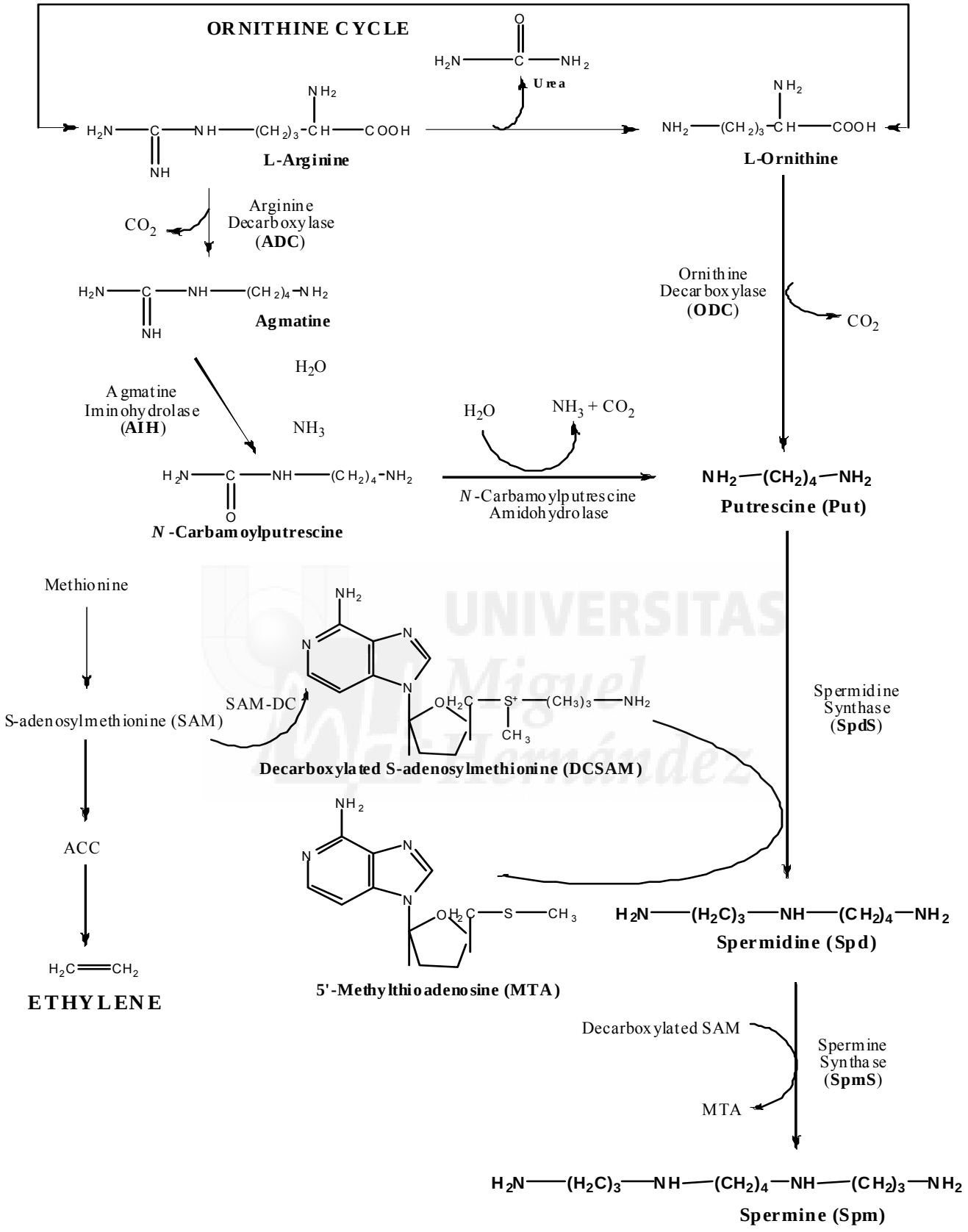


Figure 8: Polyamine biosynthetic pathway. Connection with ethylene biosynthesis (schematic) is also provided (Valero and Serrano, 2010).

Accordingly, postharvest application of PAs, by immersion or vacuum infiltration, has been reported to delay fruit ripening and extend shelf life in some fruits, including 'Golden Delicious' and McIntosh' apple (Kramer et al., 1991), 'Kesington Pride' mango (Malik and Singh, 2005), 'Babygold-6' peach (Martínez-Romero et al., 2000), 'Mauricio' apricot (Martínez-Romero et al., 2002), blueberry (Basiouny, 1996), 'Golden Japan', 'Black Diamond', 'Black Star', 'Santa Rosa' and 'Angelino' plums (Pérez-Vicente et al., 2002; Serrano et al., 2003; Khan et al., 2008) and 'Mollar de Elche' pomegranate (Mirdehghan et al., 2007a). These effects could be attributed to the fact that Put treatments led to inhibition of ethylene production as has been reported in several *Prunus* species, such as apricot, peach and four plums cultivars, which was inversely correlated with de maximum ethylene production at the climacteric peak, and was dependent on the fruit type. This inhibitory effect of exogenous PAs in ethylene production may be ascribed to both, the competitive biosynthesis mechanism between ethylene and PAs and to the inhibition of ACC synthase and ACC oxidase.

Accordingly, postharvest Put application markedly slowed softening during ripening at ambient temperature of 'Angelino' plum, this effect being higher as increased Put concentration from 0.1 to 2 mM (Khan et al., 2008). Put treatment at 10 mM was also effective on delay softening in blueberry, while no effect was observed with 1 mM Spd treatment (Basiouny, 1996). Several mechanisms have been postulated to explain the increased fruit firmness after Put treatment. One is supported by decreased activity of ethylene biosynthetic enzymes by PA, ACS and ACO, as well as cell wall-related enzymes, such as the inhibition of the action of endo- and exo-PG, EGases and PME involved in softening. Other mechanism would involve the PA capacity to cross-link pectic substances in the cell wall, producing rigidification (Valero et al., 1999; Martínez-Romero et al., 2002; Pérez-Vicente et al., 2002). This binding also blocks the access of such degrading enzymes reducing the rate of softening during storage (Valero et al., 2002a, and cites therein).

Another effect of PA infiltration is to ameliorate chlorophyll breakdown in several plant organs, including fruit, such as lemon and apricot, since Put treatment delayed the colour change during storage, which is an indicator of reduced senescence rate (Martínez-Romero et al., 2002; Valero et al., 1998c). Also, exogenous PAs retarded chlorophyll loss in muskmelon by reducing the hydrolytic activities acting on chloroplast thylakoid membranes (Lester, 2000). Similarly, Put-treatments reduced colour a* value after 3 and 6 weeks of low temperature storage in 'Angelino' plum, the effect being also attributed to lower chlorophyll degradation and delay in the senescence process (Khan et al., 2008).

Taking into account data of the commented parameters related to fruit quality, it could be concluded that Put treatment increased the fruit shelf life in different plum and peach cultivars. In addition, in different plum cultivars, the effect of Put treatment on increasing shelf life was highly correlated with the fruit firmness at harvest, that is, as

higher was plum firmness at harvest longer increase in plum shelf life was achieved (Martínez-Romero et al., 2000; 2002; Serrano et al., 2003; Valero and Serrano, 2010).

PAs are also involved in reducing CI due to their ability to preserve membrane integrity, both by lowering the membrane phase transition temperature fluidity and by retarding lipid peroxidation, resulting in increased cell viability, due to their membrane-binding capacity and/or antioxidant properties. This hypothesis is supported by the fact that exogenous PA treatments after harvest but before cold storage decreased CI in chilling sensitive fruits, such as apple (Kramer et al., 1991), zucchini (Martínez-Téllez et al., 2002), mango (Kondo et al., 2003; Nair and Singh, 2004) and pomegranate (Mirdehghan et al., 2007a). The reduction of CI symptoms was correlated with increased PA endogenous levels, especially Spd concentration after Put treatment. These evidences suggest an activation of the PA biosynthesis pathway, with part of the exogenous Put being used to be transformed to Spd using DCSAM, while the conversion of Spd to Spm did not occur, since not significant increase in Spm were found (Mirdehghan et al., 2007a).

3.5. 1-Methylcyclopropene

The discovery of 1-methylcyclopropene (1-MCP) as an ethylene inhibitor began in the late 1980s with research by Blankenship and Sisler as reported in the review of Blankenship and Dole (2003). The scientific research on this compound has shown that is a powerful inhibitor of ethylene action and capable of maintaining postharvest quality in many fresh horticultural products. 1-MCP was approved by the Environmental Protection Agency (EPA) in 1999 for use on ornamentals and marketed as Ethylbloc® and under the trade name SmartFreshTM for edible horticultural products with global use rights of Rohm & Haas and now transferred to Agrofresh Inc., which is the company in charge to commercialize 1-MCP at industrial level worldwide. In 2005, the European Union approved the use of 1-MCP within the Member States as plant growth regulator and established a maximum residue limit (MRL) as 0.01 mg kg-1, since 1-MCP has a non-toxic mode of action, negligible residue and is active at very low concentrations.

Since 1997 year researching about the application of this compound has increased progressively with more than 650 research papers on the topic 1-MCP and fruit (**Figure 9**, **inner graph**) while 50 papers dealt on vegetables. The most studied fruit has been apple followed by banana, tomato, pear and plum (**Figure 9**). Other important fruits with contrasted 1-MCP efficacy are avocado, peach, nectarine, apricot, papaya, melon and kiwifruit. Then, the impact of 1-MCP on postharvest science and technology has two approaches. First, it provides the potential to maintain fruit and vegetable quality after harvest. Second, 1-MCP provides a powerful tool to gain insight into the fundamental processes that are involved in ripening and senescence.

Table 6 shows 1-MCP concentration, duration for treatment and temperature application with positive effects in retarding the ripening process in a wide range of fruit commodities, as well as the main observed adverse effects that sometimes occur. From this **Table 6**, it can be inferred that for a particular fruit the appropriate 1-MCP concentration, duration of treatment and temperature need to be established to get positive results in terms of reducing the ripening process by inhibiting the ethylene production.

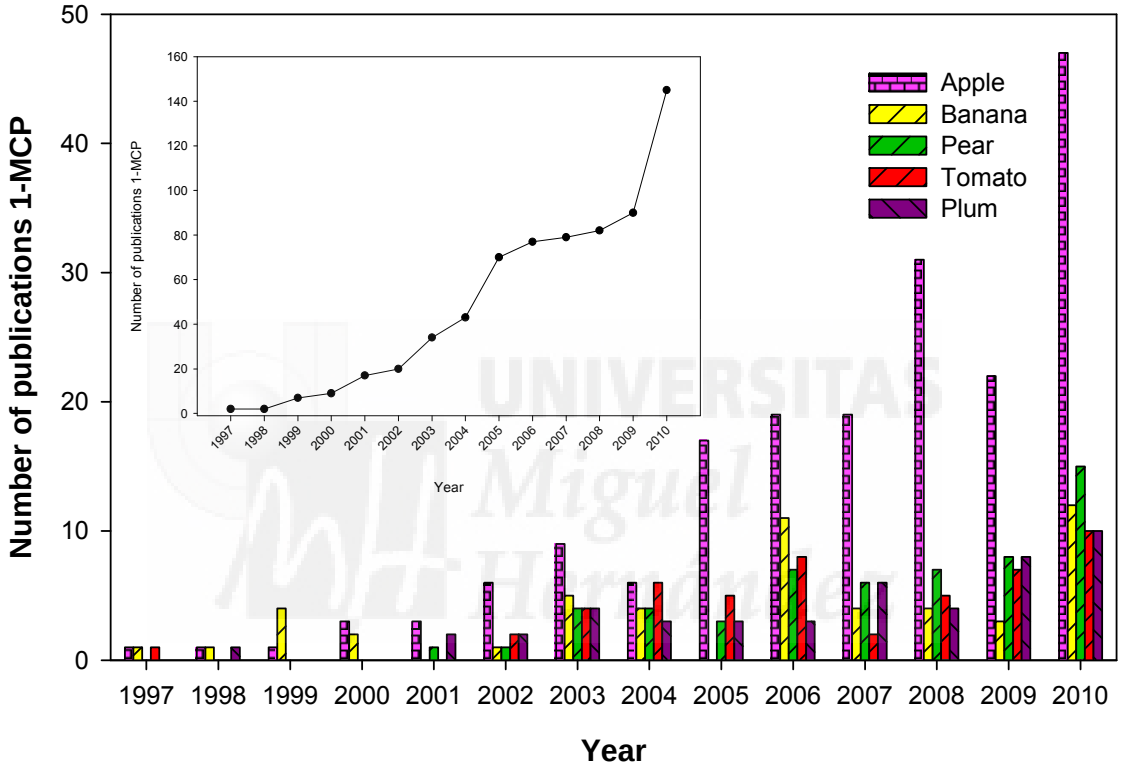


Figure 9: Evolution of number of publications per year about 1-MCP on the fruits in which 1-MCP has been most studied in the last 20 years. Insert graph shows the total number of publications per year about the use of 1-MCP in fruits and vegetables.

In apple, 1-MCP dramatically inhibits fruit ripening, the increase in ethylene production and internal ethylene concentration associated with the climacteric ripening stage, the extent of these inhibitions being related to cultivar, temperature for 1-MCP application, type of storage, temperature and duration of storage (Watkins et al., 2000). Banana has been also another fruit with good results in terms of ethylene inhibition during postharvest storage, with increased 'green life' after 1-MCP treatment, which was function of concentration × exposure time (Golding et al., 1998).

Table 6. Fruit types, 1-MCP concentration, duration for treatment and temperature with positive effects in retarding the ripening process, as well as the main adverse effects (1).

Fruit	1-MCP (μL L^{-1})	Duration (h)	Temperature (ºC)	Adverse Effects
Apple	0.5-10	12	20	Reduced volatiles
Banana	0.01-1	6-24	20	
Pear	0.1-4	12-24	0, 20	
Tomato	0.1-100	1-24	0-25	
Peach	0.02-0.5	18-24	20,24	Internal browning
Apricot	0-05-0.75	6-48	3,5,20,22	
Nectarine	0.2-1	12-24	20,24	Flesh woolliness
Plum	0.1-40	6-24	2,20	
Mango	1-100	6-14	20	Decay
Avocado	0.1-25	6-48	20-24	
Kiwifruit	0.5-5	16-20	20	
Papaya	0.5-10	4-24	20	

(1) Data obtained from Blankenship and Dole (2003); Watkins (2006); Martínez-Romero et al. (2007a); Guillén (2009).

Accordingly 'd'Anjou' pears treated with 1-MCP (0.1-1 μL L^{-1}) extended pre-climacteric period with low ethylene production and respiration rates, although total inhibition was not observed (Argenta et al., 2003). In stone fruits, such as peach, apricot, nectarine and plum, 1-MCP applied at 0.5 μL L^{-1} was also effective in reducing the ethylene production compared with control non-treated fruits (**Figure 10**), although the extension in ethylene inhibition was dependent on fruit specie and even cultivar. Thus 'Reina Claudia' plum and 'Currot' apricot showed the lowest percentages of ethylene inhibition compared with 'Golden Japan' and 'President' plums.

Moreover, it is interesting to point out that the percentage of ethylene inhibition by 1-MCP was correlated inversely to the maximum value of ethylene production at the climacteric peak of each particular fruit (**Figure 10, insert**). Thus, fruit with the lowest ethylene production ('Golden Japan' plum, <0.5 nL g^{-1} h^{-1}) showed the highest rate of ethylene inhibition (over 95%). Conversely, in 'Currot' apricot which had a climacteric peak production rate of >40 nL g^{-1} h^{-1}, an inhibition below 40% was observed. Thus the fruit type, the cultivar and the maximum ethylene production at the climacteric peak should be taken into account to explain the different effect of 1-MCP on ethylene production inhibition (Martínez-Romero et al., 2007a). In addition, the effects of 1-MCP are generally dose dependent, with maximum responses occurring at the highest 1-MCP concentration applied (Valero and Serrano, 2010).

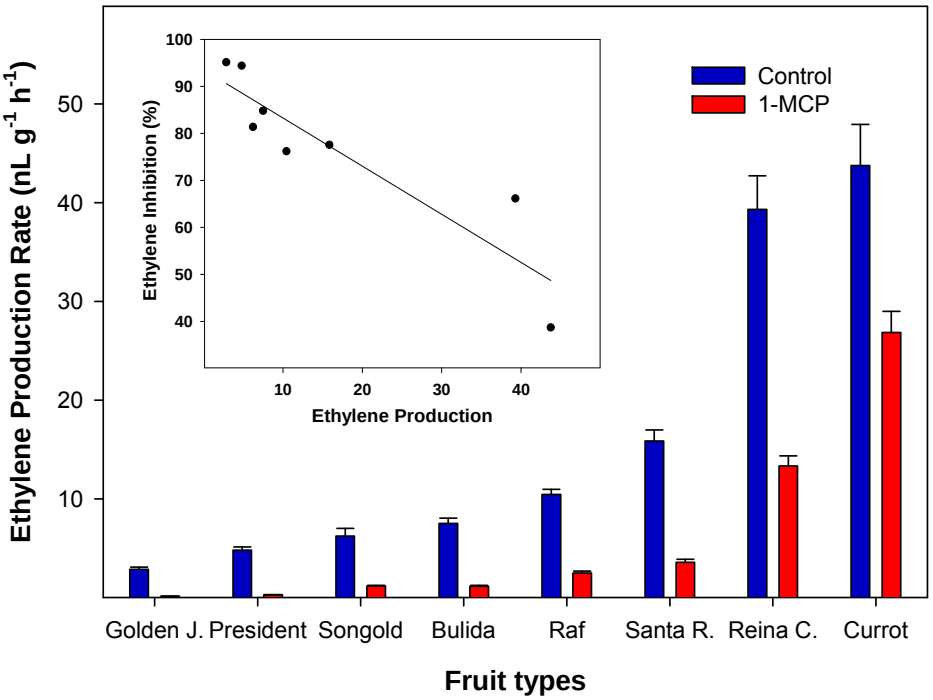


Figure 10: Ethylene production at the climacteric peak in control plums (GJ-'Golden Japan', 'President', SG-'Songold', SR-'Santa Rosa' and RC-'Reina Claudia'), apricots ('Bulida' and 'Currot') and tomato ('Raf') and those obtained in their 1-MCP (0.50 µL L-1 for 24 hours) corresponding treated fruits. Insert graphic shows the relationship between ethylene production at the climacteric peak and the inhibition caused by 1-MCP (Adapted from Martínez-Romero et al., 2007a).

Specifically in plums, 1-MCP has been shown to be equally effective on inhibiting the ethylene production in plums harvested at commercial ripening stage or in those picked 10 days later, although in the more mature ones a slight increase in ethylene was detected after 5 weeks of storage, which could be associated with some kind of ethylene receptor generation (Valero et al., 2003). Moreover, when 1-MCP was applied to fruit handled and packaged in perforated cardboard boxes, ethylene production was totally inhibited during all storage periods, while in those plums treated with 1-MCP in bulk (before handling and packaging), ethylene production increased after 3 and 4 weeks of cold storage plus 7 days at 20 °C, the differences being attributed to the higher gas diffusion around the fruit when they are packaged in small-perforated boxes (Valero et al., 2004). In addition, positive effects of 1-MCP on CI in plums have been recently observed in both climacteric and suppressed-climacteric types (Candán et al., 2008).

Different combinations of 1-MCP doses (0.5 or 1 µL L-1) and duration (3, 6, 12 or 24 h) were used on tomatoes harvested at the mature-green stage, with main conclusion being that 0.5 µL L^{-1} for 24 hours induced the maximum benefit in terms of ethylene inhibition and retarding the ripening process (Guillén et al., 2007a). Based on this result, these authors studied the effect of tomato cultivar and ripening stage at harvest and

concluded that for both stages, 1-MCP-treatment blocked the tomato receptors and absence of sharp increase in ethylene production or respiration rate was obtained, indicating that typical autocatalytic ethylene biosynthesis was also inhibited in tomatoes at both ripening stages (Guillén et al., 2006). The inhibition of both ethylene production and respiration rate by 1-MCP was negatively correlated with the maximum ethylene value reached for each of the cultivars and ripening stage assayed.

According to the delay and/or inhibition of ethylene production in climacteric fruits, all the quality parameters that are dependent on ethylene, such as firmness, colour, soluble solids concentration and loss of acidity are retarded, together with the reduced weight loss (Valero and Serrano, 2010). Thus, colour change is another fruit attribute which evolution is being also retarded following the application of 1-MCP, although little is known about the effects of 1-MCP on pigment metabolism, except its effect on delaying chlorophyll degradation in a wide range of fruits including apple, pear, green plum, kiwifruit and avocado. One clear effect of 1-MCP has been the delay of acidity losses that occurs during postharvest storage, as reported for apricot, plum, avocado, pear, tomato, etc., while the effect of 1-MCP on TSS is unclear (Martínez-Romero et al., 2003a; Valero et al., 2003; Watkins, 2006; Egea et al., 2010).

Since volatile production can be greatly affected by ethylene, the decreased and/or altered volatile production in 1-MCP treated fruits compared with untreated ones may impact negative product acceptability by consumers. However, other fruits, certain aromas are associated with over-ripening and therefore their inhibition is desirable, or aroma concentrations may be less important than texture and acid/sugar levels.

Literature exists about 1-MCP application on several non-climacteric commodities such as sweet cherry, citrus and strawberry. Thus, 1-MCP treatments on strawberries led to maintenance of fruit firmness and colour, but increased disease development (Jiang et al., 2001). In sweet cherry, 1-MCP transiently stimulated ethylene evolution but did not have impact on respiration rate, softening or colour changes (Gong et al., 2002). 1-MCP maintained, or delayed loss of greenness in citrus fruits, including orange, mandarin and lime. In the latter, this is with special importance since this is desirable for better commercialization, since as the green colour fades, fruit acceptance gradually decreases. Therefore, ethylene seems to be involved in some maturation-related events in non-climacteric fruits, such as chlorophyll degradation of the citrus skin, and it is possible that the skin green colouration loss in some citrus cultivars can be delayed by the application of 1-MCP, because of its influence on the ethylene action (Jomori et al., 2003). Accordingly, 1-MCP effectively inhibited ethylene responses in 'Shamouti' oranges as indicated by its inhibition of the degreening process, the most effective concentrations of 1-MCP being 50-100 nL L^{-1} (Porat et al., 1999), while 1-MCP had no effects on the loss of fruit weight and firmness.

3.6. Modified Atmosphere Packaging (MAP)

Modified atmosphere packaging (MAP) consists on sealing a certain quantity of fruit or vegetables by the use of plastic films with a particular permeability to gas diffusion. Then, respiration of commodities increases CO_2 concentration and decreases O_2 concentrations inside the package, while transpiration rate increases vapour pressure. The reduction in O_2 partial pressure and the increase in CO_2 partial pressure, as a consequence of commodities respiration rate, create gradients that, according to Fick's law, cause O_2 to enter and CO_2 to exist the package until the steady state is reached. Thus, steady-state O_2 levels are achieved in the package when the O_2 uptake by the product is equal to that permeating into the package, a situation that exists only when the respiratory rate is constant. As for O_2, the steady-state CO_2 in the package are achieved when CO_2 production by the product equals CO_2 escape from the package. The steady-state levels for both O_2 and CO_2 are dependent on the interaction of respiration of the produce (respiration rate and mass of product in the package) and the size and permeability properties of the packaging film (Kader et al., 1989).

For continuous film, since the permeability of CO_2 is usually 2-8 times higher than that for O_2, the decrease in O_2 inside the package is higher than the increase in CO_2, and the sum of CO_2 plus O_2 is lower than 20-21%, unless than the respiration quotient (RQ) is of the same magnitude or greater than the ratio of CO_2 to O_2 permeability. However, for perforated films, since the permeability of perforations to CO_2 is only a 20% less than to O_2, the sum of O_2 and CO_2 concentration is usually only slightly less than 21%, unless than RQ is significantly greater than 1, and in that case the sum will be larger than 21%. This effect of film type on MA composition is displayed in **Figure 11** for broccoli stored at 1 ºC in film packages of continuous and micro-perforated polypropylene, in which the concentration of CO_2 at the steady-state was 2.0-2.5% in micro-perforated film and ca. 6% in the continuous one, while those concentrations for O_2 were ca. 14 and 5%, respectively.

Recommended gaseous concentration for equilibrium atmospheres and ranges of optimal temperatures for many whole and fresh-cut horticultural commodities stored under MAP conditions are reviewed by. For each particular fruit or vegetable, the recommendations for O_2 and CO_2 optimum concentrations generally represent the conditions that will result in maximum storage life of each commodity, being in an extremely wide range (Beaudry 1999; 2000; Watkins, 2000; Artés et al., 2006; Sandhya, 2009). However, if the level of O_2 drops below its critical value (extinction point), aerobic respiration finishes and anaerobic respiration becomes important and when the level of CO_2 rises above a critical value, the produce develops physiological disorders. In this sense, maintenance of the desired atmosphere composition inside the packages depends on rigorous temperature control, since for a given temperature change, large differences between changes in produce respiration rate and in film permeability occurs leading to an

accumulation of CO_2 and a decrease in O_2 inside the packages with detrimental effects on fruit quality.

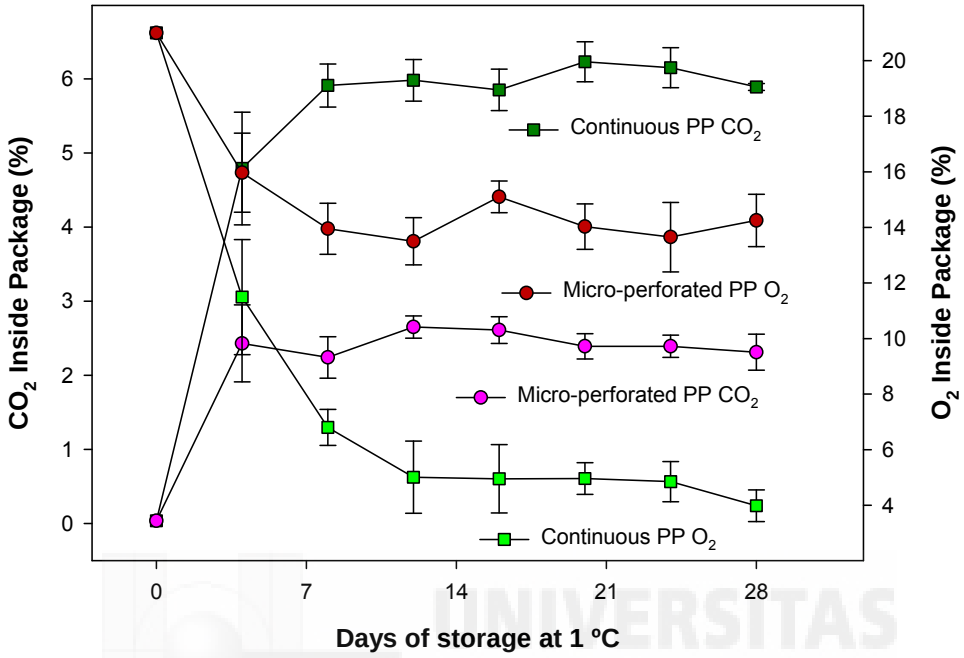


Figure 11: Evolution of CO_2 and O_2 concentration during cold storage of broccoli inside MAP with continuous and micro-perforated polypropylene (PP 20 µ thickness) films (Adapted from Serrano et al., 2006b).

The use of MAP as a supplement to proper temperature maintenance in the effort of delay fruit ripening and vegetable senescence, and associated physiological and biochemical changes, is generally beneficial for all commodities. Thus, successful applications of MAP on fruits include apples (Moodley et al., 2002), table grapes (Martínez-Romero et al., 2003b; Artés-Hernández et al., 2006), sweet cherries (Kappel et al., 2002; Serrano et al., 2005b), loquat (Amorós et al., 2008), litchi (Sivakumar and Korsten, 2006), papaya (González-Aguilar et al., 2003), strawberries and raspberries (Nielsem and Leufvén, 2008; Lange, 2000), among others.

One of the main problems during post-harvest storage of fruit and vegetables is weight loss, occurring mainly by transpiration rate, which affects its marketability, being responsible for important economic losses. In this sense, since films used in MAP have small water vapour diffusion, the internal atmosphere package becomes near saturated of water vapour pressure and then, transpiration of vegetable tissues decreases enormously, leading to low weight losses. For example, weight loss of control broccoli stored at 1 ºC in open air was 46 % of their initial weight after 21 days, while broccoli stored in non-

perforated and micro-perforated polypropylene bags lost less than 1.5 % (Serrano et al., 2006). Similar results have been obtained in loquat (Amorós et al., 2008), table grape (Martínez-Romero et al., 2003b), nectarines (Retamales et al., 2000), peaches (Akbudak and Eris, 2004) and cherries (Kappel et al., 2002; Serrano et al., 2005b).

Of the primary metabolic responses to low O_2, includes a reduction in respiration, manifested as diminution in starch degradation and sugar consumption, leading to a reduction of the rate of tissue deterioration, thereby extending storage life. A level of 50% reduction in respiration is suggested to be associated with sufficient enhancement of shelf life such that the cost of extra handling and materials resulting from MAP will be recovered (Beaudry, 2000). In addition, in climacteric fruit, such as mango and papaya, apart from reduction of respiration rate, a delay on climacteric respiration peak has been reported (Yahia, 2006; Singh & Rao, 2005). Accordingly, high CO_2 was proposed to inhibit respiration rate by feedback inhibition or by controlling mitochondrial activity including an effect on Krebs cycle intermediates and enzymes.

Of the secondary metabolic responses to low O_2, include a reduction on ethylene biosynthesis and perception. In fact, low O_2 concentration is known to inhibit ACO activity, this effect being dependent of the ACC concentration, since as ACC increase the Km of the enzyme for O_2 decreases. Oxygen has also been reported to exert an effect on ethylene perception, which is reduced as O_2 concentration decrease. Therefore, in climacteric fruits, O_2 concentrations not low enough to reduce respiration rate still reduce the rate of ripening through an effect mediated by ethylene (Abeles et al., 1992). By other hand, it is known that CO_2 is an antagonist of ethylene action and impedes its autocatalytic synthesis. In fact, CO_2 levels higher than 1% decrease or inhibit ethylene biosynthesis and consequently retard fruit ripening and deterioration, these effects being additive to those of reduced O_2 atmospheres (Artés et al., 2006).

Colour evolution associated with the postharvest ripening process is generally delayed in fruits stored under MAP conditions, as compared to those stored in open air, as has been shown in mango (Pesis et al., 2002), table grape (Martínez-Romero et al., 2003b) and loquat (Amorós et al., 2008) as well as in broccoli (Serrano et al., 2006). These effects could be attributed to the action of low O_2 on reducing chlorophyll degradation and browning mediated by the inhibition of pheophorbide oxigenase and PPO, responsible for chlorophyll loss and browning, respectively (Beaudry, 2000). In addition, colour preservation by MAP storage has been related to the delay in anthocyanin and carotenoid biosynthesis, thus preserving alteration of colour (Artés et al., 2006).

MAP is also effective on delaying the softening process and the increase of the ripening index that usually occurs during postharvest storage in a wide range of fruits, such as strawberries (García et al., 1998), apricot (Pretel et al., 1993; 1999), kiwifruit (Agar et al., 1999), loquat (Amorós et al., 2008), peaches and nectarines (Akbudak and

Eris, 2004) and table grapes (Martínez-Romero et al., 2003b). Some reports have also found an effect of MAP on reducing spoilage during storage of whole fruits. Thus, MAP decreased decay index in peaches and nectarines leading to high scores of overall appearance and taste in fruits stored under MAP conditions as compared with those stored on open air (Akbudad & Eris, 2004). Accordingly, MAP significantly reduced brown rot in sweet cherry as compared to air-stored fruits (Spotts et al., 2002).

Sensory properties are also maintained in fruit under MAP conditions with appropriate atmosphere composition. Thus, for example, scores for crunchiness and juiciness were higher in non-perforated and perforated polypropylene film packaged table grapes than in controls after 18 days of storage at 1 ºC, and even after 53 days no modifications were observed with respect to the scores at day 18 (Marínez-Romero et al., 2003a). However, negative secondary responses to low O_2 and high CO_2 may also occur, including reduced aroma biosynthesis and the possibility of off-flavour generation. The effect on aroma production is mediated by ethylene but also likely via action of O_2 on oxidative process, including respiration required for substrate production, although aroma generation is recovered when fruits are restored to normal air (Beaudry, 1999; Kader and Watkins, 2000; Artés et al., 2006).

The beneficial effects of MAP on maintaining fruit quality during postharvest storage is even greater for tropical fruits than from temperate ones, due to the reduction of chilling sensitivity by atmospheres with high CO_2 and low O_2 concentrations (Yahia, 2006; Sandhya, 2010). Thus, the increase of electrolyte leakage on papaya fruit was coincident and correlated with the occurrence of CI symptoms, both process being decreased in MAP stored fruits (Singh and Rao, 2005). MAP also alleviated CI symptoms in pepper fruits (Serrano et al., 1997) and the red spots around lenticels occurred in mangos, although this effect was attributed to both, the MA surrounding the fruits and maintenance of a high RH in the bags (Pesis et al., 2000). In peaches it has been also reported that MAP might prevent development of CI by maintaining high humidity inside the packages, since water loss cause excessive production of active oxygen species and then, the high humidity in MAP could prevent chilling-induced oxidative stress (Hodges et al., 2004).

3.7. Edible Coatings

During the last two decades, both food and packaging industries have joined efforts to reduce the amount of food packaging materials mainly due to environmental and consumer concerns. In this sense, the concept of biobased materials for food packaging was introduced at the end of the last century (Petersen et al., 1999), although these materials are not necessarily biodegradable. Biobased packaging materials include both edible films and edible coatings along with primary and secondary packaging materials.

Edible coatings may be defined as a thin layer of material that covers the surface of the food and can be eaten as part of the whole product.

The application of an edible coating onto the fruit surface modifies the internal atmosphere in the same way that does plastic films by increasing the carbon dioxide and lowering the oxygen concentrations. Success of edible coatings for fruits depends mainly on selecting films or coatings that can give a desirable internal gas composition that is appropriate for a specific product. Also, if a coating is too thick detrimental effects can result due to an internal oxygen concentration below a desirable and beneficial level and an associated increased carbon dioxide concentration above a critical tolerable level. On a general basis, oxygen permeability of most edible coatings is lower than the conventional plastic films (Park, 1999).

Biopolymer-based packaging is defined as packaging that contains raw materials originating from agricultural and marine sources. There are three such categories of biopolymers: a) extracted directly from natural raw materials, such as starch, cellulose, protein, and marine prokaryotes, b) produced by chemical synthesis from bioderived monomers, and c) produced by microorganisms such as hydroxy-butyrate and hydroxy-valerate (Cha and Chinnan, 2004). Components of edible films and coatings can be divided into three categories: hydrocolloids, lipids, and composites. Hydrocolloids include proteins and polysaccharides, such as starch, alginate, cellulose derivatives, chitosan, and agar. Lipids include waxes, acylglycerols, and fatty acids. Composites contain both hydrocolloid components and lipids. The choice of materials for a film or coating is largely dependent on its desired function.

Among polysaccharides, starch is very biodegradable and cost effective, but is also very hydrophilic. Native granular starch is converted into a thermoplastic material by conventional methods in the presence of plasticizers, such as water and glycerol and the resulting films have moderate gas barrier properties. Amylose, the linear fraction of starch, is known to form a coherent and relatively strong, freestanding film responsible for the film-forming capacity of starches, in contrast to amylopectin films, which are brittle and noncontinuous (Cha and Chinnan, 2004).

Another polysaccharide that is of high interest is chitosan, obtained from the deacetylation of chitin (poly-ß-(1→4)-N-acetyl- D-glucosamine), which is mainly obtained from crab and shrimp shells. Films and coatings based on chitosan have selective permeability to gases (CO_2 and O_2) and good mechanical properties. However, their uses are limited mainly because of their high water vapour permeability. Moreover, chitosan shows antifungal and antibacterial properties, which are believed to be originated from its polycationic nature, although the precise mechanism of its antimicrobial activity is still unknown (Srinivasa and Tharanathan, 2007).

Alginates, which are extracted from brown seaweeds of the *Phaephyceae* class, are the salts of alginic acid, a linear copolymer of D-mannuronic and L-guluronic acid monomers. The ability of alginates to react with di-valent and trivalent cations is being utilized in alginate film formation. Calcium ions, which are more effective than magnesium, manganese, aluminum ferrous, and ferric ions, have been applied as gelling agents (Vargas et al., 2008).

Proteins that can be used in the formulation of edible coatings for fruits include those derived from animal sources, such as casein and whey protein, or obtained from plant sources like corn-zein, wheat gluten, soy protein, peanut protein, and cottonseed protein (Gennadios, 2002). Proteins exhibit a wide variety of different molecular characteristics depending on their biological origin and function that will determine the ability of particular proteins to form coatings and the characteristics of the coatings formed. Casein based edible coatings are attractive for food applications due to their high nutritional quality, excellent sensory properties, and good potential for providing food products with adequate protection against their surrounding environment. Whey proteins have also been the subject of intense investigation over the past decade or so. With the addition of plasticizer, heat-denatured whey proteins produce transparent and flexible water-based edible coatings with excellent oxygen, aroma, and oil barrier properties at low relative humidity. However, the hydrophilic nature of whey protein coatings causes them to be less effective as moisture barriers.

Lipid-based edible coatings have a low affinity for water, which explains why they have low water vapour permeability, and thus the use of lipid coatings on fresh fruits and vegetables can help to control their desiccation and weight loss (Morillon et al., 2002).

Wax was the first edible coating used on fruits. The Chinese applied wax coatings to oranges and lemons in the 12th and 13th centuries. Although the Chinese did not realize that the full function of edible coatings was to slow down respiratory gas exchange, they found that wax-coated fruits could be stored longer than non- waxed fruits. Park (1999) reviewed the development of systematic means of selecting edible coatings to maximize quality and shelf life of fresh fruits and vegetables. For these products, cellulose, casein, zein, soy protein and chitosan were candidates to be used in fruits since they have the desirable characteristics of generally being odourless, tasteless and transparent. In the past few years, research efforts have focused on the design of new eco-friendly coatings based on biodegradable polymers, which not only reduce the requirements of packaging but also lead to the conversion of by-products of the food industry into value added film-forming components. Among these new materials for edible coatings *Aloe vera* gel was first used in table grape (Valverde et al., 2005b) and sweet cherry (Martínez-Romero et al., 2006) with satisfactory results in terms of reduced the weight loss and lowered the respiration rate during postharvest storage. In addition, *A. vera* gel delayed colour changes, softening and TA losses, maintaining fruit quality

together with a reduction of both mesophilic aerobics and yeast and mould counts without affecting the sensory properties of the fruits.

Traditionally, edible coatings have been used as a barrier to minimize water loss and delay the natural senescence of coated fruits through selective permeability to gases. However, the new generation of edible coatings is being especially designed to allow the incorporation and/or controlled release of antioxidants, vitamins, nutraceuticals, and natural antimicrobial agents by means of the application of promising technologies such as nano-encapsulation and the layer-by-layer assembly (Vargas et al., 2008).

In this sense, coated tomatoes with alginate or zein showed lower respiration rate and ethylene production than control ones, with a twofold lower concentration of ethylene precursor. In addition, the evolution of parameters related to tomato quality losses, such as softening, colour evolution and weight loss was significantly delayed (4–6 days on average) in coated tomatoes as compared to controls. Thereafter, sugars, organic acids (and especially ascorbic acid) and scores from sensory analysis remained at much higher levels at the end of storage in treated than in control tomatoes (Zapata et al., 2008).

3.8. Salicylic Acid and Acetylsalicylic Acid

Salicylic acid (SA) or ortho-hydroxybenzoic acid and related compounds belong to a diverse group of plant phenolics, the salicylates, which have been used in medicines since antiquity. In 1828 in Munich was isolated for the first time a small amount of salicin, the glucoside of salicyl alcohol, from willow bar. Ten years later Raffaele Piria named it SA, from the Latin word *Salix* for willow tree. The first commercial production of synthetic SA began in Germany in 1874 (Raskin, 1992a;b) and the Aspirin, a close analog of salicylic acid, was introduced by the Bayer Company in 1898 and rapidly became one of the most popular pharmaceutical preparations in the world. During the 19th century many compounds belonging to the group of salicylates were isolated from a variety of plants. Aspirin, a trade name for acetylsalicylic acid (ASA), undergoes spontaneous hydrolysis to SA and it is widely used by many plant scientists in their experiments since it has similar physiological effects than SA (Popova et al., 1997).

It was found that salicylates are distributed in many important agricultural plant species. In many plants, such as rice, crabgrass, barley, soybean, the levels of SA has been found to be approximately 1 μg g^{-1} fresh weight (Raskin, 1992a). Janssen et al. (1997) found total salicylate levels of 0-0.7 pmol/100 g in vegetables and fruits, and 2-20 μmol/100 g in herbs and spices, while levels of acetylsalicylate were lower than the limit of detection in all foods. Based on these results, they estimated that a Western diet provides about 0-15 μmol/day of total salicylates.

In the early 1960s it was suggested that in plants SA is synthesized from cinnamic acid by two possible pathways: one involves side-chain decarboxylation of cinnamic acid to benzoic acid followed by 2-hydroxylation to SA. Alternatively, cinnamic acid could be first 2-hydroxylated to *o*-coumaric acid and then decarboxylated to SA. These pathways differ in the order of ß-oxidation and ortho-hydroxylation reactions and could operate independently in plants. Two key enzymes are involved in SA biosynthesis and metabolism: benzoic acid 2-hydroxylase, which converts benzoic acid to SA, and UDP glucose: SA glucosyltransferase, which catalyses conversion of SA to SA glucoside. Later data show that the cinnamic acid → benzoic acid → SA pathway functions in rice seedlings (Silverman et al., 1995). However, more recently the shikimate pathway (**Figure 12**) has been proposed based on results obtained using Arabidopsis (Sha, 2003).

The first indications for physiological effects of SA were the discovery of flowering induction and bud formation in tobacco cell cultures (Eberhard et al., 1989) and its inhibitory effect of ethylene biosynthesis, in apple disks (Romani et al., 1989) and pear cell suspension cultures by blocking the conversion of ACC to ethylene (Leslie and Romani, 1986). Authors described SA as an effective, non-toxic and reversible inhibitor of ethylene biosynthesis at concentrations comparable to those found in some plant tissues. It is also becoming apparent that SA interacts both negatively and positively in conferring stress tolerance with other major signalling pathways including those regulated by jasmonic acid and ethylene (Raskin 1992b).

More recently, is has been reported that SA, as a plant growth regulator, can enhance disease resistance of a few growing plants or detached plant organs (Qin et al., 2003). Furthermore, exogenous application of SA has been found to enhance the efficacy of the biocontrol yeast *Cryptococcus laurentii* in pear fruit (Yu et al., 2007), in apple fruit (Yu and Zheng, 2006), and in cherry fruit (Qin et al., 2003). In peach fruit, *Rhodotorula glutinis* and SA (100 mg/mL) had potential as a biocontrol agent for the control of postharvest decay caused by *B. cinerea* (Zhang et al., 2008). In sweet cherry, bicontrol with the yeast in *Pichia membranefaciens* at a concentration of 5×107 cells ml^{-1} or in SA at 0.5mM for 10 min reduced the incidence of decay and lesion size caused by *Penicillium expansum* (Chan and Tian, 2006). Without pathogen inoculation, POD activity was enhanced in yeast-treated fruit, but activities of CAT and SOD showed a decrease in the same fruit. SA-treatment significantly inhibited CAT activity, but stimulated SOD and POD activities.

Postharvest ASA treatments alleviated CI symptoms, inhibited accumulation of superoxide free radical, and reduced PAL, CAD, and G-POD activities in loquat (Cai et al., 2006), delayed discolouration, maintained eating quality with higher content of the quality attributers, and reduced or delayed the increases in activities of PPO, POD and PAL in fresh-cut chestnut (Peng and Jiang, 2006) and reduced ethylene production and fungal decay and retained overall quality in strawberry (Babalar et al., 2007).

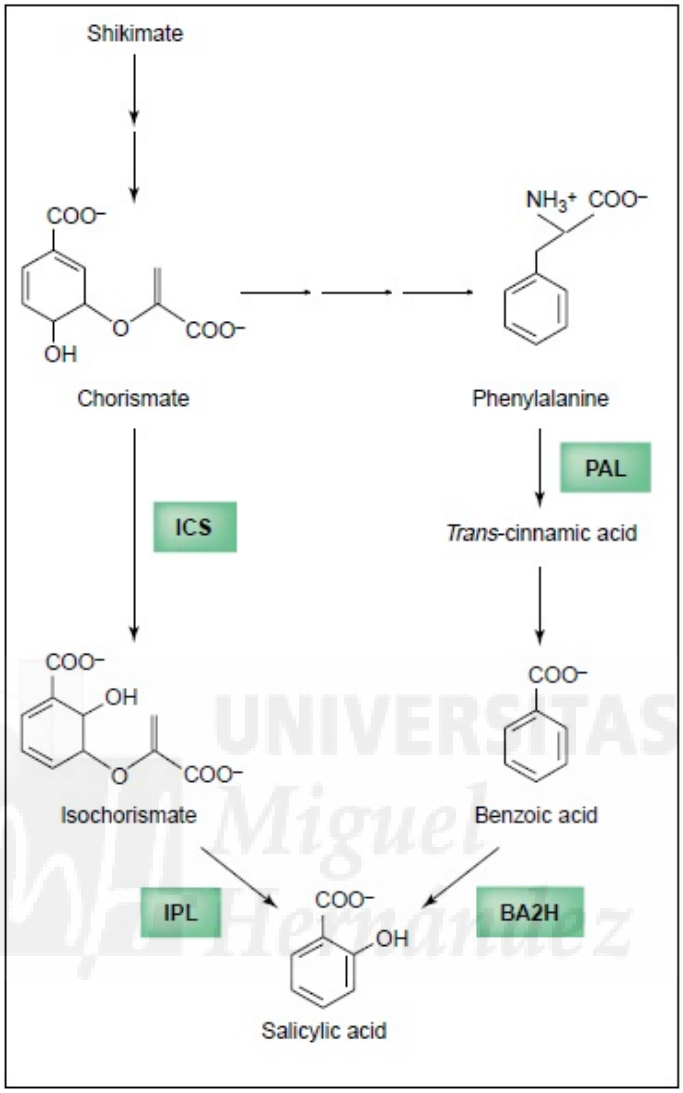


Figure 12: Proposed pathways for the biosynthesis of SA in plants. The shikimate pathway provides chorismate, which can be converted into SA (Shah, 2003).

Zhang et al. (2003) reported that postharvest treatment of kiwifruit with ASA resulted in lower ACO and ACS activities and decreased ethylene production and fruit softening, due to the conversion of ASA to SA. In apple fruit (*Annona squamosa* L.) SA treatments lowered respiration, increased activities of the antioxidant enzymes SOD, POD, CAT and ascorbate peroxidase (APX), decreased LOX activity and correspondingly lowered Malondialdehyde (MDA) contents (Mo et al., 2008).

Pre-harvest treatments with 2 mM SA significantly reduced lesion diameters on sweet cherry fruit caused by *Monilinia fructicola* compared with control post-harvest treatments and induced ß-1,3-glucanase, PAL and POD activities during the early storage

time, the efficacy of pre-harvest treatments being higher than that of post-harvest treatments, especially, at 25 ºC (Yao and Tian, 2005).

3.9. Oxalic Acid

Oxalic acid (OA) is a common constituent of plants, and several species, including some crop plants, which accumulate high levels of the simplest dicarboxylic acid. The most striking chemical impact of OA is its strong chelating ability with multivalent cations. OA has been considered as an antinutrient due to its inhibitory effect on mineral bioavailability and to its formative effect on calcium oxalate urinary stone. There are several precursors of OA including glyoxylate and L-ascorbic acid in plants. The function of OA in plants has been not completely understood, although has been reported as natural antioxidant in several systems (Kayashima and Katayama, 2002).

OA is a common acid in many plant foods, such as spinach (356–780 mg/100 g of fresh material), rhubarb (260–620 mg/100 g of fresh material) and beet root (97–121 mg/100 g of fresh material (Hodgkinson, 1977) and has a strong antibrowning activity by using a catechol-PPO model system which appeared to be competitive, with a Ki value of 2.0 mM (Son et al., 2000).

OA treatments have shown beneficial effects on fruit quality. Thus, OA at two concentrations (1 and 5 mM) applied to peach fruit reduced relative leakage rate, maintained higher flesh firmness, lower respiration, increased activities of antioxidant enzymes SOD, POD, CAT, APX and PPO and a decreased LOX activity during storage as compared with the control (Zheng et al., 2007a). In addition, the significant decreases in the production of active oxygen species such as superoxide (O_2^-), H_2O_2 and lipid peroxidation in treated fruit were found at the later time of storage. The effects of OA could therefore contribute to maintaining the membrane integrity and delaying the fruit ripening process.

Accordingly, mango fruit treated with OA resulted in delayed fruit ripening and reduced fruit decay incidence compared to the control. It was suggested that the physiological effect of oxalic acid in decreasing ethylene production was an important contributor to delaying the ripening process (Zheng et al., 2007b). In this fruit, CI was reduced by OA and SA treatments. Fruit treated with OA or SA had significantly higher reduction states of ascorbate and glutathione. Moreover, the treated fruit showed lower superoxide anion content, higher hydrogen peroxide content, lower LOX activity and higher activities of SOD, CAT, guaiacol peroxidase, APX and glutathione reductase (Ding et al., 2007). In addition, fruit firmness, total soluble solids and titratable acidity content were not obviously affected by OA and SA treatments. It was suggested that the effect of OA or SA on mango CI probably attributed to more reducing status of ascorbate and glutathione, less O_2^- accumulation and more H_2O_2 accumulation.

4. ANTIOXIDANT COMPOUNDS IN FRUITS AND VEGETABLES AND CHANGES DURING POSTHARVEST STORAGE AND PROCESSING

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Antioxidant compounds in fruits and vegetables and changes during postharvest storage and processing

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Abstract

Purpose of review: Fruits and vegetables are known to contain large amounts of bioactive compounds (polyphenols, anthocyanins, carotenoids and vitamins) that confer antioxidant activity and are related to health-benefits. This review highlights the occurrence of these phytochemicals and their changes during ripening on-tree, and examines the effect of several postharvest technologies and processing methods on modulating their concentration during postharvest storage.

Findings: Large variation exists in the content of bioactive compounds in fruits and vegetables depending on the plant species, and even among cultivars. With the ripening process, the content of polyphenols, anthocyanins and carotenoids generally increases, while it is variable for vitamins. The different postharvest technologies reported here, applied before or with cold storage (heat and calcium treatments, polyamine application, use of 1-methylcyclopropene and modified atmosphere packaging), induce beneficial effects in the stored fruits and vegetables by better maintaining the content of bioactive compounds and antioxidant properties compared with those stored under cold conditions only. Processing (blanching, freezing, canning and cooking) generally impacts negatively on phytochemical concentrations, but is dependent on temperature and processing time.

Direction for future research: Future research should focus on the use of non-toxic natural compounds, considered as environmentally friendly, to develop new preservation systems that will maintain the quality and extend the shelf-life of fruits and vegetables without compromising their safety, appearance or sensory properties, and preserving the bioactive compounds and antioxidant activity.

Keywords: phenolics; carotenoids; vitamins; storage; antioxidant activity; ripening

4.1. Introduction

Due to their perishable nature, fruits and vegetables deteriorate rapidly after harvest with significant losses in their quality attributes. To minimise these losses, a wide range of postharvest treatments has been assessed and has shown efficacy in maintaining quality. These plant foods contain non-nutrients moieties called phytochemicals which exhibit antioxidant properties. Among them, phenolic compounds, carotenoids and vitamins are the main groups. However, little attention has been paid to the impact of postharvest technologies on the content of health-promoting phytochemicals during storage. In this context, the aim of this review is to get a better knowledge of the impact of several technologies on the changes that occur in bioactive compounds. Some of the tools that will be reviewed are already being used in the horticulture industry (eg, 1-methylcyclopropene [1-MCP] and modified atmosphere packaging [MAP]), but others that are still under investigation (eg, heat, calcium treatments and polyamine [PA]) will be included. Although fruits and vegetables are consumed as raw materials, in many cases they are submitted to processing, especially heating and freezing, which can alter the composition in bioactive compounds and antioxidant properties.

4.2. Fruit Ripening and Antioxidant Compounds

Fruit ripening is a highly coordinated, genetically programmed process occurring at the later stages of fruit development and involving a series of physiological, biochemical and sensory changes leading to an edible ripe fruit with desirable quality parameters. Specific biochemical and physiological changes differ among species although generally they include altered sugar metabolism, softening, colour changes, synthesis of aroma volatiles and increased susceptibility to pathogen infection, suggesting that the underlying genetic mechanisms that regulate fruit ripening are well conserved among fruits of different species. From the physiological point of view two major classifications of fruit ripening have been classically accepted: climacteric and non-climacteric. Climacteric fruits are characterised by increased respiration and ethylene biosynthesis rates during ripening, while in nonclimacteric fruits a gradual decrease in both respiration rate and ethylene production occurs (Giovannoni, 2002, Adams-Phillips et al., 2004; Barry and Giovannoni, 2007; Valero and Serrano, 2010].

Foods from plant origin contain hundreds of non-nutrient constituents with significant biological activity, generally called “bioactive compounds” or phytochemicals with antioxidant activity. Thus, fruit and vegetable consumption has shown protective effects against several chronic diseases associated with ageing including atherosclerosis, cardiovascular diseases, cancer, cataracts, blood pressure increase, ulcerous, neurodegenerative diseases, brain and immune dysfunction, and even against bacterial and viral diseases. These protective effects have been attributed to several antioxidant

compounds, which vary widely in chemical structure and function in plant tissues and are grouped into vitamins (C and E), carotenoids, phenolic and thiol (SH) compounds (Scalbert et al., 2005; Saura-Calixto and Goñi, 2006; Lister et al., 2007; Nichenametla et al., 2006: Asensi-Fabado and Munné-Bosh, 2010; Wang et al., 2011).

4.2.1. Phenolic compounds

The terms "phenol" and "polyphenol" chemically refer to compounds that possess an aromatic ring bearing one (phenol) or more (polyphenols) hydroxyl radicals. However, from the biological point of view, plant phenolic or polyphenolics are defined as secondary natural metabolites that exhibit a very broad range of physiological roles in plants including pigmentation, growth and resistance to pathogens, among many other functions (Daayf and Lattancio, 2008).

The structure of plant phenolics and polyphenols can be either very simple (phenolic acids) or highly polymerised compounds (proanthocyanindins). In nature several thousands of different compounds have been identified with large variation of structures. The main phenolics in fruits and vegetables are classified according to their basic skeleton: C6-C1 (phenolic acids), C6-C3 (hydroxycinnamic acids), C6-C2-C6 (stilbenes) and C6-C3-C6 (flavonoids). Of all these flavonoids are the most important group with approximately 8,000 different compounds already identified. The flavonoid group can be divided in five subgroups including flavonols, flavan-3-ols, flavones, flavonones and anthocyanindins. These are known as aglycones and are usually bound through glycosidic bonds to several sugar moieties to form the glycosilated derivatives, the main sugars being glucose, rhamnose and rutinoside. The biosynthesis of flavonoids, stilbenes, hydroxycinnamates and phenolic acids involves a complex of networked routes based on the shikimate (C6-C1 moieties) and phenylpropanoid (C6- C3-C6 molecules) pathways (Crozier et al., 2009). More details on the biosynthesis of these phenolic types can be obtained from a report by Amarowicz et al. (2009).

Phenolics as a group represent the strongest antioxidants in plant foods, although the antioxidant activity of individual phenolic compounds may vary depending on their chemical structure. The antioxidant activity of phenolics is attributable to the electron delocalisation over the aromatic ring and their high redox potential, which allows them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. In addition, they have metal-chelating potential, decreasing the lipid peroxidation and can trap nitrate and prevent the formation of mutagenic N-nitroso compounds (Fernández-Panchón et al., 2008). Therefore, the inverse relationship between fruit and vegetable intake and risk of cardiovascular and neurodegenerative diseases, cancer, diabetes, or osteoporosis has partially been ascribed to dietary phenolics (Scalbert et al., 2005; Halliwell, 2007; Tucker and Robards, 2008). Total phenolics increase as maturity advances in pepper, sweet

cherry and plums (both yellow and red cultivars), while a decline occurs in tomato. This behaviour in total phenolics is reflected in the total antioxidant activity (TAA) from the hydrophilic extracts (H-TAA), which evolves in a similar way and coincides with the loss of green tonality and occurrence of fruit typical colour (red, purple or yellow). A direct relationship has been found between total phenolic compounds and H-TAA during the ripening of plums, peaches, peppers, nectarines and sweet cherries (Gil et al., 2002; Cevallos-Casals et al., 2006; Deepa et al., 2007). **Figure 13** summarises the content of total phenolics and H-TTA in a wide range of typical fruits of the Mediterranean diet at commercial harvest, in which pepper and strawberry showed the highest H-TTA, and the highest polyphenol content was found in pomegranate.

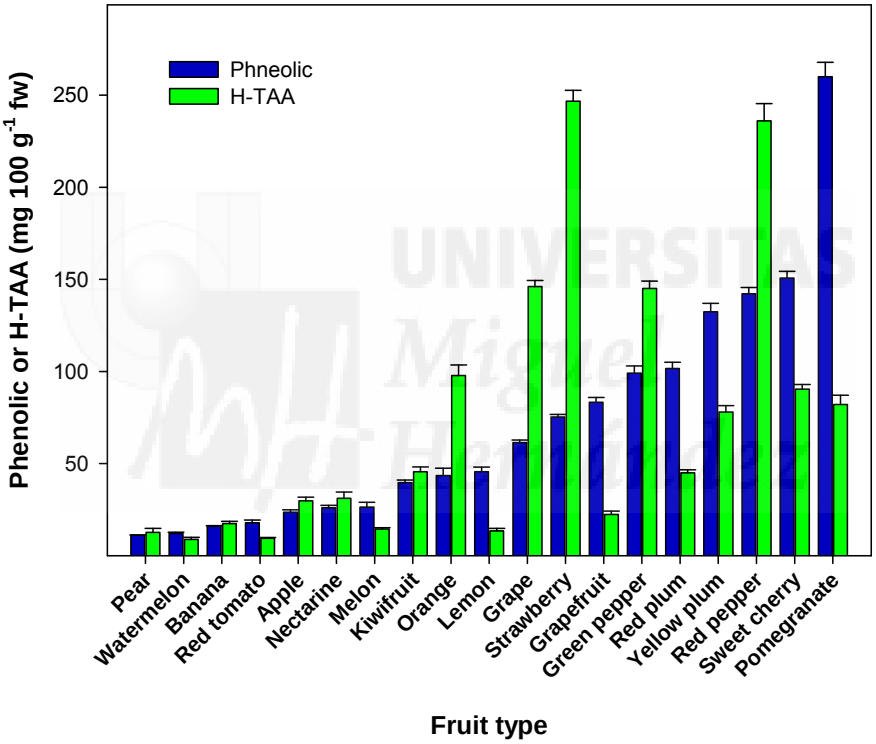

Figure 13: Total phenolic concentration and total antioxidant activity due to hydrophilic compounds (H-TAA) in the typical fruits of the Mediterranean diet at commercial harvest. Data are the mean ± SE (n=5). Total phenolics were determined by the Folin-Ciacolteau reagent (expressed as mg gallic acid eq. 100/g fresh weight) while H-TAA was estimated by the ABTS assay and expressed as mg Trolox eq. 100/g (Serrano et al., 2005a).

4.2.2. Anthocyanins

Anthocyanins are responsible for the red, blue and purple colour of some flower and fruits, and have been described as potent antioxidants. Anthocyanins are water-soluble pigments located in the vacuole and are classified as flavonoids with glycosilated

derivatives of the 3,5,7,3'- tetrahydroxyflavylium cation. The free aglycones (anthocyanidins) are highly reactive with sugars to form the glycosides and all anthocyanins are O-glycosilated. The main aglycones found in fruits are pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin while the most relevant sugars are D-glucose, L-rhamnose, D-galactose, Dxylose and arabinose (Welch et al., 2008; Castañeda-Ovando et al., 2009). In anthocyanin biosynthesis, the C6-C3-C6 skeleton is formed involving the polyketide and shikimate pathways to form flavanones which are converted to dehydro-flavonols and further transformed to leucoanthocyanindins and anthocyanins (Amarowicz et al., 2009).

Anthocyanins have shown higher antioxidant activity than other phenolic compounds, with cyanidin being the most common anthocyanidin and the 3-glucoside the most active anthocyanin with antioxidant activity. In sweet cherry and purple plum cultivars, the anthocyanin concentration increased at the latest stages of ripening on tree, showing variations between plant species and even cultivars, although for all of them, the anthocyanin accumulation in sweet cherry and in the plum skin was highly correlated with H-TAA (Valero and Serrano, 2010) indicating that anthocyanin could be the main phenolic compounds with antioxidant activity. This seems to be a general behaviour of red purple fruits, since correlations were also found between the values of the antioxidant capacity and the anthocyanin content in blackberries, red raspberries, black raspberries and strawberries (Wang and Lin, 2000). The authors also found differences attributable to ripening stage, species and cultivar. Very recently, various transgenic approaches were taken to increase the flavonoid levels in tomato fruits which started to synthesise anthocyanins at the end of the green stage and continued to accumulate these pigments during subsequent ripening, having higher H-TAA than red tomatoes (Gonzali et al., 2009).

4.2.3. Carotenoids

Carotenoids are a group of lipid-soluble natural pigments (C40 tetraterpenoids) present in fruits and vegetables that impart colours from yellow to red and which originate from eight C5 isoprenoid units joined head to tail resulting in a symmetrical molecule located in the chromoplasts. The hydrocarbon carotenoids are known as carotenes (ß-carotene, lycopene, etc) while xanthophylls are oxygenated derivatives containing at least one hydroxyl group and then being more polar than carotenes. On the other hand, carotenoids can be acyclic (eg, lycopene), monocyclic (γ-carotene) or dicyclic (α- and ß-carotene). During fruit ripening, large variations have been found not only in the pigment profile but also in the concentration, with a general increase in the ripening process for all fruits and higher contents in the skin than in the flesh (Valero and Serrano, 2010). The main physiological effect of carotenoids in humans has been classically attributed to their role as provitamin A, since those carotenes with a ß-ring end group are

converted to vitamin A (retinol) by the action of an intestinal mono-oxygenase. In addition, in the last decade several epidemiological studies have suggested that carotenoids also have important roles in a range of diseases including age related macular degradation, cataract, cardiovascular diseases, and some types of cancer due to their function in cell differentiation and proliferation regulators or cell to cell communication stimulators (Krinsky and Johnson, 2005; Voutilainen et al., 2006), as well as improving the bone and joint health by increasing alkaline phosphatase activity and osteopontin in osteoblastic cells (Lister et al., 2007) due to their antioxidant properties.

The most studied carotenoids are ß-carotene, lycopene, lutein and zeaxanthin. Lycopene, a carotenoid with non-provitamin A activity, has been found to have both greater antioxidant capacity and stronger inhibition of cancer cells proliferation than other carotenoids (Omoni and Aluko, 2005). The antioxidant ability of several carotenoids (carotenes and xanthophylls) follows the sequence from high to low: lycopene > ß-cryptoxanthin ≈ ßcarotene > lutein ≈ zeaxanthin > α-carotene > canthaxanthin. Carotenogenesis occurs parallel to the loss of chlorophyll during fruit ripening and rends the yellow, orange and red colour of several fruits such as tomato, pepper, yellow plums, peaches, nectarines, apricots, etc. When the antioxidant capacity of fruits and vegetables is examined separately on hydrophilic and lipophilic extracts the antioxidant activity derived from lipo-soluble molecules (L-TAA) has been correlated with total carotenoids in both flesh and peel of plum cultivars (Valero & Serrano, 2010) as well as in tomato fruits (Lenucci et al., 2006), vegetables and legumes (Cho et al., 2007).

4.2.4. Vitamins

Vitamins are a class of nutrients that are essentially required by the human body for its biochemical and physiological functions. Vitamins are subdivided into fat-soluble and water soluble, the vitamins A, D, E and K being lipophilic, while C and B are hydrophilic. Tocopherols (vitamin E) and carotenoids (pro-vitamin A) are the major lipid-soluble antioxidant vitamins in fruits and vegetables, while vitamin C is the major hydrophilic antioxidant vitamin, although recent evidence indicates that vitamin D could also have a role as an antioxidant (Asensi-Fabado and Munné-Bosh, 2010).

Natural vitamin E is comprised of eight different forms, namely α-, ß-, γ- and δ-tocopherols and the α-, ß-, γ- and δ-tocotrienols. The tocotrienols have an unsaturated isoprenoid side chain, while the tocopherols contain a trimethyltridecyl tail. These compounds are exclusively synthesised in photosynthetic tissues, but accumulate predominantly in the seeds. Thus, plant oils represent the major sources of vitamin E in the human diet, α-tocopherol being predominant in olive and sunflower oils, γ-tocopherol in corn oil and δ- tocopherol in soybean oil, whereas the tocotrienols are the major vitamin E components of palm oil.

In fact, vitamin E has been recognized as one of the most important antioxidants due to its capacity to scavenge directly reactive oxygen species (ROS) and nitrogen species and to up-regulate the activities of antioxidant enzymes. In this sense, vitamin E inhibits ROS induced generation of lipid peroxyl radicals, thereby protecting cells from: a) peroxidation of polyunsaturated fatty acids in membrane phospholipids, b) oxidative damage of plasma very low-density lipoprotein, cellular proteins and DNA, and c) membrane degeneration. Accordingly, a dietary deficiency of vitamin E reduces the activities of hepatic catalase (CAT), glutathione peroxidases and reductases, induces liver lipid peroxidation, and causes neurologic and cardiovascular disorders, which can be reversed by dietary vitamin E supplementation (Zingg, 2007).

Vitamin C was first isolated in 1928 by the Hungarian biochemist and Nobel Prize Szent-GyorGyi. Ascorbic acid (vitamin C) is a familiar molecule due to its dietary significance since a diet devoid of it causes scurvy. Ascorbic acid has four isomers but only the L-ascorbic and L-dehydroascorbic acids have physiological activity as vitamin C. Since dehydroascorbic acid can be easily converted into ascorbic acid in the human body, it is very important to measure both isomers to determine the vitamin C capacity, for which dehydroascorbic supposes ca. 10% of total vitamin C.

Apart from the vitamin role of ascorbic acid, vitamin C has great importance as an antioxidant, being highly effective in inhibiting lipid peroxidation initiated by peroxyl radical and acting as radical scavenger of ROS. Ascorbic acid acts as an antioxidant at two levels: a) in the cytosol, in which ascorbate acts as a primary antioxidant to scavenge free radical species that are generated as by-products of cellular metabolism, and b) in cellular membranes, in which ascorbic acid may play and indirect antioxidant role to reduce the α-tocopherooxyl radical to α-tocopherol, and thus recycling the latter. Thus, these two vitamins (C and E) can be effective partners in reducing the destructive processes of lipid peroxidation (Asensi-Fabado and Munné-Bosh, 2010).

The strong antioxidant activity of ascorbic acid is considered responsible for its effect in lowering the risk of some degenerative and cardiovascular diseases. Ascorbic acid is widely distributed in nature, the main food sources of vitamin C being fruits, with large variations among the different species. Thus, citrus species, kiwifruit, pepper and other leafy vegetables are considered to have the highest content of vitamin C (100–200 mg 100/g), compared with the 1–10 mg 100/g found in pomegranate, pear, stone fruits and apple (Valero and Serrano, 2010).

The ascorbic acid pool is affected by synthesis, transport, catabolism and recycling processes contributing specifically in the different plant tissues (Ishikawa et al., 2006). It is generally accepted that immature green fruits contain higher concentrations of ascorbic acid than mature ones, in which the diminution of ascorbic acid coincides with the beginning of the colour changes, as has been observed in tomato (Serrano et al., 2008b),

although in pepper fruit ascorbic acid concentration increased as did the developmental process and colour changes from green to red (Serrano et al., 2010); the same occurred in apricot, peaches and papayas (Lee and Kader, 2000).

4.2.5. Gluosinolates

Other phytochemicals with contrasted antioxidant activity are those compounds with sulphur radicals, such as glucosinolates present in cruciferous species, the most important being *Brassica* vegetables such as broccoli. More than a hundred different glucosinolates have been identified although all of them share a common structure comprising a sulphonated moiety, a ß-D-thioglucose and a variable side-chain with amino acid nature. The three major classes of glucosinolates are aliphatic (derived from L-methionone homologues), aromatic (derived from L-phenylalanine homologues and L-tyrosine) and indolic (derived from L-tryptophan). The most important glucosinolates found in *Brassica* vegetables are those derived from methionine (Moreno et al., 2006).

The glucosinolates are chemically stable and biologically inactive while they remain sequestered within sub-cellular compartments throughout the plant. However tissue damage caused by pests, harvesting, food processing or chewing initiates contact with the endogenous enzyme myrosinase (thioglucoside glycohydrolase). This leads to rapid hydrolysis of the glucosidic bond, releasing glucose and an unstable intermediate, which undergoes a spontaneous rearrangement to form a complex variety of breakdown products, the most important being isothiocyanates followed by indoles, nitriles and thiocyanates. These autolytic breakdown products have exhibited protective effects against many types of cancer, in both *in vivo* and *in vitro* studies, by modulating the induction of detoxification enzymes and the inhibition of activation enzymes (Cartea and Velasco, 2008; Traka and Mithen, 2009). The main factors influencing the levels of glucosinolates in vegetables are genetic, ecophysiological (such as the climate parameters of irradiation, temperature, water and nutrition supply), storage and processing conditions (Moreno et al., 2006).

4.3. Health-related Interactions of Fruit Phytochemicals

From the above sections, it can be concluded that there is a wide range of phytochemical compounds with antioxidant properties and health-benefits. However, recent reviews have shown that there are additive and synergic effects of bioactive compounds in fruits and vegetables, which confer altogether more antioxidant and anticancer activity, among other diseases, than the sum of the individual components. Moreover the dietary supplements with a particular phytochemical do not have the same health-benefits as a diet rich in fruits and vegetables, since taking alone, the individual

antioxidants studied in clinical trials do not appear to have consistent preventive effects. One of the most documented examples is the relationship between lycopene and prostate cancer, for which tomato fruit ingestion confers cancer chemoprotective effects, but it is not clear if lycopene alone is able to have a similar effect (Lila and Raskin, 2005; Seifried et al., 2007).

4.4. Changes in Phytochemicals during Postharvest Storage

4.4.1. Cold storage

Storage at low temperatures is the main postharvest treatment used to reduce produce metabolism, maintain quality and prolong storability in those perishable fruits and vegetables considered as non-chilling sensitive. Some evidence exists for the changes in bioactive compounds and antioxidant activity during cold storage although no general tendency has been found. Thus, loss of health-beneficial compounds (phenolics and ascorbic acid) has been found in table grapes (Serrano et al., 2006a), broccoli (Serrano et al., 2006b), pomegranate (Sayyari et al., 2010) and apple (Carbone et al., 2011), in which the loss of phenolics was highly dependent on cultivar. However, increases in phytochemicals were reported for sweet cherry and plum cultivars during cold storage (Valero and Serrano, 2010), which were related to the advancement of the ripening process. Accordingly, increase in anthocyanin concentration has been found in red orange during storage at 4°C due to an enhanced expression of the structural genes involved in anthocyanin biosynthesis, such as phenylalanine ammonia lyase (PAL), chalcone synthase, dehydroflavonol 4-reductase and UDP-glucose flavonoid glucosyl transferase (Amarowicz et al., 2009).

4.4.2. Heat treatment

Information on the influence of heat treatments on nutritional and bioactive compounds is scarce. In this sense, heat treatments have been postulated as physical elicitors that affect the biosynthesis of phytochemicals and antioxidant properties of horticultural crops (Schreiner and Huyskens-Keil, 2006). For example, arils from heat-treated pomegranates (45°C for 4 min) exhibited higher H-TAA than controls after 45 days of cold storage, which was correlated primarily with the high levels of total phenolics and to a lesser extent to ascorbic acid and anthocyanin contents (Mirdehghan et al., 2006). Accordingly, in peaches, air heat treatment (38°C, 12 h) significantly increased the content of total phenolics after 3 and 4 weeks of storage and also prevented the increase in polyphenol oxidase (PPO) activity and accelerated the decrease in peroxidase (POX) activity that usually occurs in non-heated fruits (Jin et al., 2009).

Hot water treatment in tomato (35°C, 12 h), melon (55°C, 5 min) and mango (42°C, 24 h) inhibited PPO and POX activities leading to delayed anthocyanin degradation and protection of colour pigment changes, by maintaining the anthocyanins in their red-pigmented form with high antioxidant activity during postharvest storage (Schreiner and Huyskens-Keil, 2006).

Increases in total carotenoids, ß-carotene and lycopene during storage were found in tomato heated with air at 34°C for 12 h, which might indicate that heat treatment induced faster fruit ripening once the heat stress was removed and a promotion of the tomato antioxidant system (Yahia et al., 2007). In contrast, total carotenoids decreased during storage of kumquat, and lower levels of total carotenoids were recorded in heat-treated fruits in comparison to their initial values (Schirra et al., 2008).

The effect of heat application on vitamins during storage caused either increases or decreases, or did not affect vitamin content. Thus, tomato heat-treated with air (34°C, 24 h) exhibited lower ascorbic acid and α-tocopherol than control fruits, the diminution being enhanced when 38°C and 12 h were used, which was related to acceleration of the ripening process (Yahia et al., 2007). In kumquat, the levels of ascorbic acid remained unchanged during storage at 17°C while vitamin E (α-tocopherol and γ-tocopherol) increased, but hot water treatment (50°C, 2 min) did not modify this behaviour (Schirra et al., 2008). The application of the heated air (45°C, 3h) to strawberry resulted in higher antioxidant capacity than the control immediately after harvest and lasted until day 7 of storage, which was correlated with the increase in ascorbic acid in response to heat treatments (Vicente et al., 2006).

In summary, with this simple and non-contaminant technology, the functional and nutritive properties, after long periods of storage, could then be even greater than in recently-harvested fruits, thus providing a high content in health-beneficial compounds to consumers after the intake of these fruits.

4.4.3. Calcium treatment

The effect of calcium (Ca^{2+}) treatments on bioactive compounds with functional properties has been only addressed in few reports and in very recent years. Thus, Ca^{2+} applied as a preharvest treatment in litchi fruits increased anthocyanin concentration in the skin of fruits from trees sprayed with Ca^{2+} at the fruit set and fruitlet stage, the concentration of anthocyanin being correlated with the calcium concentration of the skin (Cronje et al., 2009). In pomegranate arils this treatment induced an increase in ascorbic acid (Ramezanian et al., 2009).

In contrast, in green and red pepper carotenoid and provitamin A contents were lower when Ca^{2+} was increased from 2 to 8 mmol/L, but the content of total phenolics was

enhanced (Marín et al., 2009). With respect to postharvest treatments, Ca^{2+} treatments on papaya fruit led to maintenance of higher ascorbic acid concentrations during storage as compared with that in nontreated fruits, this effect being greater with vacuum infiltration than with dipping treatment (Mahmud et al., 2008). Then, due to the antioxidant properties of anthocyanins, ascorbic acid and lycopene, these results would lead to contradictory conclusions, that is, a possible role of Ca^{2+} treatments is that it both increases and decreases of functional properties of fruits, and thus, more research is needed to clarify this issue.

4.4.4. Polyamine treatments

There is little information about the effect of PA treatment on the concentration of bioactive compounds in fruits. The first evidence of the in vivo role of PAs in the fruit ripening process was obtained with transgenic tomatoes. Engineering of tomato fruit with the yeast SAMDC1 gene under a fruitspecific promoter led to an increase in spermidine (Spd) and spermine (Spm) concentrations throughout the fruit growth cycle, and red ripe transgenic tomatoes accumulated 200–300% more lycopene than did the red fruits from the parental lines, which is of special significance as tomato fruits are an important source of lycopene for human consumption (Mehta et al., 2002). Recently, was reported that in pomegranate arils, the application of 1 mM of putrescine (Put) or Spd, either by pressure infiltration or immersion, was effective in maintaining the concentration of total anthocyanins and total phenolics compounds as well as the H-TAA at higher levels than in control fruits during storage (Mirdehghan et al., 2007c). The mechanism by which Put and Spd induces these effects is still unknown, although they may be related to their antisenescence and antioxidant effects (Valero et al., 2002a; Valero and Serrano, 2010).

In contrast, pre- and postharvest Put application to 'Angelino' plum led to linear reduction in the levels of ascorbic acid, carotenoids and TAA during postharvest storage, which was more pronounced with increased concentrations of Put and storage periods, these effects being ascribed to increased ascorbate oxidase activity (Khan et al., 2008). Thus, more research is needed to clarify the effects of PA treatments on fruit functional compounds. Finally, it is interesting to point out that although most papers dealing with PA postharvest treatments in fruits have reported on the use of pressure infiltration in order to ensure the PA intake, Mirdehghan et al. (2007c) showed that the immersion method could be considered, as it is associated with lower cost, is easier to handle than pressure infiltration and could be incorporated as a continuous process in the horticultural industry.

4.4.5. 1-Methylcyclopropene treatments

1-MCP is a powerful inhibitor of ethylene action that is capable of maintaining postharvest quality in many fresh horticultural products since it binds to the ethylene receptor with 10 times more affinity than ethylene itself, being more active at much lower concentrations (Valero and Serrano, 2010). However, there is little information about how 1-MCP can modulate the content of the bioactive compounds and antioxidant activity during postharvest storage of vegetable products. Generally, 1-MCP slowed the decrease of ascorbic acid (Vitamin C) in peach, pineapple, quince and tomato, but ascorbic acid in apple showed lower content in 1-MCP-treated than in control fruits (Vilaplana et al., 2006; Valero and Serrano, 2010). With respect to TAA, both H-TAA and L-TAA, 1- MCP increased the antioxidant capacity in sweet cherry by increasing the content of total phenolics or flavonoids, although hydroxycinnamic acids and anthocyanins were unaffected (Mozetiĉ et al., 2006). Conversely, 1-MCP delayed the increase in total phenolics and anthocyanins occurring during ripening of strawberry, which was associated with lower PAL activity (Jiang et al., 2001). In mango, 1-MCP inhibited the production of H_2O_2 and maintained higher ascorbic acid during storage (Wang et al., 2009). The authors also found that 1-MCP inhibited the activities of antioxidant enzymes including CAT, superoxide dismutase (SOD) and ascorbate peroxidase, suggesting that 1-MCP could play a positive role in regulating the activated oxygen metabolism balance. In air-stored apple fruits, total phenolic concentration was higher in the peel of 1-MCP-treated fruits than in the controls, but slightly lower in the flesh of 1-MCP treated fruit (Vilaplana et al., 2006). Thus, further research is warranted on the effects of 1-MCP on various non-enzymatic and enzymatic antioxidant systems to better understand how 1-MCP can enhance TAA.

4.4.6. Modified atmosphere packaging (MAP)

MAP storage has been shown to have a beneficial effect on maintaining bioactive compounds of fruits and vegetables, although currently there are only a few reports about this issue. Broccoli heads lost half of their initial H-TAA, phenolics and ascorbic acid after 21 days of cold storage, while these losses were minimised in broccoli packaged with micro- perforated and non-perforated polypropylene (PP) films. In addition, H-TAA has been correlated in broccoli with total phenolics and to a lesser extent with ascorbic acid (Serrano et al., 2006b). Similarly, important losses in ascorbic acid occurred in loquat stored at 2ºC, while levels at harvest were maintained in loquat stored under MAP conditions (Amorós et al., 2008). In papaya, MAP helped in maintaining the antioxidant potential of fruits by retaining acceptable levels of antioxidants, such as ascorbic acid and lycopene (Singh and Rao, 2005).

In plums, total phenolic, total anthocyanins and antioxidant activity increased during the first weeks of cold storage and decreased when fruit was over ripe and in

senescence. This evolution was delayed by the use of MAP packages, due to the effect of MAP (low O_2 and high CO_2) on retarding the postharvest ripening process, as can be inferred by the reduced ethylene production, fruit softening, colour evolution and acidity loss (Valero and Serrano, 2010). In addition, the possible effect of low O_2 and high CO_2 on the delay in PAL, a key enzyme in the biosynthesis pathway of phenolic compounds, or the reduced PPO activity (the enzyme responsible of polyphenol degradation) should not be discharged.

Anthocyanins tend to increase with ripening during postharvest storage in many fruits, such as apples, sweet cherries, strawberries, blueberries and raspberries; lycopene also tends to increase with ripening in tomato and watermelon. However, under MAP conditions, these increases have been reported to be lower than in control fruits stored in open air, due to the effect of MAP on delaying the evolution of the postharvest ripening process (Jones, 2007).

4.4.7. Processing

Most fruits and vegetables are consumed as raw commodities although for centuries humans have transformed these perishable products into more stable, delicious and safer dishes. The most used unit operations include blanching, freezing, canning and cooking (boiling, frying, baking or microwaving). These operations affect the nutritional content of fruits and vegetables and, in general, there is significant loss of nutrients as well as of bioactive compounds with antioxidant activity.

4.4.8. Blanching, freezing and canning

Fresh produce contains enzymes which cause loss of colour and nutrients, flavour changes, and colour changes in canned and frozen fruits and vegetables. Blanching is the exposure of the vegetable to boiling water or steam for a brief period of time to inactivate naturally occurring enzymes but also helps to destroy microorganisms on the surface. Most reports dealing on antioxidant compounds losses studied the combination of blanching + freezing and blanching + canning.

Ascorbic acid (vitamin C) has been used as a model to evaluate nutrient losses during fruit and vegetable processing by calculating its retention, since it is considered the least stable of the bioactive compounds. In general, Vitamin C content decreases during thermal processing conditions, although large variations exist among vegetable products, ranging from 90% in carrots to 8% in beets (Rickman et al., 2007).

Ascorbic acid remained in the drained liquid but is usually rapidly oxidized and its concentration is negligible. Generally, higher losses of ascorbic acid have been found after the canning operation compared with freezing for the same commodity. During prolonged

storage, vitamin C is also degraded, the losses being higher (2-3 fold) in frozen than in canned products. Microwave cooking did not affect ascorbic acid concentration of some vegetables such as broccoli, carrot or green beans (Howard et al., 1999).

Phenolic compounds decreased after thermal processing mainly due to leaching into the brine or syrup in the caning process. Thus in cherries, 50% of total phenolics was lost during canning and was found in the syrup transferred from the fruit (Chaovanalikit and Wrolstad, 2004). During freezing the content of total phenolics is largely dependent on fruit type. Thus, no losses were found in peaches, while 40% was lost in cherries after 3 months of storage. Another way to loose total phenolics is the peeling process before canning, since it is known that peel contain higher content of phenolics, as has been reported for peach and nectarine (Legua et al., 2011).

4.4.9. Cooking

Cooking is an important factor that can impact the antioxidant capacity of fruits and vegetables, although this issue has been poorly studied. Generally, cooking is considered destructive to antioxidant compounds. However, potato (baked) and broccoli, carrot or tomato (boiled) exhibited a different behaviour when H-TAA and L-TAA were determined compared with the raw material. H-TAA and L-TAA were reduced in the cooked broccoli and carrot, and the opposite occurred for tomato, while baked potato showed H-TAA but decreased L-TAA (Wu et al., 2004b). However, steam processing elevated both H-TAA and L-TAA (Roy et al., 2009) in broccoli.

Losses of ascorbic acid (15–55%) were reported depending on the cooking method (Goyal, 2000). Similarly, several studies have shown that lycopene or ß-carotene degrades during cooking, mainly attributed to isomerisation and oxidation. In the case of carrot, heating does not impact negatively on antioxidant compounds. In pepper, boiling led to decreased (28%) capsacinoid content but grilling enhanced (90%) their content (Ornelas-Pas et al., 2010). In other studies lycopene increased with cooking and processing operations, and also its bioavailability, since heating resulted in the promotion of trans-cis lycopene isomerization due to formation of cis-lycopene, which is known to be more bioavailable than all-trans-lycopene, the predominant form of lycopene in plants (Jones, 2007). However, in this fruit, total phenolics decreased with thermal processing, the diminution being directly related to the increase in temperature (Pérez-Conesa et al., 2009). In peppers, boiling (96°C) and grilling (210°C) induced significant losses in total phenolics, with the magnitude being dependent on the cultivar (Ornelas-Pas et al., 2010).

Anthocyanins are easily degraded during thermal processing, affecting both colour quality and nutritional properties (Patras et al., 2010). Anthocyanin and other flavonoid content tends to decline when cooking with water as reported for blueberry, bilberry and maize, among others (Ornelas-Pas et al., 2010), although they remain in the boiling water.

However, the magnitude and duration of heating has a strong influence on anthocyanin degradation after thermal processing, with logarithmic course degradation related to arithmetic increased temperature. In addition, during storage of thermally processed commodities, anthocyanin showed continuous degradation affecting colour and antioxidant capacity (Sadilova et al., 2007).

Glucosinolates from broccoli heads decreased after boiling or microwave cooking with losses about 40% less leaking to the water, although the rate of loss depended on type and time of cooking and water volume, although generally steam boiling induced the lowest losses (Ornelas-Pas et al., 2010).

5. THE PLUM AND SWEET CHERRY *Prunus* SPECIES

5.1. Plum Taxonomy and Cultivars

The scientific taxonomy of plums is:

Kingdom: *Plantae*

Division: Magnoliophyta

Class: Magnoliopsida

Order: Rosales

Family: *Rosaceae*

Subfamily: *Maloideae* or *Spiraeoideae*

Genus: *Prunus*

Subgenus: *Prunus*

The two most cultivated plum species are *Prunus salicina* Lindl., Japanese plum, and *Prunus domestica* L., European plum. Plums are nutritious stone fruit, and plum tree is grown in cool subtropical to temperate regions throughout the world. Japanese plums are mainly consumed as fresh fruit, while European types are popular for processing. The European plum probably originated in Eastern Europe or western Asia around the Caucasus and the Caspian Sea. It has been known in Europe for more than 2000 years. Prunes are the most important subgroup. Others are the greengage, yellow egg, imperatrice, and lombard groups. The Japanese plum originated in China and was domesticated in Japan.

There are as many as 140 plum fruit varieties that can be found across all the continents, except Antarctica. Among which approximately 20 varieties are grown for commercial purposes, which differ in agronomical characteristics, crop yield and fruit size and quality attributes. There are many varieties of plums that may vary in size. Plums come in a variety of colours like blue-black, red, orange and yellow. The juicy flesh is generally light orange or pinkish in colour. The most commercial plum cultivars are described next:

Angeleno: The heavily-planted Angeleno plum yields purple to black fruit with good size if thinned. The sweet, yellow flesh of this variety is meaty, but can be somewhat dry near the freestone pit. Angeleno is harvested the 1st week of September, and requires a mid-season-blooming pollenizer. This variety has an estimated chilling requirement of 400-450 hours.

Blackamber: The Blackamber plum is a firm dark purple to black fruit that resembles the Friar plum. The Blackamber tree grows rapidly and bears a heavy crop in the 4th week of

June. This heavily planted variety blooms mid-season and requires a pollenizer. Blackamber has an estimated chilling requirement of 400 hours.

Larry Ann: This plum is a mid-late cultivar (mid August) with red-purple skin colour and yellow flesh colour and produces good caliber fruits.

Black Diamond: The plums are large and firm with black skin and pink-to-red flesh and have been developed to have juiciness and mildly-sweet flavour that is superior to other plums of the season. It is harvested beginning summer.

Golden Japan: Large fruit, light yellow straw, glowing skin thick and tough, meat very juicy and pleasant which belongs to the Japanese plum. The tree is vigorous and high fertility. It is grown in the U.S., France, Italy and South Africa, to be available from January to May and June to August. In Spain, the harvest is in mid-June.

Songold: Bred by Infruitec in South Africa from a cross between Wickson and Golden King. It was released in 1970. The fruit is very good, yellow flesh, semi-melting and firm texture and clingstone. The fruit is large, conical shape and light-red when ripe. It is a late harvested cultivar (end of September).

Golden Globe: A distinct variety of plum tree characterized by its large size, vigorous upright growth and being a regular and productive bearer of large, firm, yellow flesh, clingstone fruit with good flavour and eating quality.

The marketing standards for plums are divided in the next categories (COMMISSION REGULATION (EC) No 1168/1999):

Extra class: Plums in this class must be of superior quality. In shape, development and colouring they must be characteristic of the variety. They must be:

— Practically covered by their bloom, according to variety.

— Of firm flesh.

They must be free from defects, with the exception of very slight superficial defects, provided these do not affect the general appearance of the produce, the quality, the keeping quality and presentation in the package.

Class I: Plums in this class must be of good quality. They must be characteristic of the variety. The following slight defects, however, may be allowed, provided these do not affect the general appearance of the produce, the quality, the keeping quality and presentation in the package:

— A slight defect in shape.

— A slight defect in development.

— Slight defects in colouring.

— Skin defects of elongated shape must not exceed in length one third of the maximum diameter of the fruit. In particular, healed cracks may be allowed for 'Golden gage' varieties.

— Other skin defects of which the total area affected must not exceed one sixteenth of the whole surface.

Class II: 10% by number or weight of plums satisfying neither the requirements of the class nor the minimum requirements, with the exception of produce affected by rotting, market bruising or any other deterioration rendering it unfit for consumption.

5.2. Sweet Cherry Taxonomy and Cultivars

The scientific classification of sweet cherry is:

Kingdom: *Plantae*

Division: Magnoliophyta

Class: Magnoliopsida

Order: Rosales

Family: *Rosaceae*

Subfamily: *Prunoideae*

Genus: *Prunus*

Subgenus: *Cerasus*

Cherry is a fruit belonging to the genus *Prunus* in the Rosaceae family, which contains over several hundred species distributed across northern temperate regions. The sweet cherry (*P. avium* L.) is native to Europe and western Asia, while the tart cherry (*P. cerasus*) is produced from the Montmorency cultivar. The cherry fruit is considered a nutrient dense food with a relatively low caloric content and a significant amount of important nutrients and bioactive food components.

Sweet cherries are very attractive fruits and one of the few non-surplus fruit crops in Europe (Esti et al., 2002); and their production is therefore not limited only to the areas with ideal environmental conditions. In many of the European sweet cherry production areas, rain occurring in the period of cherry ripening causes crop losses. Fruit cracking, fruit softening and rapid decay after harvest are major problems which cause crop losses in sweet cherry production. This is the reason for frequent premature picking of sweet cherry fruits of lower quality. Early-harvested cherries show insufficient size, low content of soluble solids and moderate colour (Usenik et al., 2005).

In recent years, the number of fresh-market sweet cherry cultivars (varieties) produced in the world has grown from a few, dominated by one ('Bing'), to a dozen or more. 'Lapins' was among the first new cultivars to gain significance in the mid-1990s, followed by 'Sweetheart', 'Chelan', 'Tieton', and others. Some of these early selections have lost favor with growers as production problems have become apparent and breeding programs release superior alternatives.

Even with the inevitable struggles and growing pains of an industry rapidly adopting new, largely untested cultivars, the potential benefits are significant. With multiple varieties, bloom times are staggered, reducing the risk that the entire crop will be affected by poor weather during pollination. In addition, producing early- and late-ripening cultivars lengthens the growing season, potentially providing greater early- and late season financial returns when supplies are limited. An extended harvest season also reduces labor demand as fewer pickers are needed over a longer period of time.

The most commercial sweet cherry cultivars are described next:

Sweetheart: As the name suggests, the Sweetheart cherry is a large, bright red, heart-shaped cherry. Sweetheart™ matures at the end of the season, about 5 to 7 days after Lapins and remains firm after picking. This self-fruitful Van x Newstar cross is a productive variety offering good firmness, size and flavour with moderate cracking. The Sweetheart cherry tree is spreading and precocious. Sweetheart yields heavy crops on all rootstocks, but requires pruning to maintain optimal size.

Santina: The Santina cherry is moderately large and firm with highly attractive, lustrous skin. With a flattened heart shape and medium long stems, Santina is a good choice for the early market. This tree is self-fertile, without oversetting and is considered to be moderately tolerant to splitting.

Cristalina: Cristalina is an early-maturing cherry that ripens approximately 5 days before Bing. The Cristalina cherry is heart-shaped and plump with dark red-to-black skin and firm, dark flesh. The sweet Cristalina fruit is moderately large on long, thick stems and can be picked stemless. The tree is very productive with wide, spreading branches. Cristalina requires a pollenizer and is interfruitful with Bing, Rainier and Skeena.

Newstar: Large fruited, early season cherry with yields similar to Hedelfingen but more susceptible to cracking. Ripens 1 day after Viva. Fruit is firm, but colour is lighter than Hedelfingen. Tree is very spreading, comes into production early, and fruit is well distributed.

Sunburst: Fruit are large and tend to be firm, dark red with good colour. The tree is self-fertile and sets heavy crops. Matures 3 days after Van.

Sonata: With lustrous mahogany to black skin, a plump kidney shape, and a prominent blossom dimple, the Sonata cherry is quite memorable. Sonata matures in an important

harvest window between Bing and Lapins with harvest beginning May 31st. Offering a well-balanced sweet flavour, Sonata cherries are very large with long, thick stems. The texture of this cherry is very firm and fine-grained with a moderate susceptibility to natural cracking. The upright Sonata tree is self-fertile and vigorous. It is a productive variety that bears well each year.

Brooks: Developed by the University of California, the Brooks cherry is a large, firm red cherry that tolerates hot climates. Brooks ripens ahead of Bing and requires a pollenizer such as Early Burlat, Tulare or Rainier. This variety is very susceptible to cracking in rain.

Somerset: Trees of `Somerset` have been uniquely precocious in setting fruit earlier in their life time than many other cultivars. `Somerset` trees also have had a unique branching habit wherein they have lateral branches that are more numerous than most other cherry cultivars. This branching habit produces more opportunities for flower buds to form on previous season's growth leading to more non-spur fruiting than on most other sweet cherry cultivars.

The marketing standards for plums are divided in the next categories (COMMISSION REGULATION (EC) No 214/2004):

Extra class: Cherries in this class must be of superior quality. They must be well developed and have all the characteristics and the typical colouring of the variety. They must be free from defects with the exception of very slight superficial skin defects, provided these do not affect the general appearance of the produce, the quality, the keeping quality and presentation in the package.

Class I: Cherries in this class must be of good quality. They must be characteristic of the variety. The following slight defects, however, may be allowed provided these do not affect the general appearance of the produce, the quality, the keeping quality and presentation in the package:

— A slight defect in shape — A slight defect in colouring.

They must be free of burns, cracks, bruises or defects caused by hail.

Class II: This class includes cherries which do not qualify for inclusion in the higher classes, but satisfy the minimum requirements specified above. The following defects may be allowed provided the cherries retain their essential characteristics as regards the quality, the keeping quality and presentation:

— Defects in shape and colouring, on condition that they retain the characteristics of the variety.

— Small, healed surface scars, not likely to impair significantly the appearance or the keeping quality of the fruit.

Size is determined by the maximum diameter of the equatorial section. Cherries must have the following minimum sizes:

– 'Extra' Class: 20 mm – Classes I and II: 17 mm.

5.3. Production Facts

The diversity in the available plum cultivars, wide area of distribution, and adaptability to varying conditions make it an important stone fruit throughout the world. At present, among different plum producing countries, China contributes over 48% of total plum volume in the world, followed by USA, Serbia, Romania, and Chile which contribute about 6.9%, 6.8%, 3.8% and 3% shares to world plum production, respectively.

Spain is the 7th producer country with more than 200,000 tons (**Figure 14**). Within Spain, Extremadura Region is the first producer area followed by Andalucía, Murcia, Aragón and Comunidad Valenciana (**Figure 15**).

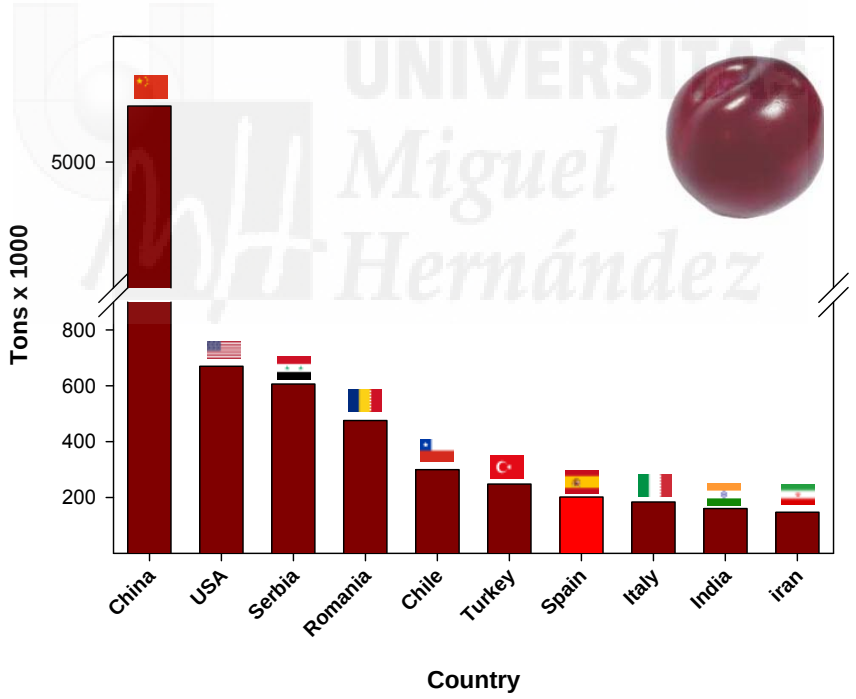

Figure 14:Total plum production by country Year 2010. Source: FAOSTAT (www.fao.org). Accesed April 2011. The first top 10 countries are displayed only.

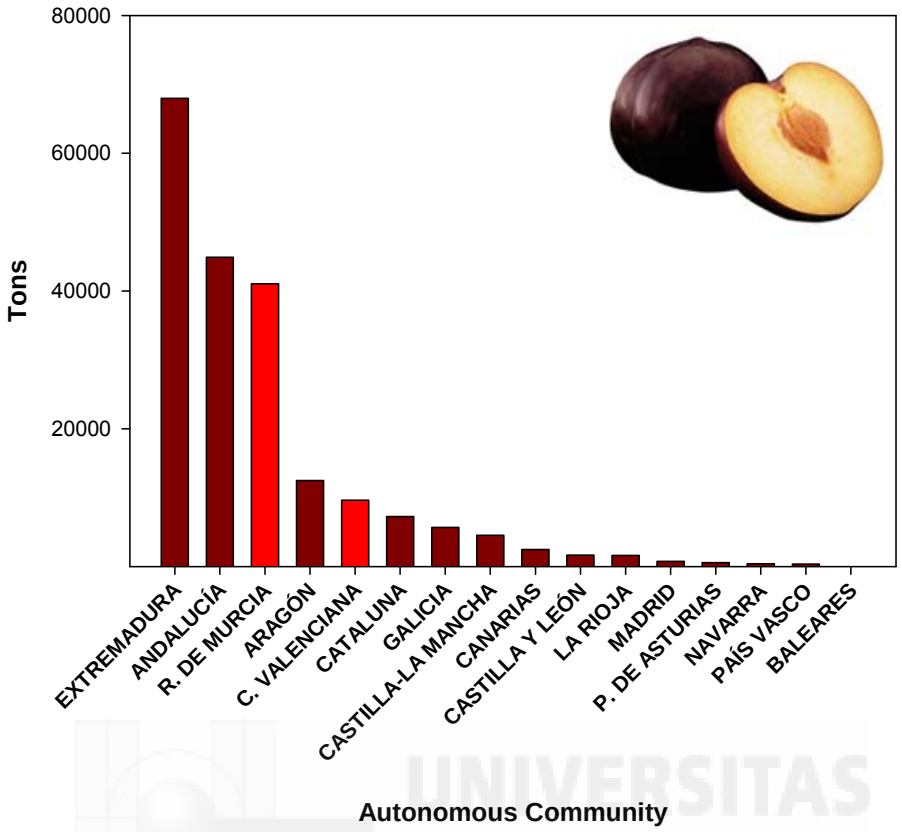

Figure 15:Total plum production by Region in Spain Year 2008. Source: MAPA (www.mapa.es). Accesed April 2011.

At present, among different sweet cherry producing countries, Turkey contributes over 25% of total cherry volume in the world, followed by USA, Iran, Italy, Russia and Syria. Spain is the 7th producer country with more than 72,000 tons (**Figure 16**). In Spain, the sweet cherry "Cereza del Jerte" is a high quality fruit of varieties from the "Jerte Valley" region, in the centre-west of Spain. Autochthonous varieties, mainly 'Ambrunés', 'Pico Negro', are hand-picked without stems (type "Picota") and marketed under the Registry of the Protected Designation of Origin "Cereza del Jerte" (Serradilla et al., 2008). Spain is one of the main cherry producers in Europe. Indeed, several regions in Spain have optimum conditions for cherry production. However, the highest prices for cherries can be obtained in other European countries, such us Norway or Sweden, due to their higher economic status and also because they do not produce cherries. Spanish producers can obtain maximum profit from their cherries if they arrive first to these northern European markets. For this, early cherry cultivars need to be grown (Remón et al., 2003). **Figure 17** shows the main production areas within Spain, in which Extremadura is the region with the highest production (about 25% of the total) followed by Aragón, Castilla León, Cataluña and Andalucía.

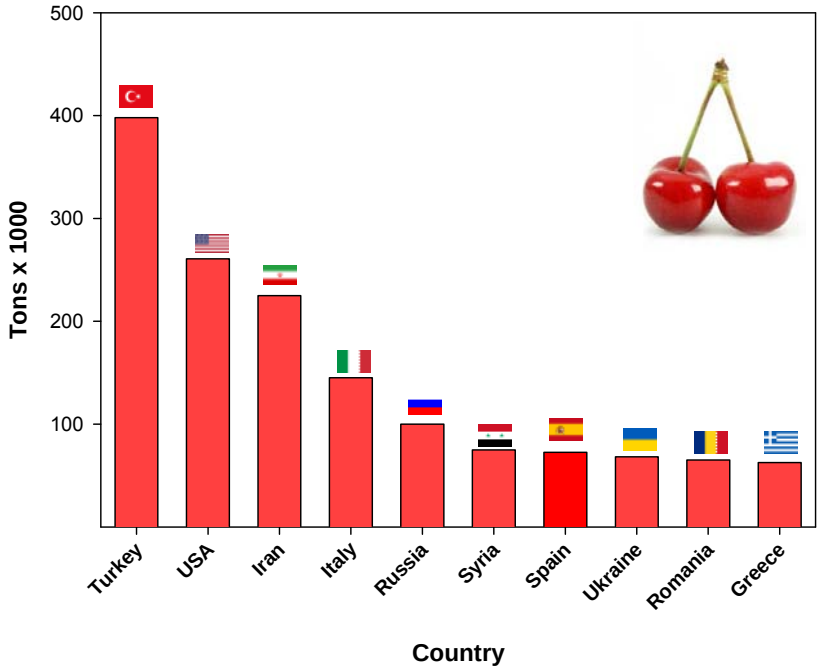

Figure 16:Total sweet cherry production by country Year 2010. Source: FAOSTAT (www.fao.org). Accesed April 2011. The first top 10 countries are displayed only.

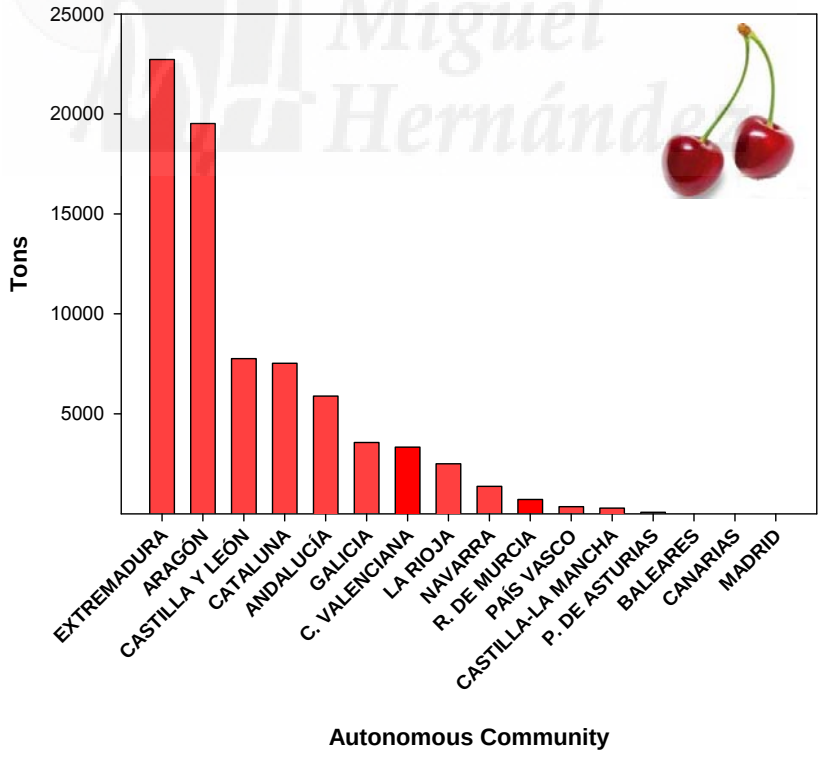

Figure 17. Total sweet cherry production by Region in Spain Year 2008. Source: MAPA (www.mapa.es). Accesed April 2011.

5.4. Chemical Composition of Plum

The Japanese plum (*Prunus salicina* Lindl.) is grown in a number of the autonomous regions of Spain and especially in Extremadura, Valencia, Murcia, Andalucía, Aragon and Catalonia. In recent years, this species has been the object of a remarkable expansion and specialisation, giving it an important role in exports, primarily to countries within Europe. There is literature about the physico-chemical parameters of the different plum cultivars, especially colour, firmness, total soluble solids and acidity. However, very few reports have studied the chemical composition of the several plum varieties.

According to USDA (2006) plums contain essential minerals, sugars and vitamins and are fruits with a good source of fiber (**Table 7**).

Table 7. Chemical composition of fresh plums (per 100 g)

Nutrient	Concentration
Energy (kcal)	83
Protein (g)	0.8
Fat (g)	0.2
Carbohydrate (g)	21
Total sugars (g)	19.4
Sucrose (g)	4.5
Glucose (g)	6.1
Fructose (g)	3.4
Sorbitol (g)	5.4
Fiber (g)	1.5
Vitamin C (mg)	9.5
Vitamin A (IU)	717
Vitamin E (mg)	0.85
Vitamin B2 (mg)	0.10
Vitamin B6 (mg)	0.08
Potassium (mg)	221
ß-carotene (µg)	430
Lutein/Zeaxanthin (µg)	240
Total anthocyanin (mg)	7.6
Total phenolics (mg)	111

Lozano et al. (2009) determined the chemical composition of 4 Japanese plum cultivars cultivated in Spain: 'Black Amber', 'Suplumeleven', 'Fortune', 'Larry Ann', 'Suplumsix' and 'Songold'. Water was the most abundant constituent of plums, ranging from 80.65% in 'Larry Ann' to 89.40% in 'Suplumeleven'. The plum has a mean sugar content of 15% (between 12.3 and 17.8%), dietary fibre 1.2% (from 0.84 to 1.50%) and a mean energy value of 255 kJ 100 g^{-1} of fresh produce (from 183 to 331 kJ 100 g^{-1}).

Nergiz and Yıldız (1997) studied the chemical composition of 11 European plum cultivars grown in Turkey: 'Baneasa 3/5', 'Fruhzwetche', 'Tuleu Timpuriu', 'Baneasa 9/13','Prune 2740','Giant Prize', 'Krikon Damson', 'Giant', 'Imperial Epineuse', 'Victoria' and 'Stanley'. Mean chemical compositions for all varieties were: moisture (837.4 g kg^{-1}), sucrose (42.4 g kg^{-1}), protein (7.5 g kg^{-1}), ash (5.5 g kg^{-1}), Na (161.5 mg kg^{-1}), K (2228.12 mg kg^{-1}), Ca (25.47 mg kg^{-1}) and Fe (4.70 mg kg^{-1}).

5.5. Chemical Composition of Sweet Cherry

The sweet cherry (*Prunus avium* L.) horticultural production chain consists of several parts: production, picking, cooling, selection, grading, packaging, transport, distribution and consumption. Quality of cherries may have a different meaning, depending on which part of the chain is considered. Consumer acceptance appears to be the most important overall characteristic of cherries (Romano et al., 2006). However, sweet cherry is a nutritious fruit due to abundance of chemical nutrients.

Serrano et al. (2005) reported that the cultivar 4-70 (Marvin Niram) had final concentrations of 6.57 and 8.43 %, for glucose and fructose, respectively, while sucrose and sorbitol concentrations were very low (0.20%) and the main organic acid being malic acid with concentration of 1.24 %.

Girard and Kopp (1998) studied the sugar, organic acid volatile constituents in 12 cultivars and selections of sweet cherries including the commercial 'Van', 'Bing', 'Salmo', 'Comp Stella', Lapins', 'Lambert', 'Summit' and 'Sweetheart' cultivars. They found glucose at 5.2-8.8 g/100 g, fructose at 4.4-6.4 g/100 g, sorbitol and mannitol at 2.2-8.0 g/100 g, and malic acid at 502.7-948.3 mg/100 g of FW. (*E*)-2-Hexenol, benzaldehyde, hexanal, and (E)-2-hexenal were the predominant flavour volatiles.

Usenik et al. 2008 reported the sugars and organic acids in fruits of 13 sweet cherry cultivars: 'Badascony', 'Burlat', 'Early Van Compact', 'Fercer', 'Fernier', 'Ferprime', 'Lala Star', 'Lapins', 'Noire de Meched', 'Sylvia', 'Vesseaux', 'Vigred' and 'Ferrador'. Generally, glucose was found to have the highest content, followed by fructose, sorbitol and sucrose. Cultivar 'Early Van Compact' had the highest (123 g/kg) and 'Sylvia' the lowest (62 g/kg) glucose content. The content of fructose varied from 47.6 g/kg FW ('Sylvia') to 102 g/kg FW ('Lala Star'). The content of sorbitol varied from 4.45 g/kg FW

('Ferprime') to 26.7 g/kg FW ('Early Van Compact'). The predominant organic acid in sweet cherry was malic acid ranging 3.53–8.12 g/kg FW followed by citric acid (0.11–0.54 g/kg FW), shikimic acid (6.56–26.7 mg/kg FW) and fumaric acid (0.97–7.56 mg/kg FW). Cultivar 'Fercer' had the highest content of malic, citric and shikimic acid and a high content of fumaric acid. The lowest content of malic acid was measured in 'Lapins' and the lowest content of citric acid in 'Vesseaux'.

According to USDA (2006) sweet cherries are fruits with a good source of fiber, potassium, and in particular anthocyanins (**Table 8**).

Table 8. Nutrient, carotenoid, anthocyanin, and quercetin content of sweet cherry fruits (per 100 grams or approx. 15 cherries)

Nutrient	Concentration
Energy (kcal)	63
Protein (g)	1.06
Fat (g)	0.2
Carbohydrate (g)	16
Total sugars (g)	12.82
Sucrose (g)	0.15
Glucose (g)	6.59
Fructose (g)	5.47
Fiber (g)	2.1
Vitamin C (mg)	7
Vitamin A (IU)	64
Vitamin E (mg)	0.07
Potassium (mg)	222
ß-carotene (µg)	38
Lutein/Zeaxanthin (µg)	85
Total anthocyanin (mg)	80
Quercetin (mg)	2.64

Objectives

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OBJECTIVES

The fruit market is very important worldwide, and the quality of organoleptic attributes represents a key role at the consumer level. This quality is related to many attributes, such as sweetness, acidity, aroma, colour, and firmness, all of which are associated with specific metabolic pathways that are typically coordinated during the ripening process. The development of these qualities depends on many factors, such as cultivar, growing conditions, ripening stage of harvest, and storage environments. During ripening, many changes in fruit composition occur; these include increase in size during development by both cell division and cell expansion and the synthesis and degradation of pigments, changes in the concentrations of organic acids and sugars, and the accumulation of volatile compounds. In this sense, the ripening stage at harvest is an important factor determining the postharvest quality of fresh produce. On the other hand, postharvest management of fruit quality has always been challenged by the paradox that quality can only be maximized when the product is harvested more mature or ripe, whereas shelf- and storage life are generally extended if it is harvested less mature or unripe. Much of the efforts of past and current postharvest physiology-based research have focused on finding such a compromise.

Fruits deteriorate rapidly after harvest and in some cases do not reach consumers at optimal quality after transport and marketing. The main causes of fruit deterioration are dehydration, with the subsequent weight loss, colour changes, softening, surface pitting, browning, loss of acidity and microbial spoilage, among others. However, deterioration rate is affected by different factors, such as intrinsic characteristics of the product and storage conditions in terms of temperature, relative humidity, storage atmosphere composition, etc. The main goal of postharvest technology is the quality optimisation and reduction of losses along the postharvest chain. Fruit consumers are not only looking for traditional quality attributes such as sugar, acidity, firmness, and colour. They also value other attributes, including nutrients and bioactive compounds availability, antioxidants, and aroma. Therefore, a major goal for growing fruits should emphasize on a good balance among the quality attributes already mentioned and by the use of appropriate postharvest technologies considered as safe and environmentally friendly.

In the present Doctoral Thesis the **Main Objective** was:

To perform a study in a wide range on plum and sweet cherry cultivars aimed to a better knowledge about the changes occurring during on-tree ripening and postharvest storage on the parameters related to fruit quality, either organoleptic, nutritive and the content of bioactive compounds with antioxidant activity.

To fulfil with this global objective the following **Specific Objectives** were:

1. To analyse the changes in organoleptic and functional properties occurring in eight plum cultivars, in order to establish the optimal harvest date to reach a high content of phytochemicals related to antioxidant activity, as well as their organoleptic quality.

2. To analyse sensory, nutritive and functional properties during the ripening on tree of 11 sweet cherry cultivars with interest in Spain, the majority of them being studied for the first time, in order to determine the appropriate harvest date for each cultivar.

3. To determine the changes in bioactive compounds during storage of a eight plum cultivars, both dark-purple and yellow pigmented as well as the total antioxidant activity determined in two separate fractions (hydrophilic and lipophilic) from peel and flesh tissues and the contribution of the several bioactive compounds in each extract.

4. To determine the changes in quality and bioactive compounds during storage of 11 sweet cherry cultivars, as well as the behavior of phenolic and anthocyanin compounds and their relationship to total antioxidant activity, 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.

5. To perform a broad study to evaluate the effect of modified atmosphere packaging (MAP) on preserving fruit quality with 4 plum cultivars, 2 with purple ('Blackamber' and 'Larry Ann') and 2 with yellow skin ('Golden Globe' and 'Songold'), by using two films with different gas permeability.

6. To study the effect of MAP on the changes in bioactive compounds (total phenolics and total carotenoids, and individual composition of anthocyanins) as well as the total antioxidant activity (H-TAA and L-TAA).

7. To analyse the effect of postharvest treatments with salicylic acid, acetylsalicylic acid and oxalic acid on the sweet cherry ripening process and parameters related to fruit quality and the content of bioactive compounds and antioxidant activity during storage.

8. To use sodium alginate applied as edible coating at three concentrations (1, 3, 5 % w/v) to preserve quality, bioactive compounds and antioxidant activity of 'Sweetheart' cherry cultivar during storage.

Results and Discussion

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Chapter 1

Changes in physicochemical and nutritive parameters and bioactive compounds during development and on-tree ripening of eight plum cultivars: a comparative study

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Abstract

BACKGROUND: There is no literature on changes in organoleptic, nutritive and functional properties during plum development and on-tree ripening. In this work a comparative study on the evolution of physical, chemical and nutritive parameters and bioactive compounds of eight plum cultivars (yellow and dark-purple) was performed.

RESULTS: The main changes related to ripening (colour, total soluble solids, acidity, firmness and bioactive compounds) started at the early stages of fruit development, with significant differences among cultivars. Colour hue angle was highly correlated with increase in anthocyanins or carotenoids (in both skin and flesh). Total antioxidant activity (TAA) was determined in the hydrophilic (H-TAA) and lipophilic (L-TAA) fractions separately, and values were always higher in the skin than in the flesh. A continuous increase in both H-TAA and L-TAA during the process of ripening occurred. H-TAA was about twofold higher than L-TAA in the dark-purple cultivars, while the opposite was found in the yellow cultivars. In addition, H-TAA was correlated with total phenolics and total anthocyanins, while L-TAA was positively correlated with total carotenoids.

CONCLUSION: In order to achieve the optimal organoleptic, nutritive and health-beneficial properties of plum consumption, it would be advisable to harvest the fruits at the fully ripe stage.

Keywords: fruit growth; harvest; anthocyanins; carotenoids; polyphenols; antioxidant activity

INTRODUCTION

Plum fruit is one of the most important commodities consumed worldwide owing to its degree of acceptance by consumers. For plums and other related *Prunus* species the degree of acceptance depends on organoleptic properties such as colour, texture, flavor and aroma,[1,2] which vary among cultivars and production areas and from season to season. In this regard it has been established that the ratio between total soluble solids and titratable acidity is more closely related to quality than total soluble solids or acidity alone.[3] There are a few papers describing changes related to plum ripening on tree, such as colour changes, softening, accumulation of soluble solids and decrease in acidity.[4-6] However, nowadays, other properties of fruits are gaining in importance, such as their antioxidant potential due to a wide range of bioactive compounds with health-beneficial effects via a putative role in decreasing risk of cancer development and cardiovascular, atherogenic and neurological diseases, among others. Some of these compounds are polyphenols (including flavonoids and anthocyanins), carotenoids, vitamin C and tocopherols, which are present at various concentrations in fruits and vegetables.[7-11]

Literature on the concentration of phytochemicals in a wide range of plum cultivars harvested at the commercial ripening stage shows that cultivar is a key factor determining antioxidant potential because of large differences in vitamin C, carotenoid, polyphenol and anthocyanin contents.[7,12-17] However, as far as we know, there are no studies on the evolution of bioactive compounds during the development and on-tree ripening of plums. Thus the objective of this research was to analyse the changes in organoleptic and functional properties occurring in eight plum cultivars, in order to establish the optimal harvest date to reach a high content of phytochemicals related to antioxidant activity, as well as their organoleptic quality.

MATERIALS AND METHODS

Plant material and experimental design

The experiment was carried out during the developmental cycle of the 2007 spring–summer period. In a commercial plot located at 'Finca Los Frutales' (Villena, Alicante, Spain), eight different plum cultivars from 15-year-old trees were selected: four yellow skinned ('Golden Japan', 'Golden Globe', 'Songold' and 'TC Sun') and four dark-purple skinned ('Angeleno', 'Black Amber', 'Black Diamond' and 'Larry Ann'). Immediately after fruit set, for each cultivar, three trees were selected and ten fruits were labelled around the equatorial perimeter of each tree. These marked fruits served to evaluate growth by measuring three linear dimensions of each fruit: polar (*P*, mm), suture (*S*, mm) and cheek (*C*, mm) diameters. The harvest date (H) for each cultivar (commercial harvest) was determined according to the Company's Technician and based on size, colour and total soluble solids. In addition, fruits were also kept 1 week longer on tree (H + 1).

Sampling schedule

For each sampling date, 30 similar fruits to those labelled on tree were collected at 7 day intervals and immediately transported to the laboratory for further analyses. Fruit weight, firmness and colour (skin and flesh) were determined individually for each fruit. Then, for each sampling date and cultivar, five subsamples of six fruits were taken at random for ethylene and respiration rate determination. In addition, when fruits were of an appropriate size (last six sampling dates), the skin and flesh were separated manually using a knife and cut into small pieces to obtain five homogeneous subsamples of both skin and flesh. A 5 g portion of the flesh was used for the determination of total soluble solids and titratable acidity. Finally, the skin and flesh of each subsample were immediately frozen in liquid nitrogen and milled for bioactive compound determination.

Fruit weight, firmness and colour

Fruit weight was determined using an ST-360Gram Precision digital balance (Barcelona, Spain). Fruit firmness was measured using a TX-XT2i texture analyser (Stable Microsystems, Godalming, UK) interfaced to a personal computer, with a flat steel plate mounted on the machine. For each fruit the diameter was measured and then a force that achieved 3% deformation of the fruit diameter was applied. Results were expressed as the ratio between this force and the covered distance (N mm^{-1}). Three colour determinations were made on each fruit using the Hunter $L*$, $a*$, $b*$ system in a Minolta CR200 colorimeter (Minolta Camera Co., Osaka, Japan). Following the recording of L∗, a∗ and b∗ values, colour was expressed as hue angle (hue = $\tan^{-1}(b/a)$).

Ethylene production

Ethylene production was measured by placing each subsample in a glass jar and sealing the jar hermetically with a rubber stopper. After 1 h, 1mL of the jar atmosphere was withdrawn with a gas syringe and its ethylene content was quantified using an HP 5890A gas chromatograph (Hewlett-Packard, Wilmington, DE, USA) equipped with a flame ionisation detector and a 3 m stainless steel column with an inner diameter of 3.5 mm containing activated alumina of 80/100 mesh. The column temperature was 90 ºC and the injector and detector temperatures were both 150 ºC. Results were expressed as nL g^{-1} h^{-1}.

Total soluble solids (TSS) and titratable acidity (TA)

TSS content in the juice obtained from each subsample of flesh tissue was determined in duplicate using an Atago PR-101 digital refractometer (Atago Co. Ltd, Tokyo, Japan) at 20 ºC. Results were expressed as g kg^{-1}. The pH of the juice was recorded and its TA was then determined by potentiometric titration with 0.1mol L^{-1} NaOH up to pH 8.1, using 1 mL of diluted juice in 25 mL of distilled water. Results were expressed as g malic acid equivalent kg^{-1}.

Bioactive compounds

Total anthocyanins were determined according to the method of García-Viguera *et al.*,[18] adapted as previously reported.[19] Total anthocyanin concentration was calculated using cyanidin-3-glucoside (molar absorption coefficient 23 900 L cm^{-1} mol^{-1}, molecular weight 449.2 g mol^{-1}) and results were expressed as mmol kg^{-1} fresh weight (FW).

Total carotenoids were extracted according to Mínguez-Mosquera and Hornero-Méndez.[20] Briefly, 1 g of skin or 2 g of flesh tissue was extracted with acetone and shaken with diethyl ether and 100 g L^{-1} NaCl to separate the two phases. The lipophilic phase was washed with 20 g L^{-1} Na_2SO4 and saponified with 100 g L^{-1} KOH in methanol. The pigments were subsequently extracted with diethyl ether, evaporated and then made up to 25 mL with acetone. Total carotenoids were estimated by reading the absorbance at 450 nm in a Helios-α spectrophotometer (Unicam, Cambridge, UK) and expressed as μmol ß-carotene equivalent kg^{-1} FW, taking $\varepsilon^{1\%}_{cm}$ = 2560. Total phenolics were extracted according to Tomás-Barberán and Espín[7] using water/methanol (2:8 v/v) containing 2 mmol L^{-1} NaF and quantified using Folin–Ciocalteu reagent.[21] Results were expressed as mmol gallic acid equivalent kg^{-1} FW. For both total carotenoids and total phenolics, five replications and two technical replications per sample were conducted.

Total antioxidant activity (TAA) was also quantified in duplicate for each subsample according to the method of Arnao *et al.*,[22] which allows the determination of TAA due to both hydrophilic (H-TAA) and lipophilic (L-TAA) compounds in the same extract. Briefly, 1 g of skin or 2 g of flesh tissue was homogenised in 5mL of 50 mmol L^{-1} phosphate buffer (pH 7.8) and 3mL of ethyl acetate and then centrifuged at 10 000 × *g* for 15 min at 4 ºC. The upper fraction was used for L-TAA and the lower fraction for H-TAA quantification. In both cases, TAA was determined using an enzymatic system composed of the chromophore 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), the horseradish peroxidase enzyme (HRP) and its oxidant substrate (hydrogen peroxide), in which $ABTS^{\bullet+}$ radicals are generated and monitored at 730 nm. The decrease in absorbance after adding the skin or flesh extract is proportional to TAA of the sample. Calibration curves (0–20 nmol) were constructed with Trolox ((R)-(+)-6-hydroxy-2,5,7,8-tetramethyl-croman-2-carboxylic acid; Sigma, Madrid, Spain) in aqueous and methanolic media for H-TAA and L-TAA quantification respectively. Results were expressed as mmol Trolox equivalent kg^{-1} FW.

Statistical analysis

Data from the analytical determinations were subjected to analysis of variance (ANOVA). Sources of variation were cultivar and developmental sampling date. Mean comparisons were performed using Tukey's highest significant difference (HSD) test to examine if differences were significant at $P < 0.05$, $P < 0.01$ or $P < 0.001$. Linear regressions (skin or flesh) were performed between colour hue angle and anthocyanin or carotenoid

concentration as well as between functional compounds and H-TAA or LTAA. The regressions were carried out for each cultivar individually and taking into account data for all cultivars and sampling dates. All analyses were performed with the SPSS Version 12.0 (SPSS Inc., Chicago IL, USA) for Windows software package.

RESULTS AND DISCUSSION

Changes in parameters related to fruit growth and ripening

Important changes occurred in the physiological and physicochemical parameters of all plum cultivars during development and on-tree ripening (**Tables 1 and 2**). As an example, **Fig. 1** shows the results for 'Golden Globe'. The parameters related to fruit growth (volume, polar, suture and cheek diameters and fruit weight) followed a double-sigmoid pattern (data not shown), which is typical for *Prunus* species, in which four distinct stages (S1–S4) could be established according to fruit weight evolution. S1 is the first exponential growth phase and characterised by cell division and elongation. S2 shows little or no fruit growth, but the endocarp hardens to form a solid stone. S3 is the second exponential growth phase due to cell enlargement, while in S4 the fruit ripening or climacteric stage occurs.[5,23,24]

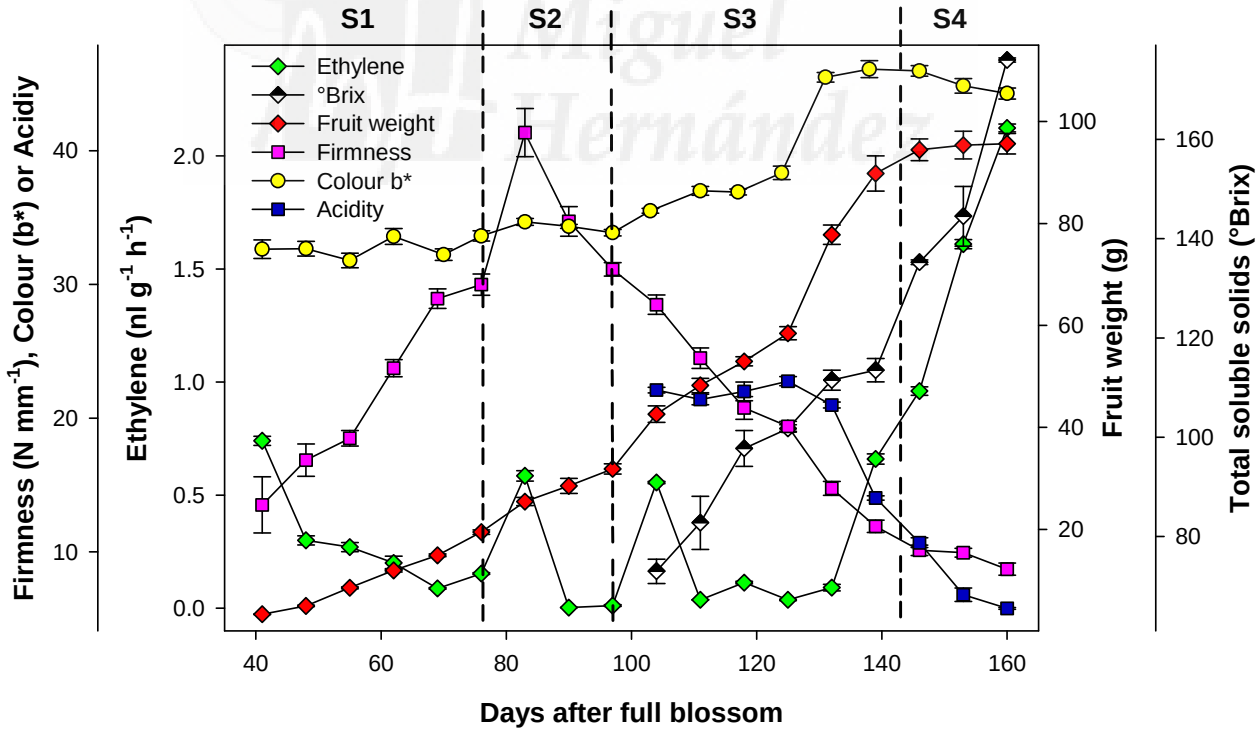

Figure 1. Evolution of some physical, chemical and physiological parameters during fruit development and ripening on tree of 'Golden Globe' plum cultivar. Data are mean ± standard error. S1–S4 denote developmental stages according to fruit weight evolution.

However, fruit weight at commercial harvest was cultivar-dependent and no significant differences were found when fruits were kept 1week longer on tree (H + 1) (**Table 1**).

Table 1. Fruit properties at commercial harvest date (H) and after 1 week of further ripening on tree (H + 1) for eight plum cultivars.

	Fruit weight (g)		Fruit Firmness (N mm^{-1})	
	Harvest (H)	H + 1 week	Harvest (H)	H + 1 week
Golden Japan	59.2±1.7 a A	59.9 ± 2.9 a A	7.2 ± 0.5 a A	5.7 ± 0.3 b A
Blackamber	117.4 ± 1.3 a B	120.0 ± 2.3 a B	6.7 ± 0.4 a A	5.6 ± 0.2 b A
Black Diamond	119.5 ± 2.5 a B	120.0 ± 1.8 a B	9.9 ± 0.3 a B	7.8 ± 0.5 b B
Golden Globe	95.4 ± 2.7 a C	95.7 ± 2.0 a C	9.9 ± 0.3 a B	8.7 ± 0.4 b B
TC Sun	113.8 ± 3.1 a B	113.0 ± 3.1 a B	8.9 ± 0.2 a C	8.1 ± 0.2 b B
Larry Ann	97.1 ± 4.3 a C	98.0 ± 2.5 a C	8.3± 0.2 a C	5.4 ± 0.5 b A
Songold	148.4 ± 4.9 a D	148.6 ± 4.0 a D	6.2 ± 0.2 a A	5.7 ± 0.1 b A
Angeleno	89.0 ± 2.0 a C	88.9 ± 2.9 a C	9.5 ± 0.2 a B	8.7 ± 0.2 b B
	TSS (°Brix)		TA (g 100 g^{-1})	
	Harvest (H)	H + 1 week	Harvest (H)	H + 1 week
Golden Japan	10.6 ± 0.1 a A	10.9 ± 0.1 a A	1.60 ± 0.01 a A	1.39 ± 0.04 b A
Blackamber	13.1 ± 0.1 a B	15.0 ± 0.1 b B	1.25 ± 10.2 a B	0.86 ± 0.01 b B
Black Diamond	12.4 ± 0.1 a C	14.0 ± 0.1 b C	0.74 ± 0.01 a C	0.57 ± 10.01b C
Golden Globe	14.5 ± 0.2 a D	17.6 ± 0.1 b D	0.68 ± 0.05 a C	0.58 ± 0.01 b C
TC Sun	15.8 ± 0.1 a E	16.7 ± 0.2 b E	0.75 ± 0.01 a C	0.73 ± 0.01 a D
Larry Ann	14.6 ± 0.1 a D	14.9 ± 0.2 a B	1.05 ± 0.01 a D	0.72 ± 0.01 b D
Songold	13.4 ± 0.2 a B	14.0 ± 0.2 b C	0.88 ± 0.04 a E	0.72 ± 0.03 b D
Angeleno	13.7 ± 0.1 a B	13.9 ± 0.1 a C	1.02 ± 0.06 a D	0.84 ± 0.02 b B

For each parameter and cultivar, different lowercase letters indicate significant differences at $P < 0.05$ between harvest dates. For each parameter and harvest date, different capital letters indicate significant differences at $P < 0.05$ among cultivars.

In addition, although the full blossom dates were very close among cultivars (from 2 to 14 March), the harvest date ranged from 4 July for 'Golden Japan' to 6 September for 'Angeleno', and, in turn, the necessary days to achieve the commercial ripening stage differed greatly among cultivars, from 112 to 185 days for 'Golden Japan' and 'Angeleno' respectively (data not shown).

At the beginning of each phase the occurrence of ethylene peaks was detected, although its physiological role is markedly different. Thus at S1 ethylene is associated with cell division, at S2 with pit hardening due to stimulation of lignin synthesis by increasing phenylalanine ammonia lyase (PAL) activity, at S3 with colour changes and at S4 with climacteric ethylene production. This confirms the climacteric ripening pattern for most plum cultivars and the role of ethylene as the plant hormone responsible for the acceleration of physicochemical changes during fruit ripening.[25-27] However, no increase in climacteric ethylene production was found in 'Golden Japan' during S4 (data not shown), confirming other reports that this cultivar is of the suppressed climacteric type during both on-tree development and postharvest storage,[5,28] as also are 'Shiro' and 'Rubyred' cultivars.[4] At transcriptomic level, such differences within plum cultivars have been attributed to differences in the accumulation patterns of mRNAs implicated in ethylene perception and signal transduction components.[24]

Table 2. ANOVA of dependent variables for cultivar and developmental days.

	Cultivar	Days
External Colour Hue	***	***
Flesh Colour Hue	***	***
Skin Anthocyanins	***	***
Flesh Anthocyanins	***	***
Skin Carotenoids	**	**
Flesh Carotenoids	**	**
Skin Phenolic compounds	**	***
Flesh Phenolic compounds	**	***
Skin H-TAA	**	***
Flesh H-TAA	**	***
Skin L-TAA	**	***
Flesh L-TAA	**	***

Significance levels: *** $P < 0.001$; ** $P < 0.01$.

Differences in fruit firmness, TSS and TA were found among cultivars (**Table 1**), with values being within the ranges reported for other plums.[1,3,6,29] However, no relationship between TSS or TA and harvest season can be established, although there is a tendency for early season plums to have lower TSS than late season plums.3 For consumers the levels of TSS in plums have special importance for their acceptability, since TSS has been correlated with perception of sweetness, flavour and aroma. In this regard, plums harvested at H + 1 would be more appreciated by consumers, since higher levels of TSS and lower TA are achieved for all cultivars (**Table 1**).

For comparative purposes, colour hue angle was chosen to show the changes in both external and flesh colour during fruit development and on-tree ripening. For all cultivars, external hue angle remained high (120–115°) between S1 and the beginning of S3, which means that the plums had a dark-green colour.

From this moment, hue angle decreased sharply in the purple/black-pigmented cultivars, while in the yellow pigmented cultivars the decrease was slower (**Fig. 2**). For flesh colour the same behaviour was detected, although the decrease in hue angle occurred at the later developmental stages (**Fig. 3**). Intriguingly, flesh hue angle in 'Black Diamond' reached values of ~20° at the ripe stage, which indicates a purple flesh, while the other dark-purple cultivars had higher hue angle values owing to their orange/yellow flesh.

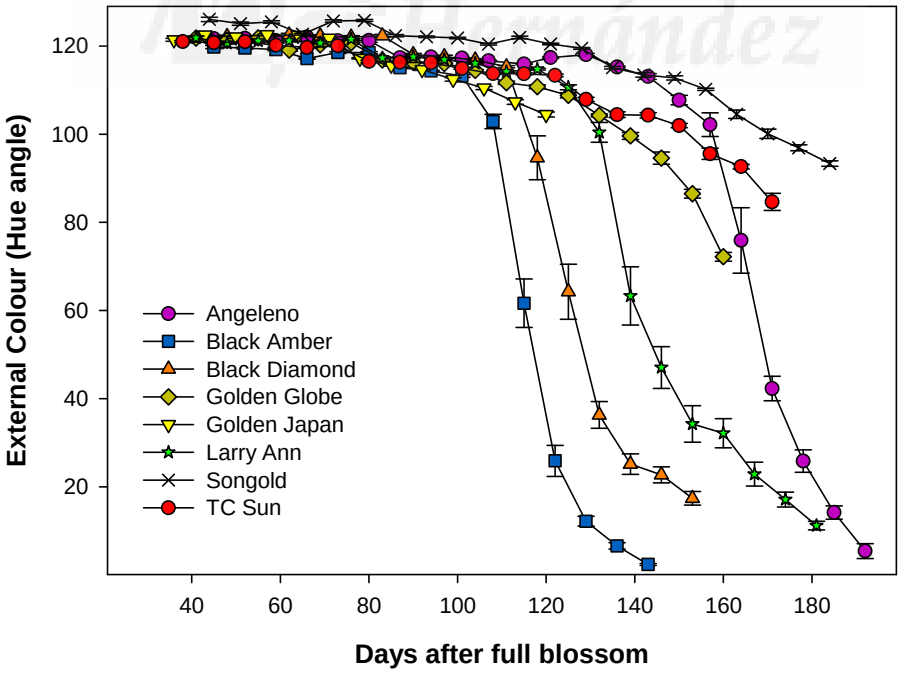

Figure 2. Evolution of external colour (hue angle) during fruit development and ripening on tree of eight plum cultivars. Data are mean ± standard error.

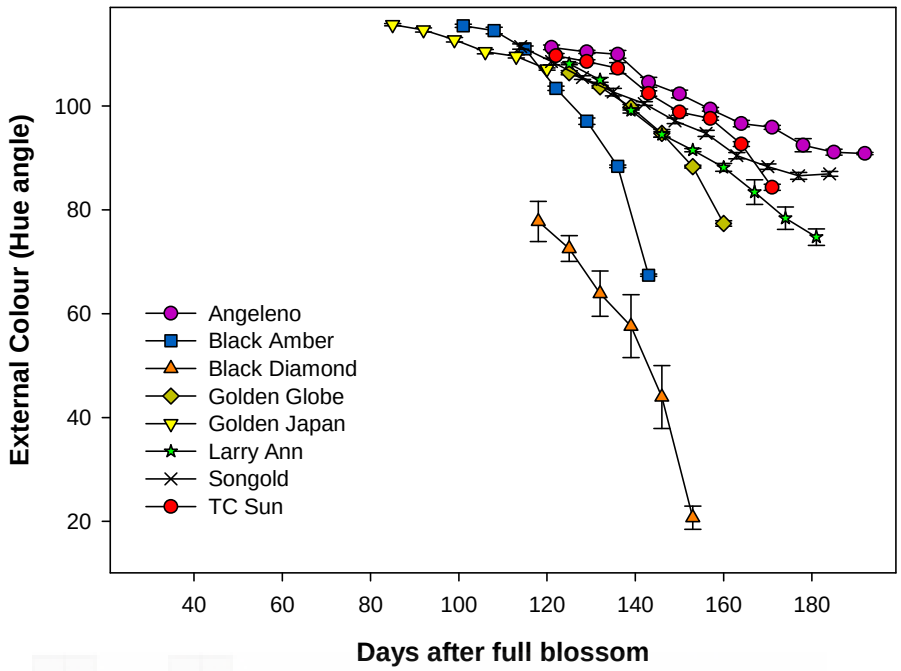

Figure 3. Evolution of flesh colour (hue angle) during fruit development and ripening on tree of eight plum cultivars. Data are mean ± standard error.

Changes in bioactive compounds

In the dark-purple cultivars the levels of anthocyanins were analysed in both skin and flesh. The results revealed that anthocyanins started to accumulate first in the skin, then in the flesh, and showed a continuous increase until the last sampling date (H + 1), although significant differences ($P < 0.001$) were found among cultivars. For all cultivars the anthocyanin concentration was significantly higher (~50-fold) in the skin than in the flesh. Among cultivars, 'Black Amber' and 'Black Diamond' had the highest (18.89 ± 0.67 mmol kg^{-1}) and lowest (5.93 ± 0.22 mmol kg^{-1}) skin anthocyanin concentrations respectively. In the flesh, these values ranged between 0.06 and 0.36 mmol kg^{-1} for 'Angeleno' and 'Black Diamond' respectively (**Fig. 4**). The anthocyanin concentrations are within the range reported for other red/purple plum cultivars.[7,16,17,30] The main anthocyanin in plum cultivars is cyanindin- 3-glucoside, followed by cyanindin-3-rutinoside, while peonidin derivative and peonidin-3-glucoside are found in minor concentrations.[7,14] For all cultivars, in both skin and flesh, total anthocyanins were negatively correlated (r^2 = 0.86–0.99) with hue angle (**Table 3**), showing that these pigments are responsible for the colour changes associated with ripening. However, the correlations were exponential in the skin, indicating that higher anthocyanin accumulation occurred during the last 2weeks of ripening, while the linear correlations in the flesh showed a continuous increase during on-tree ripening.

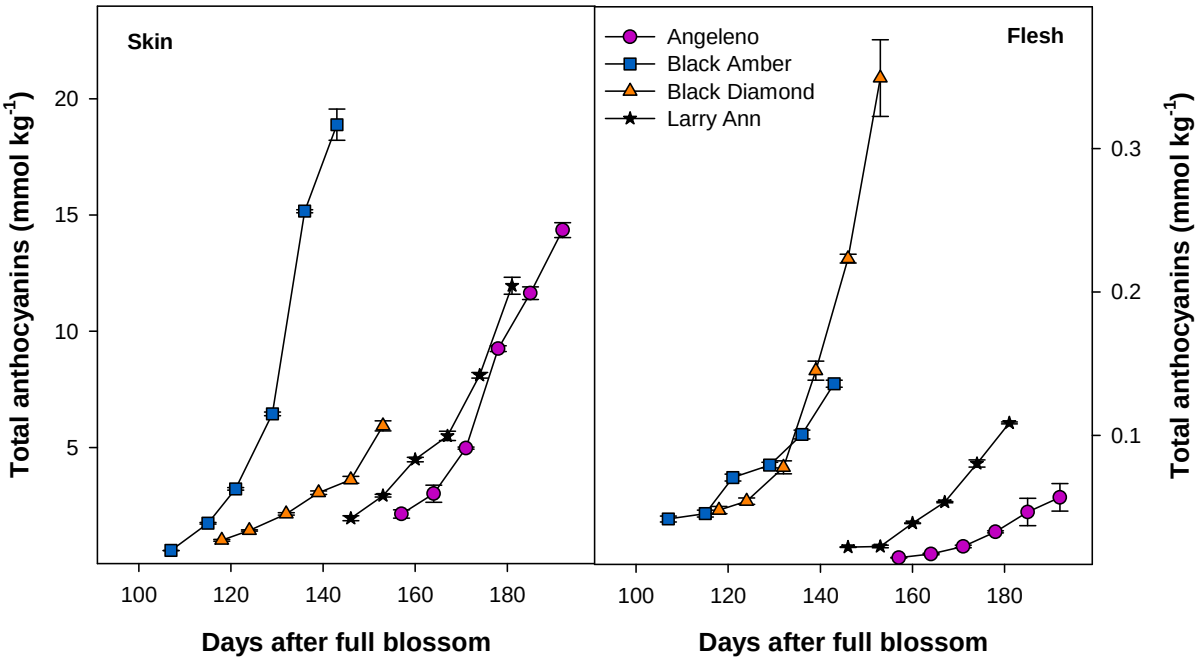

Figure 4. Evolution of total anthocyanin content in skin and flesh during last 6 weeks of fruit development and ripening on tree of four dark-purple plum cultivars. Data are mean ± standard error.

Table 3. Correlations between bioactive compounds and colour Hue angle, or H-TAA or L-TAA either in the skin or the peel.

		Anthocyanins vs Hue	Carotenoids vs Hue	Phenolics vs H-TAA
Golden Japan	Skin	–	* * *	* * *
	Flesh	–	* * *	* * *
Blackamber	Skin	* * *	NS	* * *
	Flesh	* * *	* * *	* * *
Black Diamond	Skin	* * *	NS	* * *
	Flesh	* * *	NS	* * *
Golden Globe	Skin	–	* * *	* * *
	Flesh	–	* * *	* * *
TC Sun	Skin	–	* * *	* * *
	Flesh	–	* * *	* *
Larry Ann	Skin	* * *	–	* * *
	Flesh	* * *	* * *	* * *

Sungold	Skin	–	* * *	* * *
	Flesh	–	* *	* * *
Angeleno	Skin	* * *	NS	* * *
	Flesh	* * *	* * *	* * *
All cultivars	Skin	* * *	*	* * *
	Flesh	* *	NS	* * *

		Anthocyanins vs H-TAA	Carotenoids vs L-TAA
Golden Japan	Skin	–	* * *
	Flesh	–	* * *
Blackamber	Skin[a]	* * *	* * *
	Flesh	* * *	* * *
Black Diamond	Skin[a]	* * *	* * *
	Flesh	* * *	* *
Golden Globe	Skin	–	* * *
	Flesh	–	* * *
TC Sun	Skin	–	* * *
	Flesh	–	* * *
Larry Ann	Skin[a]	* * *	* * *
	Flesh	* * *	* * *
Sungold	Skin	–	* * *
	Flesh	–	* *
Angeleno	Skin[a]	* * *	* * *
	Flesh	* * *	* * *
All cultivars	Skin	*	* *
	Flesh	NS	* *

For all parameters the correlations were linear, except for [a] anthocyanins vs hue in the skin which was exponential. Significance levels of correlation coefficients (R^2): *** P < 0.001; ** P < 0.01; * P < 0.05. NS, no correlation: – , absence of anthocyanin data.

For all plum cultivars the levels of total carotenoids were determined in both skin and flesh. Similarly to anthocyanins, total carotenoids in both skin and flesh reached their maximum concentration at the last sampling date, although they started to accumulate earlier (**Fig. 5**). However, the absolute concentration of total carotenoids was cultivar-dependent, with the highest skin carotenoid concentration among the yellow-pigmented plums being found in 'Golden Globe' (141.93 ± 3.57 μmol kg^{-1}) and the lowest in 'Golden Japan' (32.04 ± 0.75 μmol kg^{-1}). For these cultivars a similar behaviour was found for total carotenoids in the flesh. Interestingly, 'Black Amber' and 'Larry Ann' (dark-purple plums) also showed high levels of total carotenoids in both skin and flesh (~150 and ~20 μmol kg^{-1} respectively), close to those found in 'Golden Globe'.

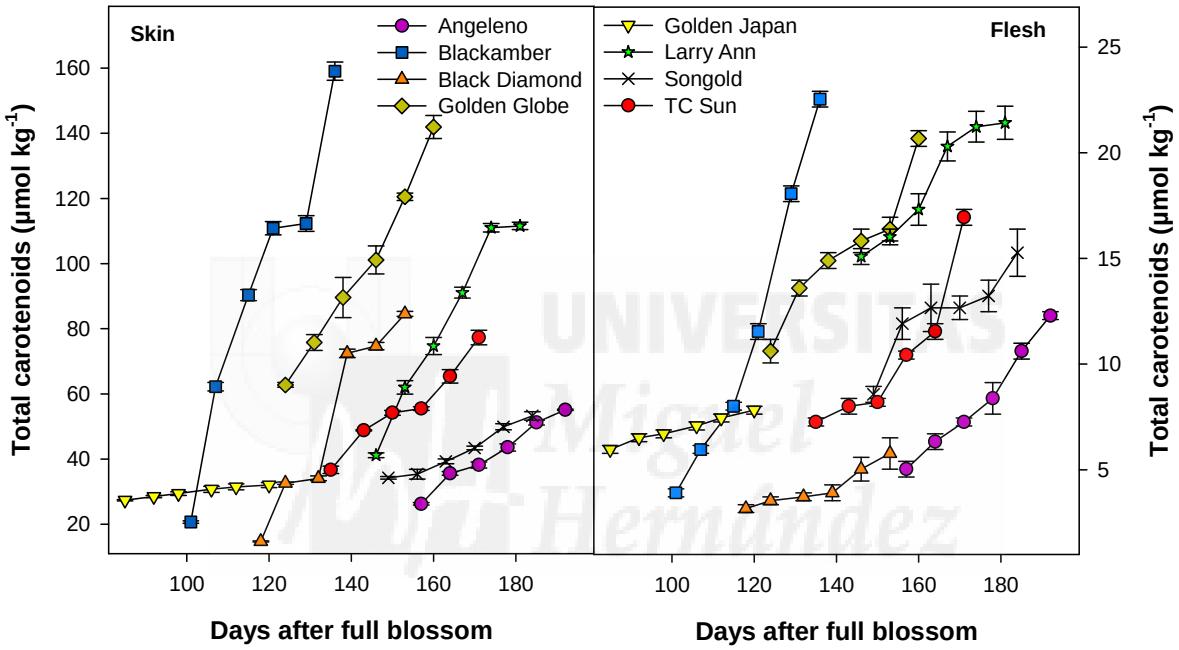

Figure 5. Evolution of total carotenoid content in skin and flesh during last 6weeks of fruit development and ripening on tree of eight plum cultivars. Data are mean ± standard error.

A high linear correlation was found between total carotenoids and colour hue angle for the yellow-pigmented cultivars (**Table 3**) in both skin and flesh (r^2 = 0.75-0.99), showing that carotenoids are the main compounds responsible for the colour changes from green to orange/yellow occurring during plum ripening. Among the carotenoids, ß-carotene has been found as the major carotenoid pigment in the skin and flesh of yellow or dark-purple plums, while ß-cryptoxanthin occurred at much lower concentrations.[12,31] It is interesting to point out that, among the yellow plum cultivars assayed in this study, 'Golden Globe' had a high carotenoid concentration compared with other yellow cultivars such as 'Wickson',[12] 'Golden Japan', 'TC Sun' and 'Songold'. In a study of 45 (light-red and red flesh) plum genotypes, carotenoids were also present,[17] with concentrations similar to those reported for 'Black Amber' and 'Larry Ann' in the present work.

The content of total phenolics showed a similar pattern for all cultivars, with progressive increases throughout the ripening process (**Fig. 6**). In addition, the concentration of total phenolics was about threefold higher in the skin than in the flesh, although important differences were found among cultivars. At the last sampling date the final concentration of total phenolics in the skin ranged from 50.85 ± 0.90 mmol kg^{-1} ('Black Amber') to 7.53 ± 0.32 mmol kg^{-1} ('Golden Japan'), while in the flesh it varied it between 17.82 ± 0.36 mmol kg^{-1} ('Black Amber') and 3.94 ± 0.09 mmol kg^{-1} ('Larry Ann'). The contents of total phenolics in the eight plum cultivars are within the range found in other cultivars, and the dark-purple cultivars generally had higher phenolic contents than the yellow cultivars.[12,15,32] The main phenolic compound in plum cultivars is 3-O-caffeoylquinic acid, followed by cyanidin-3-rutinoside, while quercetin-3-rutinoside occurs in lower concentrations.[14]

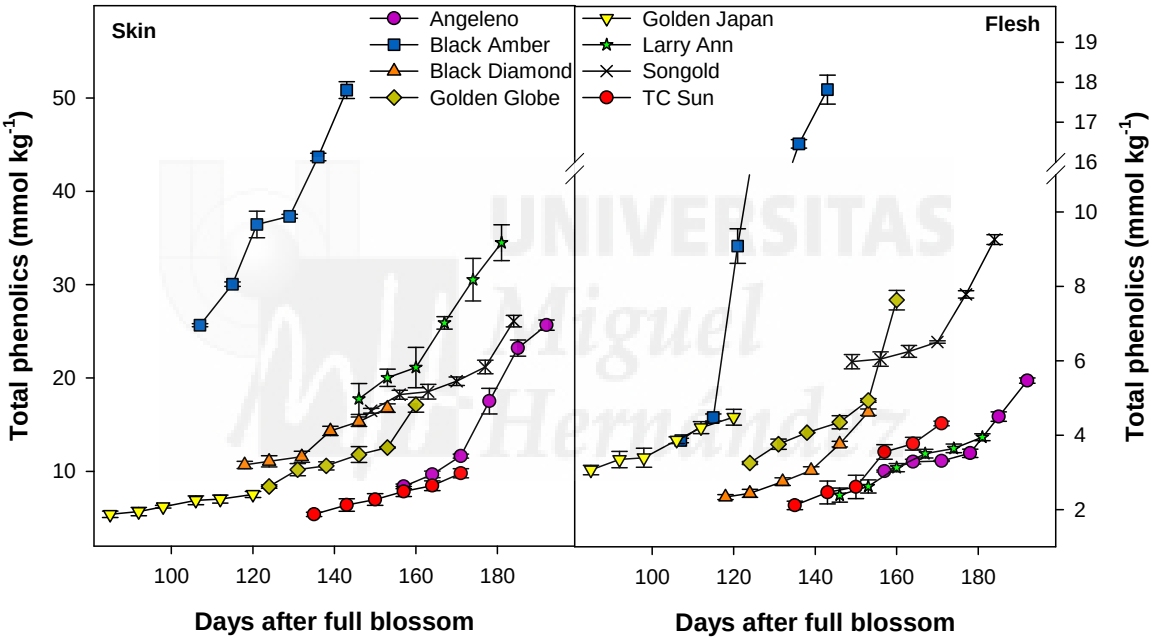

Figure 6. Evolution of total phenolic content in skin and flesh during last 6weeks of fruit development and ripening on tree of eight plum cultivars. Data are mean ± standard error.

TAA was assayed in hydrophilic (H-TAA) and lipophilic (L-TAA) fractions. As shown above for the bioactive compounds, both H-TAA and L-TAA increased throughout the ripening process on tree in both skin and flesh tissues for all plum cultivars (**Figs 7 and 8**). TAA (H-TAA + L-TAA) of the skin was generally higher in the dark-purple plums than in the yellow plums. H-TAA was about twofold higher than L-TAA in the dark-purple cultivars, while the opposite was found in the yellow cultivars, with the exception of 'Songold' which had a threefold higher H-TAA than L-TAA. H-TAA was always lower (~1.5–2-fold) than L-TAA in the flesh, except for 'Black Diamond'. In addition, H-TAA was highly correlated (r^2 = 0.76–0.99) with total phenolics in the skin and flesh of all cultivars (**Table**

3), indicating that phenolics are important bioactive compounds contributing to hydrophilic antioxidant activity, in accordance with previous reports on other plum, peach and nectarine cultivars.[12,14-16]

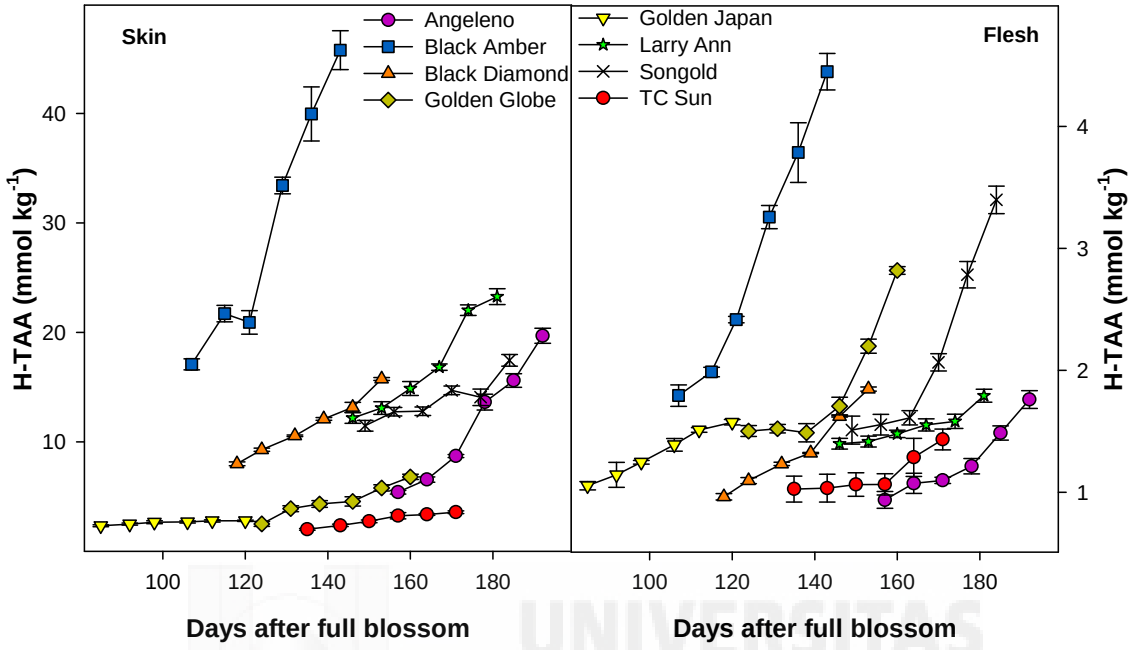

Figure 7. Evolution of total antioxidant activity in hydrophilic fraction (H-TAA) of skin and flesh during last 6weeks of fruit development and ripening on tree of eight plum cultivars. Data are mean ± standard error.

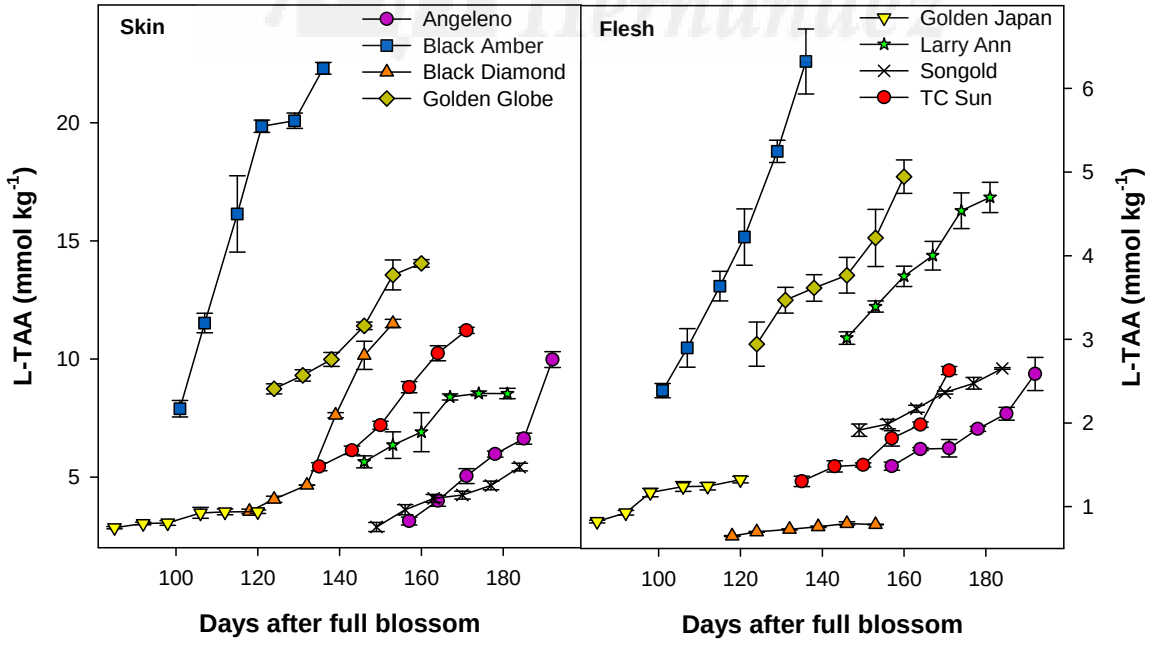

Figure 8. Evolution of total antioxidant activity in lipophilic fraction (L-TAA) of skin and flesh during last 6weeks of fruit development and ripening on tree of eight plum cultivars. Data are mean ± standard error.

In addition, in the dark-purple cultivars, total anthocyanins were also highly correlated with H-TAA, showing that anthocyanins might contribute to antioxidant activity more than other phenolic compounds. Finally, LTAA was highly correlated with total carotenoids (r^2 = 0.73–0.99) in both skin and flesh tissues. As far as we know, this is the first study in which H-TAA and L-TAA have been independently assayed in plums, and thereafter L-TAA has been correlated with total carotenoids, although evidence exists about this issue for tomato.[33] Among the cultivars assayed in this work, 'Black Amber' had the highest antioxidant activity in both skin and flesh (for both H-TAA and L-TAA) and also had the greatest accumulation of total bioactive compounds (phenolics, anthocyanins and carotenoids), although the differences found in these compounds among cultivars could not justify the large differences in antioxidant activity. For example, 'Black Amber' had an approximately 2.5-fold higher total antioxidant activity than 'Larry Ann', while its concentration of bioactive compounds was only 1.5-fold greater. This behaviour would indicate a synergistic effect of all the compounds contributing to TAA.

In conclusion, plums harvested at the commercial maturity stage do not reach their maximum concentration of bioactive compounds, since keeping them 1 week longer on tree led to significant increases in these compounds and the corresponding total antioxidant activity (by 10–20% on average). Thus, in order to achieve the maximum health-beneficial effects of plum consumption and their optimal organoleptic and nutritive properties, it would be advisable to harvest the fruits at the fully ripe stage.

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Chapter 2

Sensory, nutritive and functional properties of sweet cherry as affected by cultivar and ripening stage

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In this article 11 commercial sweet cherry cultivars were selected to evaluate sensory, nutritive and functional properties over the maturation process on tree. Fruit quality was significantly different among cultivars and maturity stages, with the highest quality scores being found in the harvest which was 4 days beyond current commercial harvest maturity for all the cultivars tested. Taking into account all of the measured parameters (weight, firmness, color, acidity and total soluble solids), 'NY-6479', 'Prime Giant' and 'Sunburst' could be classified as having the highest quality in terms of sensory attributes. However, 'Cristalina' and 'Sonata' had the highest functional quality, as determined by the measurement of bioactive compound content and antioxidant capacity. We conclude that a delay of a few days in harvesting of sweet cherries would lead to achieve maximal nutritional (highest sugar and organic acid contents), sensory (greatest firmness and color development) and functional (greatest phenolics content, anthocyanins and antioxidant capacity) quality to provide both eating enjoyment and health benefits to the consumer.

Key Words: sweet cherry, ripening, antioxidants, phenolics, quality

INTRODUCTION

Sweet cherry is one of the most appreciated fruits by consumers due to its precocity and excellent quality. Spain is one of the main cherry producers in Europe, with a production of 115,000 ton in 2003, which represents 20% of the total in the European Union (FAO, 2005). The concept of 'quality' depends on the product itself and the consumer's preferences, and for sweet cherry it is widely accepted that the main characteristics related to fruit quality are fruit weight, color, firmness, sweetness, sourness, flavor and aroma (Romano et al., 2006). In sweet cherry, the ripening process is characterized by color changes, from green to red, which can be followed by the evolution of L*, a* and b* parameters and the color indices Chroma and Hue. However, the industry has a standard color chart used for this purpose, the most common being that from the Centre Technique Interprofessionel de Fruits et Légumes (CTIFL, Paris). Red color development in sweet cherry is used as indicator of quality and ripening, and is due to accumulation and profile of anthocyanins (Gao and Mazza, 1995; Mozetiĉ et al., 2004; Serrano et al., 2005a). Sweetness in cherry fruit is mainly due to glucose and fructose, while sourness is primarily due to the presence of malic acid (Serrano et al., 2005a; Usenik et al., 2008). Fruit firmness is also appreciated by consumers, together with 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 (Crisosto et al., 2003).

Nowadays, especially in developed countries, fruits and vegetables are appreciated not only by their sensory and nutritional properties, but also by their additional health benefits. In fact, critical and epidemiological studies have established an inverse correlation between the intake of fruit and vegetables and the occurrence of several degenerative diseases, such as cancer, cardiovascular illness and even Alzheimer's disease, due to their content in some bioactive compounds (Kris-Etherton et al., 2002; Scalbert et al., 2005; Schreiner and Huyskens-Keil, 2006). Among these compounds there are vitamins (A, C and E), carotenoids and phenolics, including anthocyanins, due to their antioxidant properties (Kaur and Kapoor, 2001; Tomás-Barberán and Espín, 2001).

There are some papers about sensory, nutritive and functional properties of sweet cherry at harvest time, showing important differences among cultivars (Girard and Koop, 1998; Gonçalves et al., 2004; Kim et al., 2005; Usenik et al., 2008). In addition, some parameters related to fruit quality, such as fruit weight, color and anthocyanins, firmness, and sugar and acid content have been also evaluated on different cultivars during fruit development on tree (Crisosto et al., 2002; Mozetiĉ et al., 2004; Usenik et al., 2005; Muskovics et al., 2006). Harvesting is usually performed based on the attainment of acceptable fruit size, color and concentration of soluble solids. However apart from our previous paper, with sweet cherry cultivar '4-70' (Serrano et al., 2005a), there is no available information about the changes in the content of health-promoting compounds during sweet cherry development and ripening on tree. In this sense, the aim of this work

was to analyze sensory, nutritive and functional properties during the ripening on tree of 11 sweet cherry cultivars with interest in Spain, the majority of them being studied for the first time. This information could be useful to pick each cultivar with the maximum overall quality, in order to satisfy the demand of consumers for taste, nutrition and health beneficial effects. In addition, results would also serve as a basis for selection of sweet cherry cultivars with both high quality fruits and health beneficial effects, since in the last decades cultivars have been screened mainly on the basis of field growth factors and on a few fruit quality attributes such as size, color, texture and flavor.

MATERIALS AND METHODS

Materials

Plant Material and Experimental Design

The experiment was carried out along the developmental cycle during the 2007 spring period, in a commercial plot located at 'Finca Los Frutales' (Villena, Alicante, Spain). Eleven different sweet cherry cultivars (Table 1) from 10 year old trees on 'Santa Lucía' rootstock were selected. After fruit set, three trees were selected for each cultivar and then 10 fruits were labeled around the equatorial perimeter of each tree. These marked fruits served to evaluate the growth by measuring three linear dimensions of the fruit: polar, suture and cheek diameters. After 3-4 day intervals along the development process, 30 similar fruits to those labeled on tree were taken, and then immediately transferred to laboratory for further analytical determinations. The commercial harvest date (CH) for each cultivar was determined according to the Technician's company and based on size and color using the CTIFL chart. Thus, the scores at CH were 3 for 'Brooks' and 'Somerset', 5 for 'Cristalina' and 'Sonata' and 4 for the remaining cultivars. In addition, some fruits were kept 4 days further on tree to take the last sample (CH+4 days). Fruit weight, firmness, and color were measured individually in each fruit, and data are the mean±SE (n=30). When the fruit had an appropriate size (the last six sampling dates) five subsamples of six fruits were made at random, and then the edible portion was cut in small pieces to obtain five homogenous subsamples for each cultivar and sampling date. Five grams were used for total soluble solids and titratable acidity determination and the remaining tissue was immediately frozen in liquid N_2 and milled for total phenolics, anthocyanins and antioxidant activity determination at the last four sampling dates.

Methods

Fruit Weight, Firmness and Color

The weight for each fruit was determined using a digital balance (ST-360 Gram Precision) with two significant figures and results were the mean±SE. Fruit firmness was determined using a TX-XT2i Texture Analyzer (Stable Microsystems, Godalming, UK) interfaced to a personal computer, with a flat steel plate mounted on the machine. For each fruit, the cheek diameter was measured and then a force that achieved a 3% deformation of the fruit diameter was applied. Results were expressed as the ratio between this force and the covered distance (N/mm) and were the mean±SE. This determination of firmness as the slope of the force-deformation curve has been chosen as the most characteristic parameter for textural changes in cherry fruits (Serrano et al., 2005a; Muskovics et al., 2006). Three color determinations were made on each fruit at 120º interval along the equatorial perimeter using the Hunter Lab System (L*, a*, b*) in a Minolta colorimeter CR200 model (Minolta Camera Co., Osaka, Japan). In addition, a/b, Chroma index (Chroma=$(a^2+b^2)^{1/2}$) and Hue angle (Hue=arctan (b/a)) were calculated. Results were the mean±SE.

Total Soluble Solids and Total Acidity

Total Soluble Solids (TSS) were determined in duplicate from the juice obtained from each subsample with a digital refractometer Atago PR-101 (Atago Co. Ltd., Japan) at 20 ºC and results expressed as ºBrix. Total acidity (TA) was determined from the above juice by potentiometric titration with 0.1 N NaOH up to pH 8.1, using 1 mL of diluted juice in 25 mL distilled H_2O and results were the mean±SE expressed as g of malic acid equivalent per 100 g fresh weight.

Total Anthocyanins, Total Phenolics and Antioxidant Activity

Total anthocyanins were determined as previously reported (Serrano et al., 2005a). Two grams of fruit tissue were homogenized in 4 mL methanol and left 1 h at -18 ºC. Extracts were centrifuged at 10,000 x g for 15 min at 4 ºC and the supernatant was loaded onto a C18 Sep-Pak® cartridge, previously conditioned with 5 mL methanol, 5 mL pure water and then with 5 mL 0.01 N HCl. Cartridge was washed with 5 mL pure water and then eluted with acidified MeOH (0.01% HCl). Absorbance of the collected fraction was measured at 530 nm and total anthocyanins were calculated using cyanidin-3-glucoside (molar absorption coefficient of 23,900 L/cm . mol and molecular weight of 449.2 g/mol). Results were expressed as mg cyanindin 3-glucoside equivalent per 100 g fresh weight, and were the mean±SE of determinations made in duplicate in each one of the five subsamples. Total phenolics were extracted according to Tomás-Barberán et al. (2001)

using water: methanol (2:8) containing 2 mM NaF (1:5 w/v) and quantified using the Folin-Ciocalteu reagent (Singleton et al., 1999). Briefly, a suitable volume (25-100 µL) of extracts was mixed with 2.5 mL of water-diluted Folin-Ciocalteau. The mixture was incubated for 2 min at room temperature and 2 mL of sodium carbonate (75 g/L) were added and shaken. Finally, mixture was incubated at 50 ºC for 15 min and absorbance was measured at 760 nm. A calibration curve was performed with gallic acid and results were expressed as mg gallic acid equivalent per 100 g fresh weight. Results were the mean±SE of determinations made in duplicate in each one of the five subsamples.

For antioxidant activity quantification 1 g of cherry flesh was homogenized with 5 mL of 50 mM Na-phosphate buffer pH 7.5 and 3 mL of ethyl acetate, centrifuged at 10,000 x g for 15 min at 4 ºC and then the aqueous and organic phases were separated and used to quantify hydrophilic and lipophilic total antioxidant activity (H-TAA and L-TAA), respectively, according to Arnao et al., (2001). The method is based on the capacity of different fruit components to scavenge the $ABTS^{\bullet+}$ radicals (2,20-azino-bis- (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), which have been previously generated by the horse radish peroxidase enzyme (HRP) and its oxidant substrate (hydrogen peroxide). The reaction mixture contained 1.5 mM ABTS, 15 mM hydrogen peroxide and 0.25 mM HRP in a total volume of 2 mL of 50 mM glycine-HCl buffer (pH 4.5), for H-TAA or in ethyl acetate for L-TAA. The assay temperature was 25 ºC 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 cherry fruit extract was added and the observed decrease in absorbance was determined. A calibration curve was performed with Trolox ((R)- (+)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), as standard antioxidant for both H-TAA and L-TAA and results are expressed as mg of Trolox equivalent per 100 g fresh weight. Results were the mean±SE of determinations made in duplicate in each one of the five subsamples.

Statistical Analysis

Data for the analytical determinations were subjected to a two-way analysis of variance (ANOVA). Sources of variation were cultivar and developmental sampling dates. LSDs ($p<0.05$) were calculated for mean separations and are shown in the Figures. Polynomial linear or quadratic regressions were performed between color parameters and anthocyanin concentration, as well as among anthocyanins or phenolics and H-TAA. The regressions were carried out taking into account data for all cultivars and sampling dates. All analyses were performed with SPSS software package v. 12.0 for Windows (2001).

RESULTS AND DISCUSSION

Changes in Sensory and Nutritional Parameters

It is known that environmental factors and orchard management (choice of rootstock, pruning, fertilization and irrigation) affect cherry fruit quality, in terms of different concentration of nutritive and bioactive compounds (Predieri et al., 2004; Gonçalves et al., 2006). However, in this work all cherry cultivars were in the same farm, under similar environmental conditions and cultural practices and even on similar rootstocks and tree age. Then, differences in quality parameters among cultivars that will be commented below should be attributed to genetic characteristics of each cultivar.

Cherry fruit weight increased along development on tree, as it is shown in **Figure 1** for 'Sonata' as an example, in which the second and third phases of double sigmoid curve for stone fruit growth can be observed.

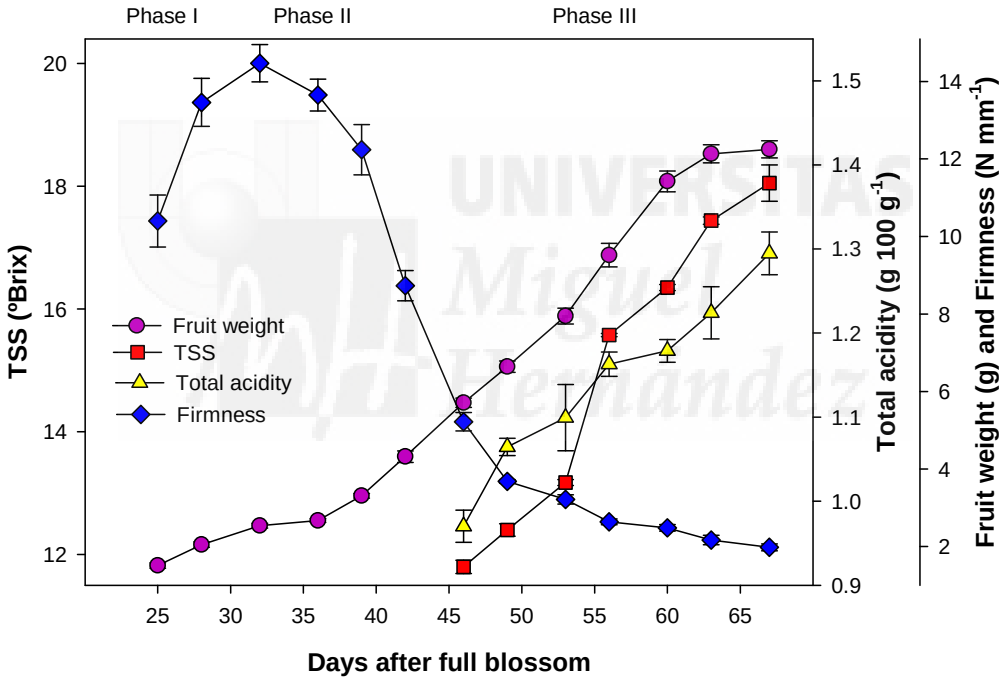

Figure 1. Evolution of fruit weight, firmness, total acidity and TSS content during development and ripening on tree of 'Sonata' cultivar. Data are the mean±SE (n=30 for firmness and fruit weight; n=5 for acidity and TSS). $LSD_{(P<0.05)}$=0.14 for fruit weight, 0.21 for total acidity, 0.27 for TSS and 0.28 for firmness. (●) Fruit weight, (□) TSS, (Δ) Total acidity, (◊) Firmness.

The final fruit weight and the time from full blossom to harvesting were significantly different depending on cultivar (**Tables 1 and 2**). Thus, 'Sunburst' had faster development, with only 57 days from full blossom to harvesting, while 'Prime Giant' had the slowest with 83 days. Final fruit weight was also different among cultivars, ranging from 9 g in 'Sweetheart' to 14 g in 'Sunburst' and 'No. 57', although for all cultivars

similar values were obtained in CH and CH+4 days. This means that at CH all cherry fruits had reached their final size (**Table 2**), which were higher as than the majority of other cultivars studied by others (Usenik et al., 2008). According to morphological properties, Beyer et al. (2002) distinguished five typical shape characteristics of cherries: kidney, flat-round, round, oblong and chordate. In this study, most cultivars had chordate shape ('Santina', 'Somerset', 'No. 57', 'NY-6479', 'Prime Giant' and 'Sunburst'), 3 had round shape ('Newstar', 'Sonata' and 'Sweetheart'), while 'Brooks' and 'Cristalina' had kidney and flat-round shape, respectively.

Table 1: Dates of full blossom and harvesting and total days from full blossom to reach harvest ripening stage for sweet cherry cultivars in 2007 year.

Cultivar	Date of full blossom	Date of commercial harvesting	Total days
Brooks	23 March	4 June	74
Cristalina	12 April	14 June	63
Newstar	24 March	4 June	73
Nº 4 or Santina	8 April	11 June	60
Somerset or Nº 52	6 April	11 June	66
Nº 57 or 13N 7-19	9 April	14 June	66
NY-6479 or Picota	3 April	14 June	69
Prime Giant	20 Mach	11 June	83
Sonata	12 April	14 June	63
Sunburst	15 April	14 June	57
Sweetheart	30 March	21 June	79

Texture is one of the most important attributes in sweet cherry and it is often used as quality assessment, although there are considerable genotypic differences, as can be observed in **Table 2**. In previous reports, it has been found that late cultivars were generally firmer than early ones (Chistesen, 1995; Esti et al., 2002). This was true for 'Sweetheart', which was both the firmest (3.15±0.12 N/mm at CH) and the latest cultivar, while 'Brooks' and 'Newstar' were early-season cultivars and showed an intermediate firmness, and the softest was 'No 57', with 1.85±0.10 N/mm at CH and considered mid-season cultivar (**Tables 1 and 2**). For all cultivars, fruit firmness reached the highest value at the second phase of fruit growth, which has been associated to pit hardening in sweet cherry (Muskovics et al., 2006). After that, fruit firmness decreased sharply as fruit weight increased, as it is shown in **Figure 1** for 'Sonata', which simply reflects cell enlargement during fruit growth. However, softening in the last days of ripening has been attributed to increases in ß-galactosidase activity (Gerardi et al., 2001), unlike in most of fruits, in which softening is dependent on pectin depolymerization due to polygalacturonase activity (Batisse et al., 1996).

For all sweet cherry cultivars, TSS and TA started to increase when fruit had around 40-50% of its final size and went on until the last sampling date, as it is shown for 'Sonata' in **Figure 1**. However, significant differences were found among cultivars and between CH and CH+4 days for each cultivar (**Table 2**).

Table 2: Fruit properties at commercial harvest (CH) and CH + 4 days.

Cultivar	Fruit weight		TSS (ºBrix)	
	CH	CH + 4 days	CH	CH + 4 days
Brooks	11.93±0.42aA	11.95±0.33aA	19.05±0.21aA	20.82±0.19bA
Cristalina	10.55±0.29aB	10.60±0.44aB	17.54±0.31aB	19.37±0.51bB
Newstar	11.75±0.33aA	11.81±0.44aA	18.14±0.24aC	20.72±0.06bA
Santina	10.14±0.33aB	10.22±0.21aB	15.95±0.15aD	16.62±0.15bC
Somerset	10.90±0.20aB	10.92±0.24aB	17.84±0.12aB	19.40±0.11bB
Nº 57	13.91±0.44aC	13.82±0.32aC	18.55±0.24aC	19.70±0.35bB
NY-6479	9.40±0.20aD	9.42±0.15aD	19.60±0.06aE	21.42±0.10bD
Prime Giant	13.36±0.53aC	13.45±0.55aC	19.55±0.14aE	19.90±0.12bB
Sonata	12.13±0.23aE	12.25±0.22aE	17.44±0.06aB	18.05±0.29bE
Sunburst	13.96±0.26aC	13.99±0.21Ca	19.90±0.12aE	20.62±0.05bA
Sweetheart	9.02±0.23aD	9.15±0.21aD	17.90±0.20aB	21.77±0.45bD
Cultivar	**Total Acidity**		**Firmness**	
	CH	CH + 4 days	CH	CH + 4 days
Brooks	0.75±0.02aA	0.84±0.02bA	2.47±0.08aA	2.38±0.10aA
Cristalina	0.85±0.03aB	0.89±0.01aB	2.26±0.09aB	2.17±0.0aB4
Newstar	1.21±0.02aC	1.29±0.02bC	2.24±0.09aB	2.10±0.04aB
Santina	0.79±0.04aA	0.81±0.03aA	2.58±0.09aA	2.27±0.11aA
Somerset	0.92±0.02aBD	0.96±0.05aBD	2.24±0.11aB	2.11±0.09aB
Nº 57	0.99±0.02aD	1.04±0.03aD	1.85±0.10aC	1.62±0.09bC
NY-6479	1.25±0.03aC	1.32±0.02aC	2.83±0.13aD	2.60±0.20aD
Prime Giant	1.12±0.01aE	1.14±0.01aE	2.42±0.10aA	2.31±0.09aA
Sonata	1.20±0.03aC	1.29±0.02bC	2.17±0.12aB	1.98±0.09aB
Sunburst	0.92±0.01aB	0.93±0.02aB	2.23±0.10aB	2.08±0.08bB
Sweetheart	1.18±0.03aCE	1.19±0.05aCE	3.15±0.12aE	2.82±0.09bE

For each cultivar and parameter different small letters between columns show significant differences at $P<0.05$ level. For each parameter and harvest date different capital letters within columns show significant differences at $P<0.05$ level.

At CH the highest TSS content was found in 'Sunburst' (19.90±0.12 ºBrix) followed by 'NY-6479' and 'Prime Giant' (≈19.5 ºBrix) and the lowest in 'Santina' (15.95±0.15 ºBrix). Nevertheless, at CH+4 days the higher levels were found in 'Sweetheart' and 'NY-6479' (≈21.5 ºBrix) while 'Santina' still had the lowest TSS (16.62±0.15 ºBrix). The TSS levels found for these cultivars were in agreement with those reported for other sweet cherries harvested at commercial ripening stage, for which values between 11 and 25 ºBrix have been reported (Girard and Kopp, 1998; Esti et al., 2002; Serrano et al., 2005a and b). The main sugars found in cherry cultivars have been glucose and fructose, followed by sorbitol and sucrose (Girard and Koop, 1998; Serrano et al., 2005a; Usenik et al., 2008).

TA reached the highest levels, close to 1.30 g per 100 g at CH+4 days in 'Newstar', 'NY-6479' and 'Sonata, while 'Brooks' and 'Santina' showed the lowest acidity, ≈0.80 g per 100 g (**Table 2**). In sweet cherry as well as in other *Prunus* species, such as plum, peach, apricot and nectarines, malic acid has been found to be the major organic acid contributing to total acidity, which differed greatly among cultivars (Crisosto, 1994; Girard and Koop, 1998; Zuzunaga et al., 2001). However, in stone fruits apart from cherries, acidity decreased over the development and ripening, while an accumulation was observed for all cherry cultivars, in agreement with the reported increase in total acidity as harvesting date was delayed in 'Lapins' and '4-70' cherries (Drake and Elfving, 2002; Serrano et al., 2005a).

The color indices (Hue angle, Chroma and a/b) of the skin showed similar evolutions for all sweet cherry cultivars which are displayed in the 'Sonata' example in **Figure 2**. Hue angle decreased sharply between 45 and 55 days from full blossom while the decrease was at much slow rate afterwards. Contrarily, Chroma index increased, reaching a plateau and decreased afterwards during the last days of ripening, which means an increase in the tonality of the fruit color. However, a/b could be a better index to describe the ripening process in sweet cherry, since it showed a continuous increase until the last sampling date.

Significant differences were found among the studied cultivars in the final values of a/b index, which ranged from 3.07±0.03 in 'Brooks' to 7.23±0.14 in 'Cristalina', which had the highest bright red (score 3 of CITFL chart) and dark red (score 5 of CITFL chart) colors, respectively (**Figure 3**). The results about parameters related to fruit ripening, such as soluble solids and total acidity accumulation, decrease in firmness and skin color changes, showed that some ripening processes in sweet cherry started to change at an early stage during development of phase III.

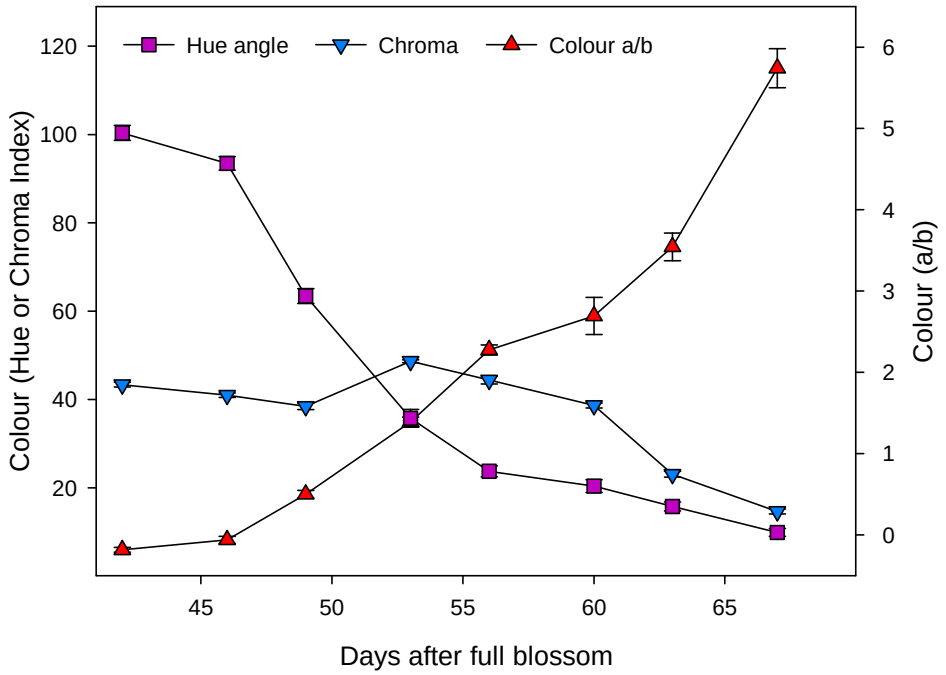

Figure 2: Evolution of color indices (Hue angle, Chroma and a/b) in 'Sonata' sweet cherry along the ripening process on tree. Data are the mean±SE (n=30). $LSD_{(P<0.05)}$=2.45 for Hue angle, 1.67 for Chroma and 0.34 for a/b. (□) Hue angle, (▽) Chroma, (△) Color a/b.

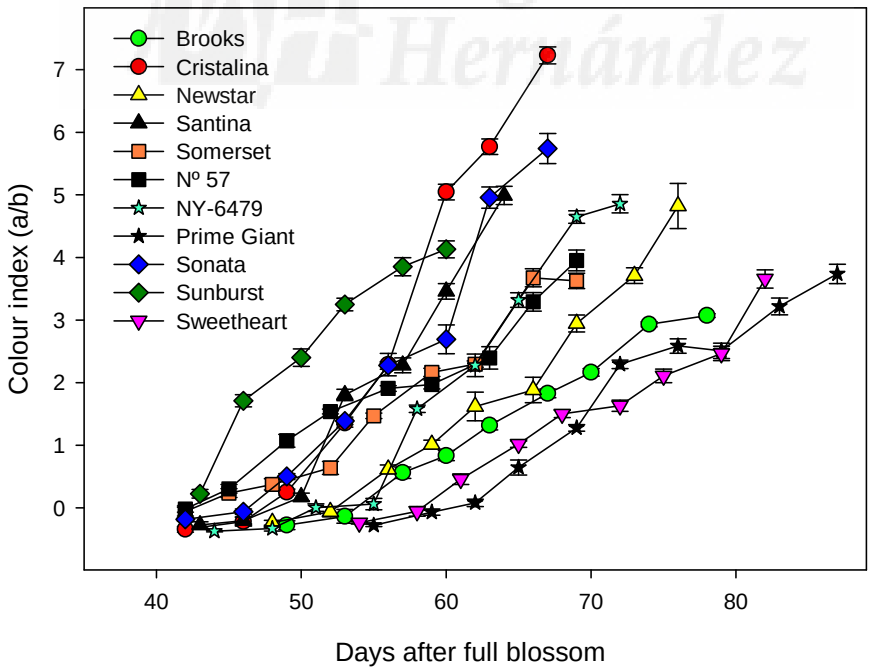

Figure 3: Evolution of color index (a/b) in the different sweet cherry cultivars along the ripening process on tree. Data are the mean ± SE (n=30). $LSD_{(P<0.05)}$ = 0.31.

Evolution on Functional Properties

Total anthocyanins were determined at the four last sampling dates in all cultivars, since it has been shown that the main anthocyanins accumulation occurred in the last two weeks of cherry development (Mozetiĉ et al., 2004). Anthocyanin concentration increased sharply in all cultivars, reaching final concentration between 39.55±2.58 and 224.65±5.57 mg cyanidin-3-glucoside per 100 g for 'Brooks' and 'Cristalina', respectively (**Figure 4**). Taking into account the data obtained at CH, the lowest anthocyanin concentration was found for 'Brooks', 'Somerset', 'Prime Giant' and 'Sweetheart' which are considered as light-colored cultivars, with a 3 score of the CTIFL color chart. 'Cristalina' and 'Sonata' had the highest anthocyanin content and were dark-colored (5 score of the CTIFL color chart), while the remaining had intermediate anthocyanin concentration and a value of 4 (medium-colored cultivars). The predominant anthocyanins in cherry are cyanidin-3-rutinoside and cyanidin-3-glucoside, while peonidin- and pelargonidin- (3-glucoside and 3-rutinoside) have been found at very low concentrations (Gonçalves et al., 2004; Mozetiĉ et al., 2004; 2006; Chaovanalikit and Wrolstad, 2004; Usenik et al., 2008).

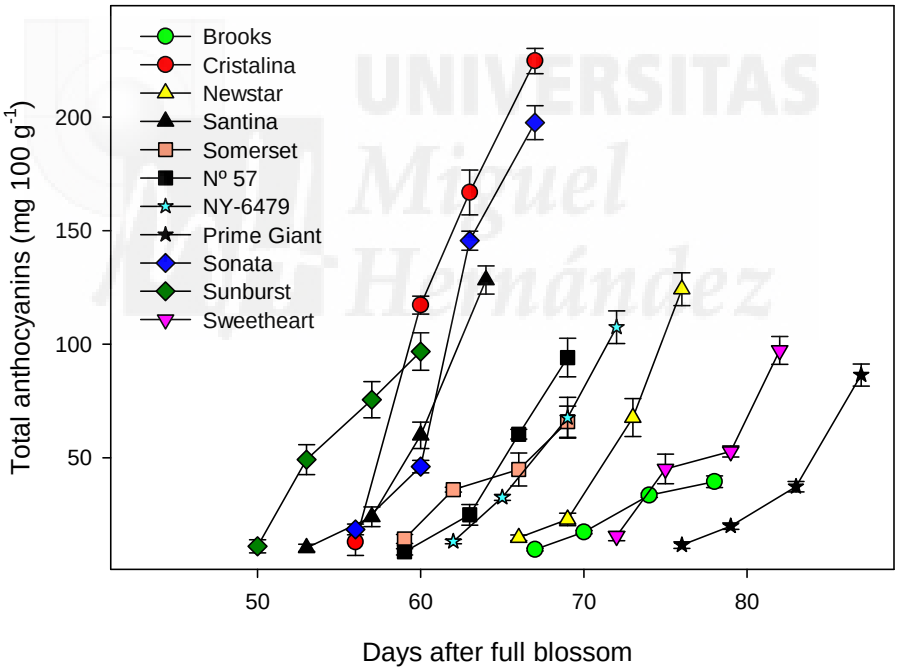

Figure 4: Evolution of total anthocyanin concentration (mg cyanidin 3-glucoside equivalent per 100 g) in the different sweet cherry cultivars along the ripening process on tree. Data are the mean ± SE (n=5). LSD(P<0.05) = 8.95.

When linear regression was performed between anthocyanin concentration and color index a/b taking into account data from all cultivars and sampling dates, a positive linear correlation was found (r^2=0.899) and this could be considered as an easy and reliable index for anthocyanin concentration in cherries, in general. In addition, the

measurement of this color index could be a good tool to predict the levels of anthocyanins in sweet cherry cultivars. Total phenolics increased in a similar way with anthocyanins. A sharp increase during the last sampling dates was observed, with significant differences among cultivars (**Figure 5**). The highest phenolic concentration at the last sampling date was found in 'Sonata' (191.90±6.23 mg gallic acid equivalent per 100 g) and the lowest in 'Brooks' (98.14±4.64 mg/100 g). These levels of total phenolics were within the same concentration range to those found in other cherry cultivars at commercial harvesting, in which concentration from 90 to 200 mg/100 g have been reported (Mozetiĉ et al., 2002; Kim et al., 2005). The major polyphenols in sweet cherry are anthocyanins followed by the hydroxycinnamic acid's derivatives neochlorogenic acid and 3'-*p*-coumaroylquinic acid (Mozetiĉ et al., 2002, Chaovanalikit and Wrolstad, 2004). Since phenolic compounds contribute to fruit quality in terms of modifying color, taste, aroma and flavor (Tomás-Baberán and Espín, 2001), those cultivars with higher phenolics content will have higher quality. In addition, taking into account data from all cultivars and the last four harvest dates, a highly positive correlation was found between total anthocyanins and total phenolics concentration using a polynomial quadratic equation (r^2=0.813). Thus, it could be concluded that in these sweet cherry cultivars, anthocyanins are the major phenolics, according to previous reports in other cultivars (Gao and Mazza, 1995; Chaovanalikit and Wrolstad, 2004).

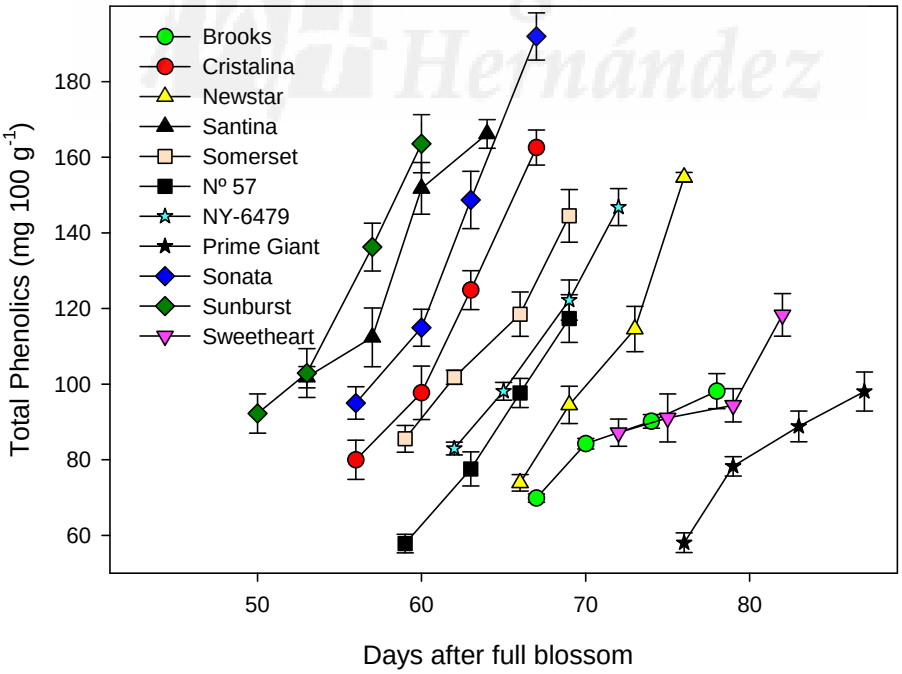

Figure 5: Evolution of total phenolic concentration (mg gallic acid equivalent per 100 g) in the different sweet cherry cultivars along the ripening process on tree. Data are the mean ± SE (n=5). LSD(P<0.05) = 7.42.

TAA was quantified in hydrophilic (H-TAA) and lipophilic (L-TAA) extracts separately. It could be observed that both H-TAA and L-TAA increased along the ripening process for all sweet cherries. For all of them, H-TAA was higher than L-TAA (ca. 80% of TAA in 'Cristalina' and ≈50% in 'Prime Giant'), showing that the major contributors to antioxidant activity are hydrophilic compounds (**Figure 6**). Nevertheless, important differences were found among cultivars. Thus, at the last sampling date, the highest levels of H-TAA were found in 'Sonata' and 'Cristalina' (≈130 mg/100 g) and the lowest in 'Brooks' (69.67±2.50 mg/100 g), while for L-TAA 'Sonata' showed the highest level (74.66±2.68 mg/100 g) and the lowest (≈35 mg/100 g) were found in 'Brooks', 'Santina', 'Cristalina' and 'NY-6479'. However, no correlations were found between H-TAA and L-TAA in these cherry cultivars. This is the first time that antioxidant activity in both hydrophilic and lipophilic extracts has been measured during sweet cherry fruit ripening on tree and no literature is available for comparative purposes. The only reports in which L-TAA and H-TAA have been quantified separately are those of Wu et al. (2004), in a wide range of fruits and vegetables at CH (including 4 cherry cultivars although no names or maturity stages were reported), and Arnao et al. (2001), in vegetable soups, showing that H-TAA contributed about 70-90% of the total antioxidant activity.

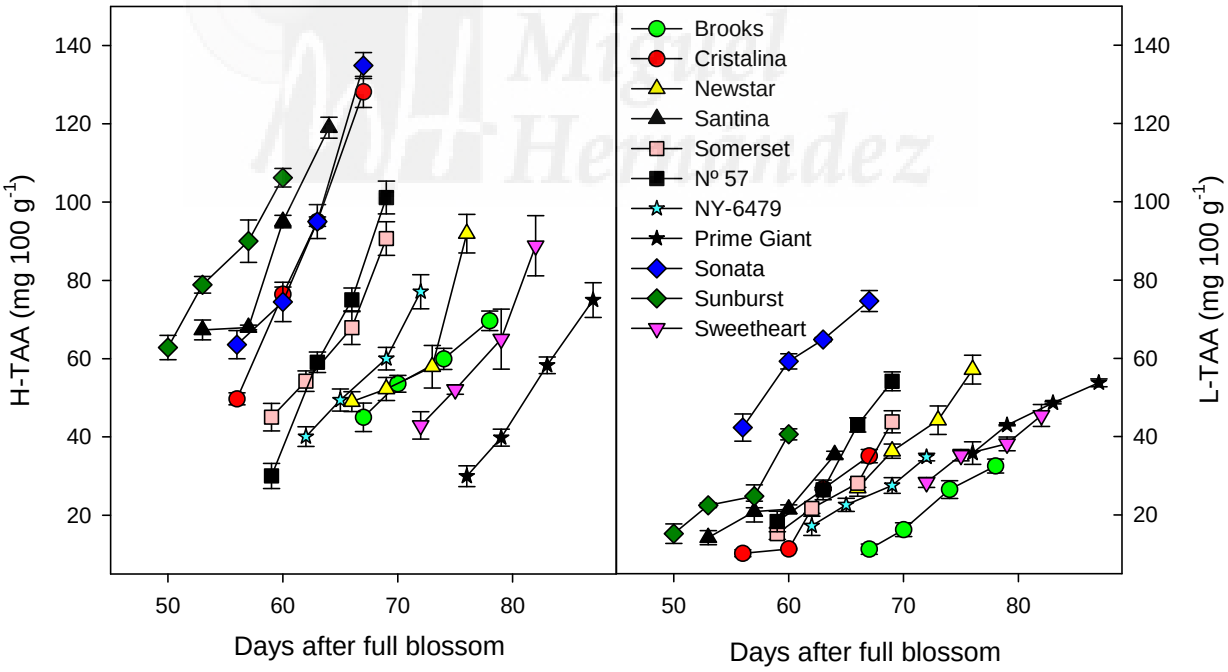

Figure 6: Hydrophilic (H-TAA) and lipiphilic (L-TAA) total antioxidant activity (mg Trolox equivalent per 100 g) evolution in the different sweet cherry cultivars along the ripening process on tree. Data are the mean ± SE (n=5). $LSD_{(P<0.05)}$ = 6.94 for H-TAA and 4.15 for L-TAA.

A high positive correlation was obtained between H-TAA and both, phenolic and anthocyanin concentrations (y=1.33 x-9.83; r^2=0.841 and y=0.41 x+45.7; r^2=0.753, respectively) taking into account data for all cultivars and the four last sampling dates. Thus, in sweet cherry the main contributors to H-TAA are phenolic compounds and especially anthocyanins. The correlation between antioxidant activity and phenolic compounds has been also found in several studies comparing a wide range of fruits and vegetables (Wang et al., 1996; Kaur and Kapoor, 2001; Wu et al., 2004). Specifically, in sweet cherry cultivars, it has also been found that there is a good correlation between total phenolics and TAA (Serrano et al., 2005a; Usenik et al., 2008), although they were determined only in hydrophilic extracts. This indicates that when sweet cherry is developing the intensity of red color, the anthocyanins and other phenolic compounds could also account for their antioxidant activity and health beneficial effects (Scalbert et al., 2005).

It has been shown that sour and sweet cherry anthocyanins have the potential to directly interfere with intestinal tumor development (Kang et al., 2003), a strong antidegenerative activity in neuronal cells (Kim et al., 2005) and a beneficial role in the treatment of inflammatory pain (Tall et al., 2004). Thus, cherry can serve as a good source of biofunctional phytochemicals in our diet, providing health beneficial effects in humans. Moreover, ascorbic acid is a hydrophilic compound with antioxidant activity which could also account for H-TAA, as has been shown in sweet cherry '4-70' (Serrano et al., 2005a) and in other fruits, such as oranges (Pretel et al., 2004).

CONCLUSIONS

Results show that there are significant differences among sweet cherry cultivars in quality parameters related to sensory, nutritive and functional properties, and between ripening stages. Taking into account data from fruit weight, color, firmness, acidity and TSS (sensory and nutritive parameters), the cultivars more appreciated could be 'NY-6479', 'Prime Giant' and 'Sunburst'. However, 'Cristalina' and 'Sonata' exhibited the highest values of total anthocyanins and total phenolic compounds that seemed to be the main responsible for antioxidant activity properties. Finally, it is interesting to point out that for all cultivars a delay in harvesting (CH+4 days) led to significant increases in functional compounds and antioxidant activity. In future, it would be necessary to determine the best conditions in handling, storage and commercialization to ensure that the overall cherry quality does not decrease until they reach the consumer.

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Chapter 3

Changes in hydrophilic and lipophilic antioxidant activity and related bioactive compounds during postharvest storage of yellow and purple plum cultivars

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Changes in hydrophilic and lipophilic antioxidant activity and related bioactive compounds during postharvest storage of yellow and purple plum cultivars

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Abstract

Eight plum cultivars (four dark-purple and four yellow) were harvested at the commercial ripening stage, and changes of fruit quality properties were evaluated during cold storage and subsequent shelf-life, with special emphasis on bioactive compounds (phenolics, anthocyanins and carotenoids) and antioxidant activity (TAA). From the eight plum cultivars, four showed the typical climacteric ripening pattern ('Blackamber', 'Larry Ann', 'Golden Globe' and 'Songold') while four behaved as suppressed-climacteric types ('Golden Japan', 'Angeleno', Black Diamond' and 'TC Sun'), the latter being described for the first time. At harvest, large variations in phytochemicals and antioxidant activity were found among cultivars in peel and pulp tissues, although phytochemical concentration and antioxidant activity were higher in the peel than in the flesh (2–40-fold depending on the bioactive compound). During storage, increases in total phenolics for all cultivars (peel and pulp), in total anthocyanin content in the peel of the dark-purple plums, and total carotenoids in the peel and pulp of the yellow cultivars were observed. This behaviour of the bioactive compounds was reflected in TAA changes, since hydrophilic-TAA (H-TAA) was correlated with both phenolics and anthocyanins, while lipophilic-TAA (L-TAA) was correlated with carotenoids. L-TAA comprised about 30–50% of the TAA in plum tissues. Carotenoids and phenolics (and among them the anthocyanins) could be the main lipophilic and hydrophilic compounds contributing to L-TAA and H-TAA, respectively. No significant loss of bioactive compounds and TAA occurred during prolonged plum storage. Moreover, for a better evaluation of the antioxidant potential of plums, the contribution to carotenoids should not be overlooked.

Keywords: Phenolics; Anthocyanins; Carotenoids; Plum; Storage

INTRODUCTION

Plums are an important group among stone fruit grown commercially in Spain. Harvest date of this crop is an essential determinant of consumer acceptability (Crisosto et al., 2004), and a number of parameters are generally used to establish the optimum ripening stage including skin colour, flesh texture, soluble solids concentration (SSC) and acidity together with developed aroma and flavour. However, large variations in these parameters can be found depending on cultivar, production area, climatic conditions and harvest season (Kader and Mitchell, 1989). In addition, two distinct patterns of ripening behaviour have been reported for plums, with cultivars showing a suppressed-climacteric phenotype such as 'Shiro', 'Rubyred' (Abdi et al., 1997) and 'Golden Japan' (Zuzunaga et al., 2001), in contrast to the typical climacteric shown for most plums.

Given the perishable nature of plum fruit, the use of cold storage, avoiding temperatures that induce chilling injury in the sensitive cultivars, is a postharvest tool to delay changes related to ripening, such as ethylene production, respiration rate, softening, pigment changes, increase in SSC and decrease in acidity (Murray et al., 2005; Guerra and Casquero, 2008). Moreover, several treatments prior to cold storage (calcium, heat, polyamines or 1-methylcyclopropene) have been reported to maintain plum quality for longer periods than low temperature alone (Valero et al., 2002a,b; Martínez- Romero et al., 2003).

Increased intake of fruit and vegetables has been associated with reduced incidence of degenerative diseases due to their antioxidant potential (Kris-Etherton et al., 2002; Prior, 2003; Schreiner and Huyskens-Keil, 2006). In this sense, plums are considered a fruit class with high amounts of bioactive compounds or phytochemicals such as vitamins (A, C and E), anthocyanins and other phenolic compounds, and carotenoids (Stacewicz-Sapuntzakis et al., 2001), which contribute to the antioxidant capacity. On the other hand, large variations in the concentration of bioactive compounds at commercial harvesting depending on cultivar have been reported (Los et al., 2000; Tomás-Barberán et al., 2001; Gil et al., 2002; Kim et al., 2003; Cevallos-Casals et al., 2006).

No literature on the changes occurring in the above bioactive compounds during storage of plums is available. The aim of this paper was to determine the changes in bioactive compounds during storage of a wide range of plum cultivars, both dark-purple and yellow pigmented. In addition, total antioxidant activity (TAA) has been determined in two separate fractions (hydrophilic and lipophilic) from peel and flesh tissues and the contribution of the several bioactive compounds in each extract will be discussed.

MATERIALS AND METHODS

Plant material and experimental design

Eight Japanese type plum cultivars (Prunus salicina Lindl.) were selected, four of them had dark-purple peel: 'Angeleno', 'Blackamber', 'Black Diamond' and 'Larry Ann', and four had yellow peel: 'Golden Japan', 'Golden Globe', 'Songold' and 'TC Sun'. Among these cultivars, seven had yellow-orange flesh while 'Black Diamond' had red flesh. The plum cultivars were harvested from a farm (Los Frutales, Villena, Alicante, Spain) at commercial ripening stage based on size, colour and firmness. For each cultivar, about 400 plums were manually picked and transferred immediately to the laboratory. Then, 240 homogeneous fruit (size, colour and with absence of any defect) were selected for each cultivar and randomly sorted into 12 lots of 20 fruit. All fruit were stored in a controlled cold chamber at 2 ºC and RH of 85% in darkness. After 0, 7, 14, 21, 28 and 35 d cold storage, two lots were taken randomly. One was immediately analysed and the other was stored for a further 4 d at 20 ºC to simulate commercial procedure (shelf-life, SL), in which the same analyses were carried out as follows. For each sampling date and cultivar, fruit firmness and colour were measured individually. Following these determinations, five subsamples of four fruit each were made from each lot, in which ethylene production was quantified. The fruit from each subsample were then manually peeled to separate the peel from the flesh. The flesh tissue was cut in small pieces and a portion used to determine soluble solids concentration and titratable acidity. The separate peel and flesh were then immediately frozen and ground in liquid N_2. The samples were stored in at −40 ºC until analysis of total phenolics, total anthocyanins,

Ethylene production

Ethylene production was measured by placing each subsample of four fruit in a 2-L glass jar hermetically sealed with a rubber stopper for 30min. One millilitre of the atmosphere was withdrawn with a gas syringe, and the ethylene was quantified using a ShimadzuTM GC-2010 gas chromatograph (Kyoto, Japan), equipped with a flame ionisation detector (FID) and a 3m stainless steel column with an inner diameter of 3.5mm containing activated alumina of 80/100 mesh. Carrier gas was helium, column temperature was 90 ºC, and injector and detector temperatures were 150 ºC. Results were the mean ± SE of determinations for each subsample and expressed as ng kg^{-1} s^{-1}.

Fruit firmness and colour

Fruit firmness was measured on the fruit shoulder using a flat steel plate coupled with a texturometer (TX-XT2i Texture Analyzer, Stable Microsystems, UK) interfaced to a personal computer. A bevelled holder prevented bruising of the opposite side. For each

fruit, the diameter was measured and then a force that achieved a 3% deformation of the fruit diameter was applied. Results were expressed as the force-deformation (Nmm^{-1}) and were the mean ± SE.

Colour was determined in both peel and flesh of each fruit using the CIE Lab Systemin aMinolta colorimeter CR200 model using D65 illuminant (Minolta Camera Co., Japan). Results were the mean ± SE of three determinations for each fruit and expressed as Hue angle.

Soluble solids concentration, titratable acidity and ripening index

Total soluble solids concentration was determined in duplicate 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 %. Titratable acidity (g of malic acid equivalent per 100 g^{-1} fresh weight) was determined also in duplicate by automatic titration (785 DMP Titrino, Metrohm) with 0.1N NaOH up to pH 8.1, using 1mL of diluted juice in 25 mL distilled H2O. The ratio between soluble solids concentration and titratable acidity was considered as the ripening index (RI).

Bioactive compounds

Total anthocyanins were determined according to García-Viguera et al. (1999) adapted as previously reported (Serrano et al., 2005). Total anthocyanin was calculated using cyanidin 3-glucoside (molar absorption coefficient of 23,900 L cm^{-1} mol^{-1} and molecular weight of 449.2 $gmol^{-1}$) and results expressed as mg kg^{-1} fresh weight, and were the mean ± SE of determinations made in duplicate in each one of the five subsamples.

Total carotenoids were extracted in duplicate according to Mínguez-Mosquera and Hornero-Méndez (1993). Briefly, 1 g of skin or 2 g of flesh tissues were extracted with acetone and shaken with diethyl ether and 10% NaCl to separate the two phases. The lipophilic phase was washed withNa2SO4 (2%), saponified with 10% KOH in methanol, and the pigments were subsequently extracted with diethyl ether, evaporated and then made up to 25 mLwith acetone. Total carotenoids were estimated by reading the absorbance at 450 nm in a UNICAM Helios-α spectrophotometer (Cambridge, UK), and expressed as mg of ß-carotene equivalent kg^{-1} fresh weight, taking into account the $\varepsilon^{1\%}_{cm}$ = 2560 and the results were the mean ± SE.

Total phenolics were extracted according to Tomás-Barberán et al. (2001) using water:methanol (2:8) containing 2mM NaF and quantified using the Folin–Ciocalteu reagent (Singleton et al., 1999) and results (mean ± SE) were expressed as mg gallic acid equivalent kg^{-1} fresh weight of determinations made in duplicate in each subsample.

Total antioxidant activity was quantified also in duplicate for each subsample according to Arnao et al. (2001), which enables the determination of TAA due to both hydrophilic (HTAA) and lipophilic (L-TAA) compounds in the same extraction. Briefly, 1 g of peel or 2 g of flesh tissues were homogenized in 5mL of 50mM phosphate buffer pH 7.8 and 3mL of ethyl acetate, and then centrifuged at 15,000 rpm for 15min at 4 ºC. The upper fraction was used for L-TAA while the lower fraction for H-TAA quantification. For 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), in which $ABTS^{\bullet+}$ radicals are generated and monitored at 730 nm. The decrease in absorbance after adding the skin or flesh extracts was proportional to TAA of the sample. A calibration curve (0–20 nmol) was performed with Trolox ((R)-(+)-6-hydroxy-2,5,7,8-tetramethylcroman-2-carboxylic acid, Sigma, Madrid, Spain) in both aqueous or methanolic media for H-TAA and L-TAA, respectively. The results are expressed as the mean ± SE in mg of Trolox equivalent kg−1 fresh weight.

Statistical analysis

Experimental data were subjected to ANOVA analysis. 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). Linear regressions were performed between total antioxidant activity (either hydrophilic or lipophilic) and the bioactive compounds taking into account all sampling data (either peel or flesh).

RESULTS

Physico-chemical parameters and ethylene production

Variables related to fruit quality such as SSC, acidity, firmness and colour (peel or flesh) were analysed at harvest and during cold storage and the subsequent SL. Since these parameters changed in a continuous way during storage, Table 1 shows the results obtained at harvest and at the end of the experiment (35 d at 2 ◦C + SL). Cultivars differed in quality variables at commercial harvest, with a range of 10–15% SSC and 0.6–1.6 g 100 g^{-1} acidity, and higher Hue values in yellow compared to dark-purple plums. During storage, acidity levels showed significant reductions for all cultivars, with losses averaging 40–45%, while SSC significantly increased with the exception of 'Golden Japan' and 'Golden Globe' plums.

Table 1. Values of soluble solids concentration (%SSC), acidity (g eq. malic acid 100 g^{-1}), ripening index (RI, SSC/acidity ratio), firmness (Nmm^{-1}) and colour of the peel and the flesh (Hue angle) at harvest and after 35 d of cold storage at 2 ºC+4d at 20ºC (SL) in eight plum cultivars.

		Angeleno	Black Amber	BlackDiamond	Larry Ann
SSC	At H	11.43 ± 0.06 aA	11.00 ± 0.07 aA	12.38 ± 0.07 aB	9.80 ± 0.15 aC
	35 + SL	13.70 ± 0.05 bA	13.03 ± 0.09 bA	13.25 ± 0.03 bA	12.83 ± 0.08 bA
Acidity	At H	1.08 ± 0.02 aA	1.24 ± 0.01 aB	0.84 ± 0.01 aC	0.92 ± 0.05 aAC
	35 + SL	0.60 ± 0.04 bA	0.67 ± 0.02 bA	0.46 ± 0.05 bB	0.57 ± 0.02 bA
RI	At H	10.56 ± 0.27 aA	8.86 ± 0.09 aB	14.67 ± 0.19 aC	10.65 ± 0.84 aA
	35 + SL	22.83 ± 1.12 bA	19.95 ± 0.76 bA	30.14 ± 1.45 bB	22.58 ± 0.99 bA
Firmness	At H	8.67 ± 0.25 aA	6.66 ± 0.45 aB	9.93 ± 0.28 aC	5.42 ± 0.56 aB
	35 + SL	3.24 ± 0.18 bA	2.18 ± 0.13 bB	2.79 ± 0.21 bA	1.75 ± 0.14 bB
Peel Hue	At H	15.45 ± 0.71 aA	9.91 ± 0.72 aB	22.69 ± 1.81 aC	19.20 ± 1.83 aD
	35 + SL	5.46 ± 0.95 bA	2.14 ± 0.49 bB	11.15 ± 1.07 bC	7.35 ± 0.44 bD
Flesh Hue	At H	91.12 ± 0.46 aA	88.37 ± 0.25 aA	43.96 ± 1.06 aB	58.95 ± 1.35 aC
	35 + SL	69.01 ± 1.86 bA	62.56 ± 1.70 bB	12.50 ± 1.47 bC	20.03 ± 1.47 bD
		Golden Japan	**Golden Globe**	**Songold**	**TC Sun**
SSC	At H	10.68 ± 0.05 aAC	13.43 ± 0.06 aD	13.95 ± 0.24 aD	14.85 ± 0.12 aE
	35 + SL	10.53 ± 0.02 aB	13.75 ± 0.17 aA	15.90 ± 0.04 bC	17.10 ± 0.14 bD
Acidity	At H	1.60 ± 0.01 aD	0.58 ± 0.01 aE	0.72 ± 0.04 aC	0.72 ± 0.01 aC
	35 + SL	0.79 ± 0.06 bC	0.27 ± 0.01 bD	0.42 ± 0.02 bB	0.45 ± 0.01 bB
RI	At H	6.68 ± 0.05 aD	23.16 ± 0.05 aE	19.42 ± 0.68 aF	20.55 ± 0.43 aF
	35 + SL	13.59 ± 0.09 bC	50.27 ± 1.03 bD	38.28 ± 1.92 bE	37.85 ± 0.72 bE
Firmness	At H	7.24 ± 0.54 aD	8.71 ± 0.45 aA	5.74 ± 0.26 aB	8.89 ± 0.34 aA
	35 + SL	2.08 ± 0.15 bB	1.62 ± 0.11 bB	1.86 ± 0.21 bB	3.07 ± 0.27 bA
Peel Hue	At H	107.29 ± 0.53 aE	88.10 ± 1.01 aF	95.39 ± 0.49 aG	93.34 ± 0.62 aG
	35 + SL	100.88 ± 0.49 bE	68.87 ± 0.92 bF	84.30 ± 0.74 bG	85.13 ± 1.09 bG
Flesh Hue	At H	104.42 ± 0.47 aD	88.63 ± 0.52 aA	94.67 ± 0.52 aE	86.30 ± 0.46 aA
	35 + SL	98.74 ± 0.68 bE	74.34 ± 0.47 bF	78.23 ± 0.72 bF	76.56 ± 0.68 bG

For each parameter, different small letters showed significant differences ($p < 0.05$) between data at harvest (H) and after 35 d of cold storage+4d at 20ºC (35 + SL) for each cultivar, while different caps letters within each row showed significant differences ($p < 0.05$) among cultivars.

For all cultivars, softening occurred during storage with firmness losses of between 60% and 80% depending on cultivar, while Hue angle significantly diminished in both peel and pulp tissues, the decrease being higher in the dark-purple than in the yellow cultivars (**Table 1**).

Fruit from four cultivars ('Angeleno', 'Black Diamond', 'Golden Japan' and 'TC Sun') had very low ethylene production (below 200 ng kg^{-1} s^{-1}) in both cold storage and SL. Of the remaining four cultivars, all had fruit with more ethylene production especially after SL, and the red-purple types were higher (>2800 ng kg^{-1} s^{-1}) than the gold types (**Fig. 1**).

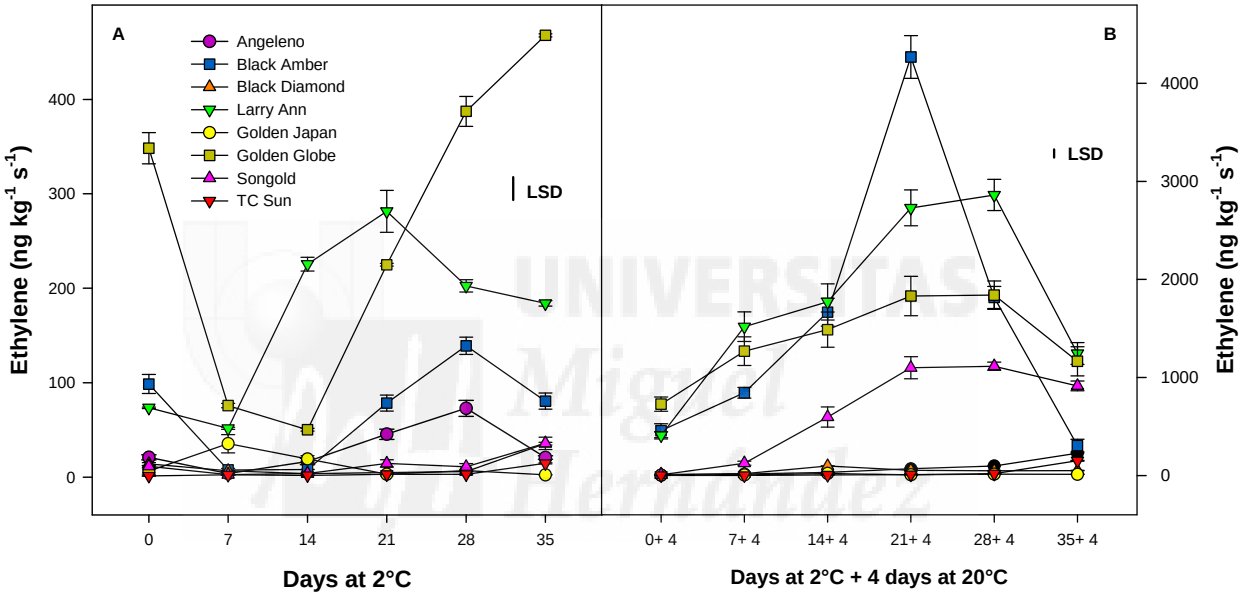

Fig. 1. Ethylene production rate during cold storage (A) and subsequent shelf-life (B) for the eight plum cultivars. Data are the mean ± SE and the LSD ($p < 0.05$) values are shown.

Bioactive compounds

The anthocyanin content was only detected in fruit of the red-purple cultivars in both tissues (peel and flesh). For all cultivars, the levels of anthocyanins were always higher in the peel (20–40- fold) than in the pulp with significant differences among cultivars. At harvest, the highest peel anthocyanin concentration was found in 'Black Amber' (4370±180 mg kg^{-1}) while the lowest occurred in 'Black Diamond' (1310±100 mg kg^{-1}). With respect to flesh, the anthocyanin levels ranged between 34±4 and 177±7 mg kg^{-1} for 'Angeleno' and 'Black Diamond, respectively (**Fig. 2**). However, a similar change during postharvest storage was found for all cultivars. Thus, significant increases were found in the peel anthocyanins for all cultivars, while they remained unchanged in the

flesh of ‘Angeleno’ and ‘Black Amber’ or with slight reductions in ‘Black Diamond’ and ‘Larry Ann’.

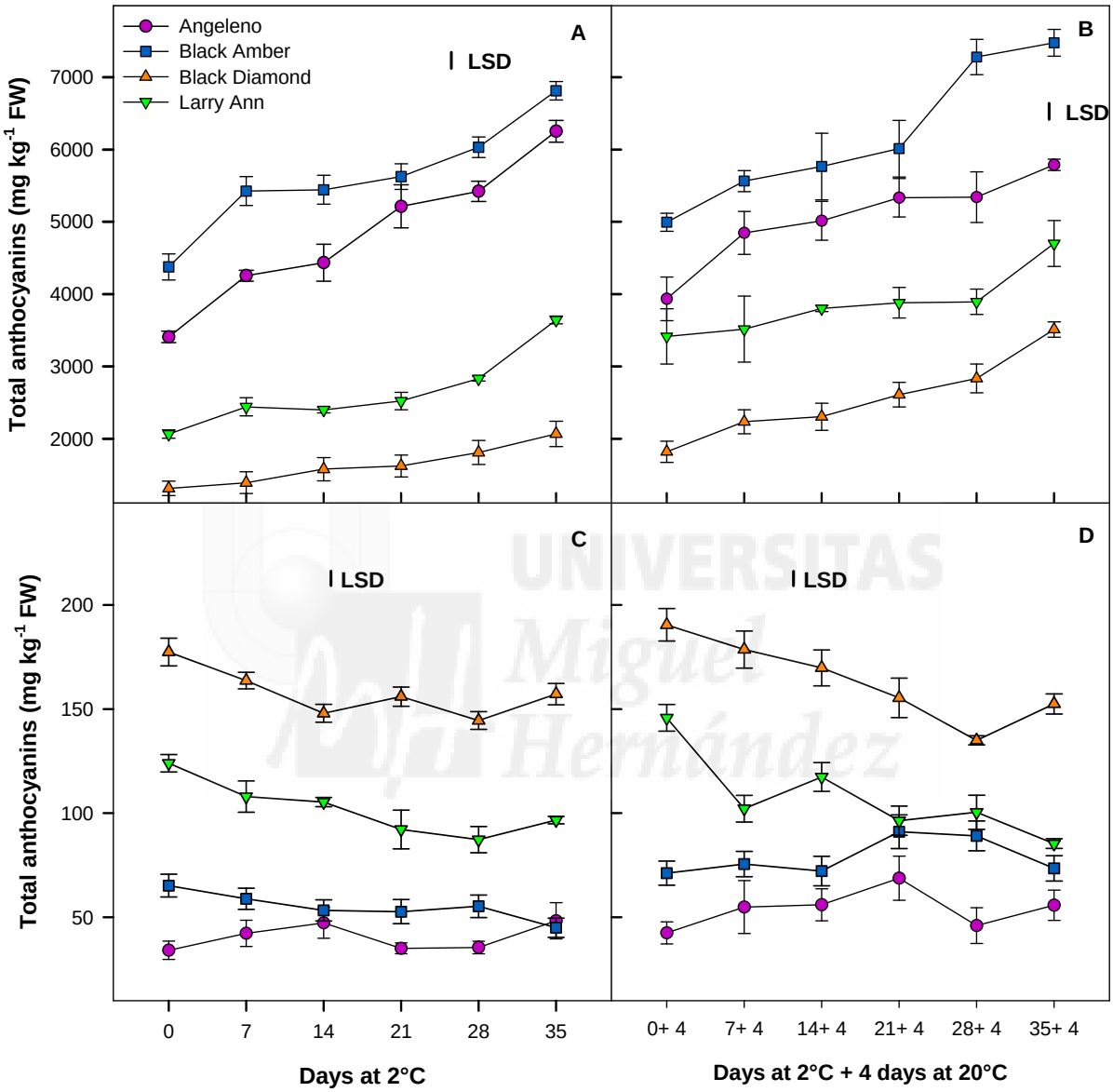

Fig. 2. Changes of total anthocyanin concentration in the peel during cold storage (A) and subsequent shelf-life (B) or in the pulp during cold storage (C) and subsequent shelf-life (D) of the dark-purple plums. Data are the mean ± SE and the LSD ($p < 0.05$) values are shown.

The content of total phenolics was generally 2–5-fold higher in the peel than in the flesh for all cultivars. In addition, the red-purple cultivars had significantly higher phenolic concentrations than the yellow plums in the peel, while in the flesh the content of total phenolics was not dependent on the fruit colour (**Fig. 3**). Among red-purple

plums, 'Black Amber' had the highest phenolic content (5210±180 mg kg^{-1}) and 'Black Diamond' the lowest (2700±130 mg kg^{-1}), while 'Songold' was the yellow cultivar with the highest concentration (1990±140mg kg^{-1}) and 'Golden Japan' with the lowest (1010±80 mg kg^{-1}) total phenolics. During storage, either in cold or after SL, the same pattern of total phenolics was found for all cultivars, that is, significant increases from the initial values to those obtained after 35 d at 2 ◦C + SL (end of the experiment). This increase was also detected in the flesh phenolics, although the differences among cultivars were not so noticeable when compared with peel.

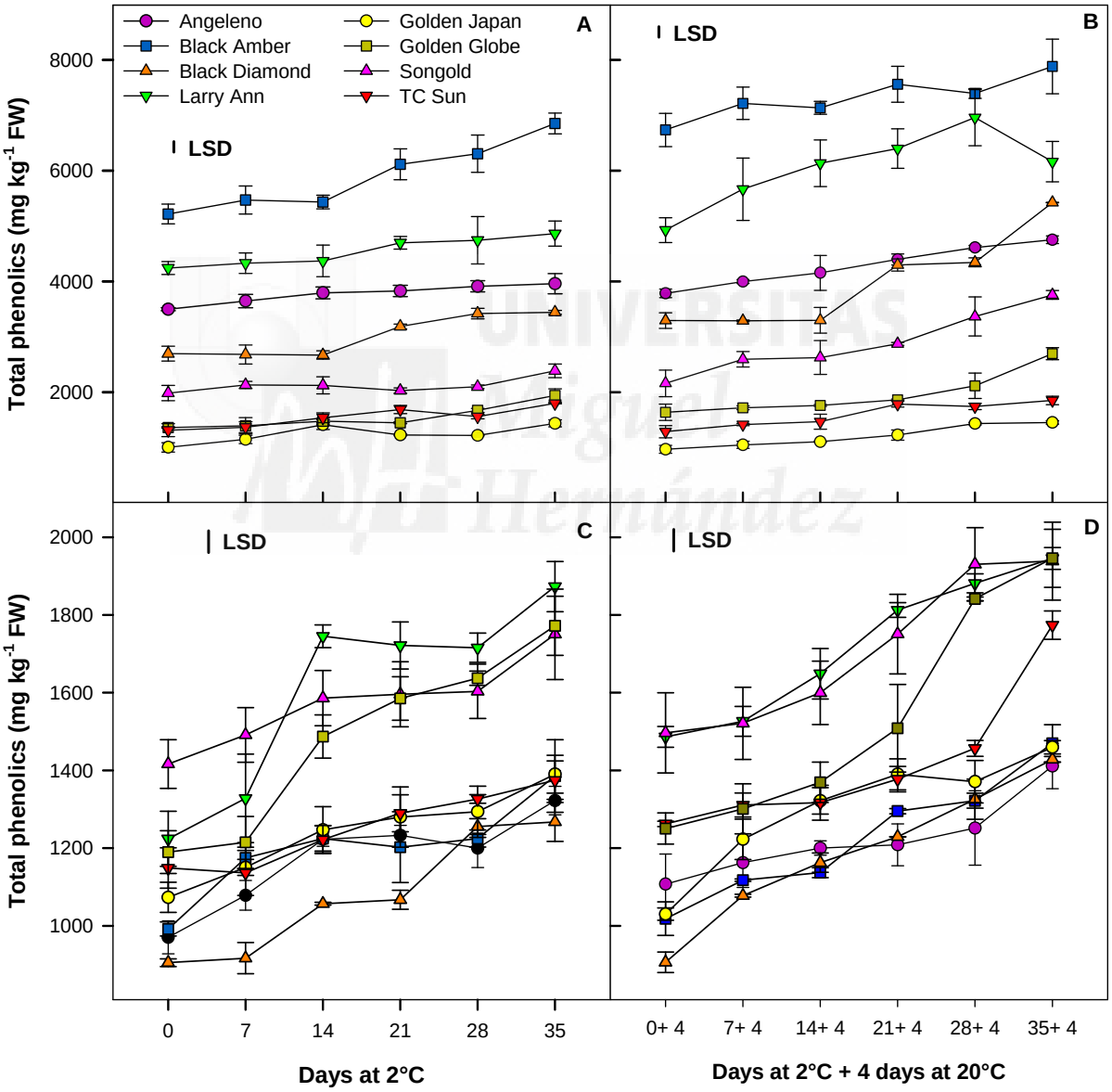

Fig. 3. Changes in total phenolic concentration in the peel during cold storage (A) and subsequent shelf-life (B) or in the pulp during cold storage (C) and subsequent shelf-life (D) of the eight plum cultivars. Data are the mean ± SE and the LSD ($p < 0.05$) values are shown.

The determination of total carotenoids (expressed as ß-carotene) showed that these pigments were present in the peel and flesh of either yellow or red-purple plums (**Fig. 4**). It is interesting to highlight that two red-purple cultivars, 'Larry Ann' and 'Black Amber', had the highest carotenoid content in both tissues: peel (99±7 and 62±5 mg kg^{-1}, respectively) and pulp (11±0.2 and 10±0.2mg kg^{-1}, respectively).

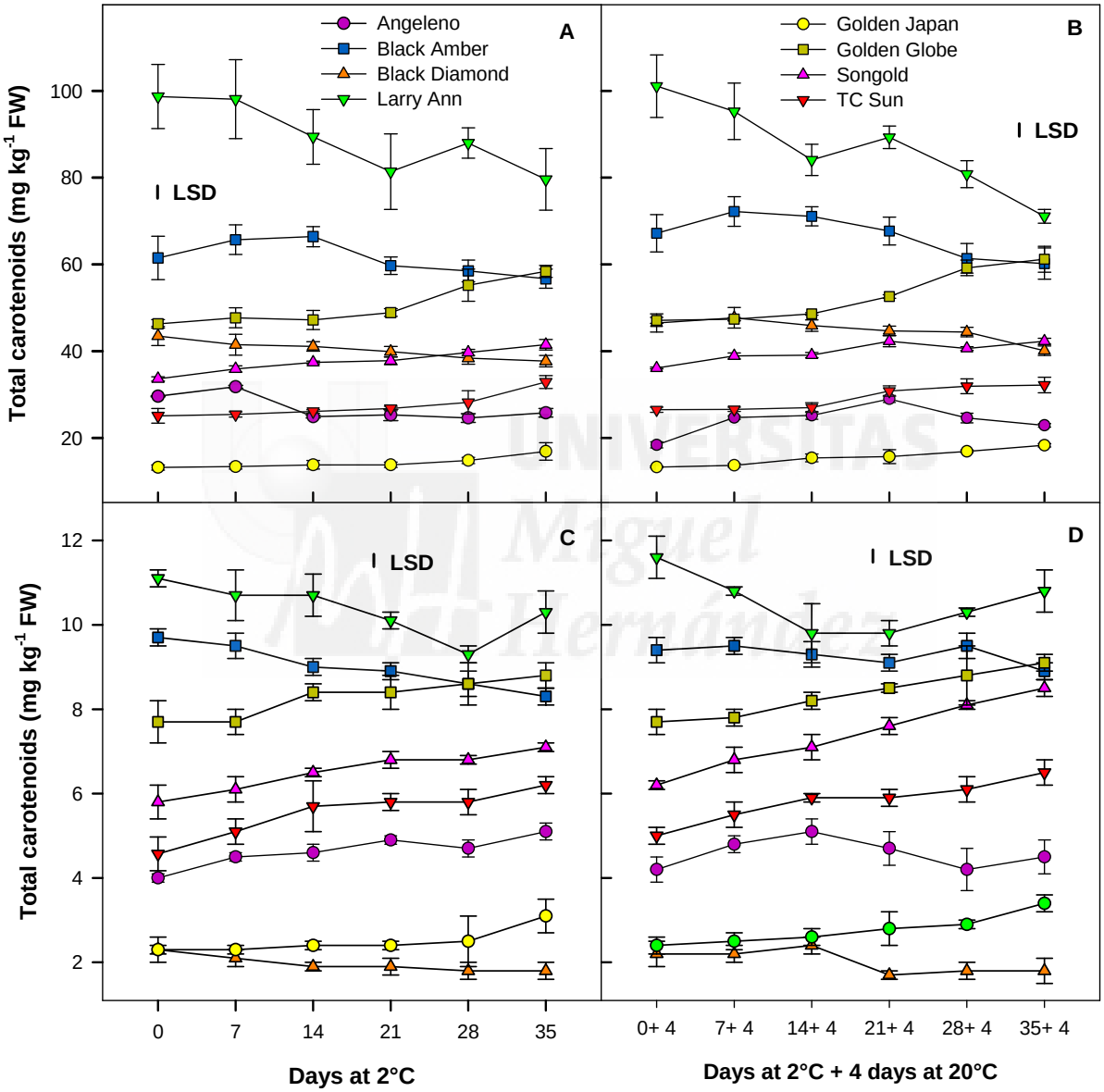

Fig. 4. Changes in total carotenoid concentration in the peel during cold storage (A) and subsequent shelf-life (B) or in the pulp during cold storage (C) and subsequent shelf-life (D) of the eight plum cultivars. Data are the mean ± SE and the LSD ($p < 0.05$) values are shown.

Among yellow plums, the highest content of carotenoids was observed in 'Golden Globe' in both peel and flesh (47±0.7 and 8±0.5 mg kg^{-1}, respectively), while the lowest was in 'Golden Japan' (13±0.6 and 2±0.1 mg kg^{-1}, for peel and flesh, respectively). During storage, the carotenoid behaviour seemed to be dependent on the cultivar peel colour, since significant increases were found in yellow plums while remaining unchanged in all dark-purple ones, for both peel and flesh tissues (**Fig. 4**).

Total antioxidant activity

The total antioxidant activity was measured separately as hydrophilic (H-TAA) and lipophilic (L-TAA) fractions and both types were higher in peel than in flesh for all cultivars. With respect to H-TAA, the levels at harvest in the peel were significantly higher in the dark-purple than in the yellow plums, ranging from 5980±560 to 594±26 mg kg^{-1}, for 'Black Amber' and 'TC Sun', respectively. However, differences in H-TAA among cultivars were lower in the flesh, since their values ranged from454±12 mg kg^{-1} in 'Larry Ann' to 260±30 mg kg^{-1} in 'TC Sun' (**Fig. 5**). During postharvest storage, H-TAA increased significantly in the peel of 'Black Amber', 'Black Diamond' and 'Larry Ann' cultivars, and in the flesh of 'Larry Ann' and 'Songold' cultivars, while no significant changes occurred in the remaining cases.

L-TAA at harvest was significantly different depending on cultivar; although for all of them the levels in the peel were 2–4 times higher than those found in the flesh (Fig. 6). Thus, the highest values in peel and flesh tissues were found in 'Larry Ann' fruit (4880±160 and 1470±90 mg kg^{-1}, respectively), while the lowest L-TAA in peel was recorded in 'Golden Japan' (720±70 mg kg^{-1}) and in the flesh of 'Black Diamond' (190±10 mg kg−1) fruit. During postharvest storage, the yellow plums 'Golden Globe' and 'Songold' exhibited significant increases in the L-TAA of the peel, while no significant changes were found for the remaining yellow cultivars. However, in the dark-purple cultivars, significant decreases occurred. With respect to the flesh, no significant changes were obtained for the majority of the cultivars, and only 'Angeleno' showed a significant increase in L-TAA from the levels at harvest (430±30 mg kg^{-1}) to those obtained after 35 d at 2 ºC + SL (950±40 mg kg^{-1}). To know which of the bioactive compounds were contributing to TAA, linear regressions were performed taking into account all data (peel and pulp) obtained from all cultivars during cold storage and after SL. As expected, the results revealed that H-TAA was highly correlated with the content of total phenolics ($y = 1.52x-163$; R^2 = 0.949) and at a lower dimension with anthocyanins ($y = 1.28x + 56$; R^2 = 0.842), while L-TAA was highly correlated with total carotenoids ($y = 46.72x + 33.55$; R^2 = 0.882).

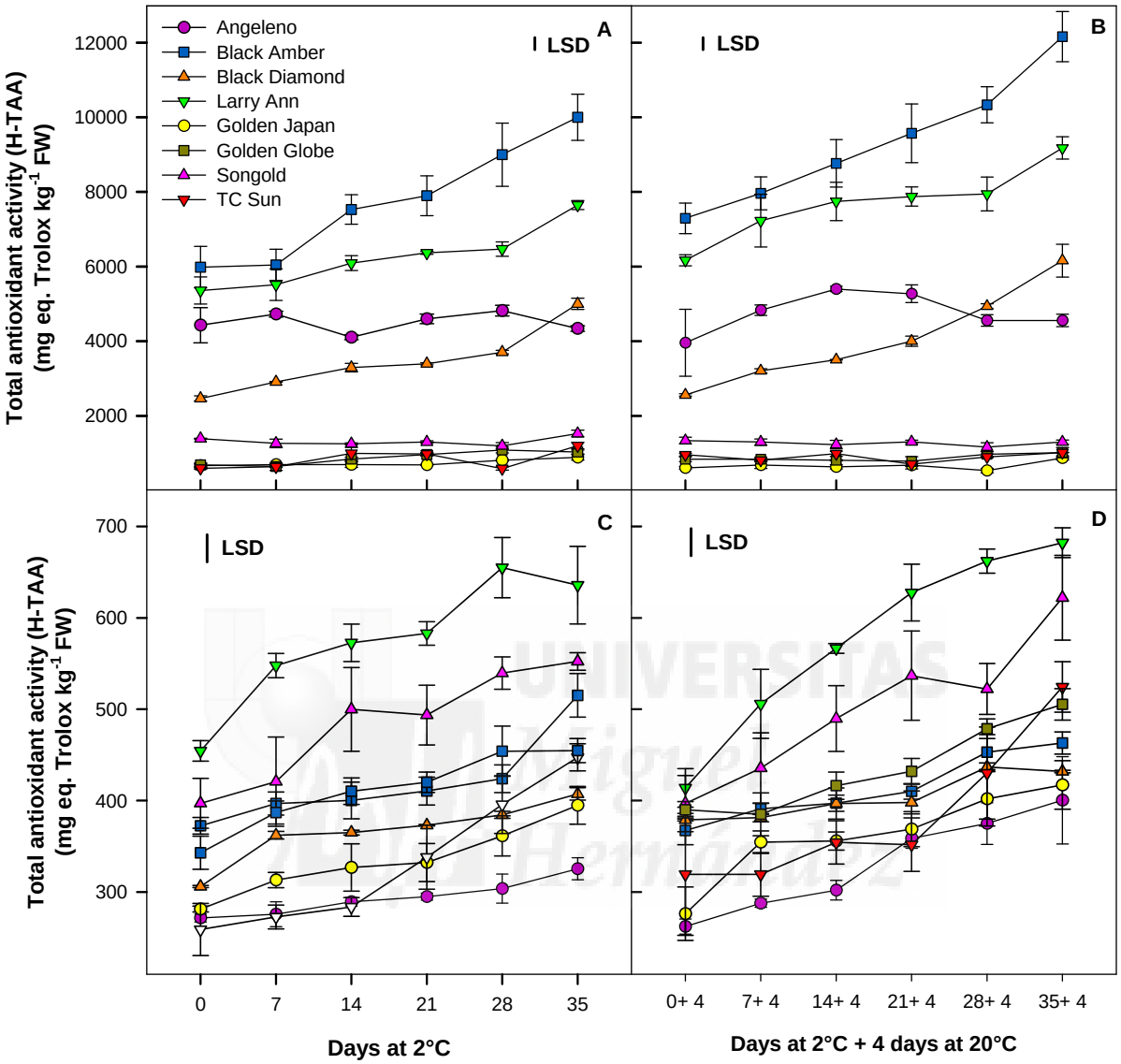

Fig. 5. Changes in total antioxidant activity from the hydrophilic extract (H-TAA) in the peel during cold storage (A) and subsequent shelf-life (B) or in the pulp during cold storage (C) and subsequent shelf-life (D) of the eight plum cultivars. Data are the mean ± SE and the LSD ($p < 0.05$) values are shown.

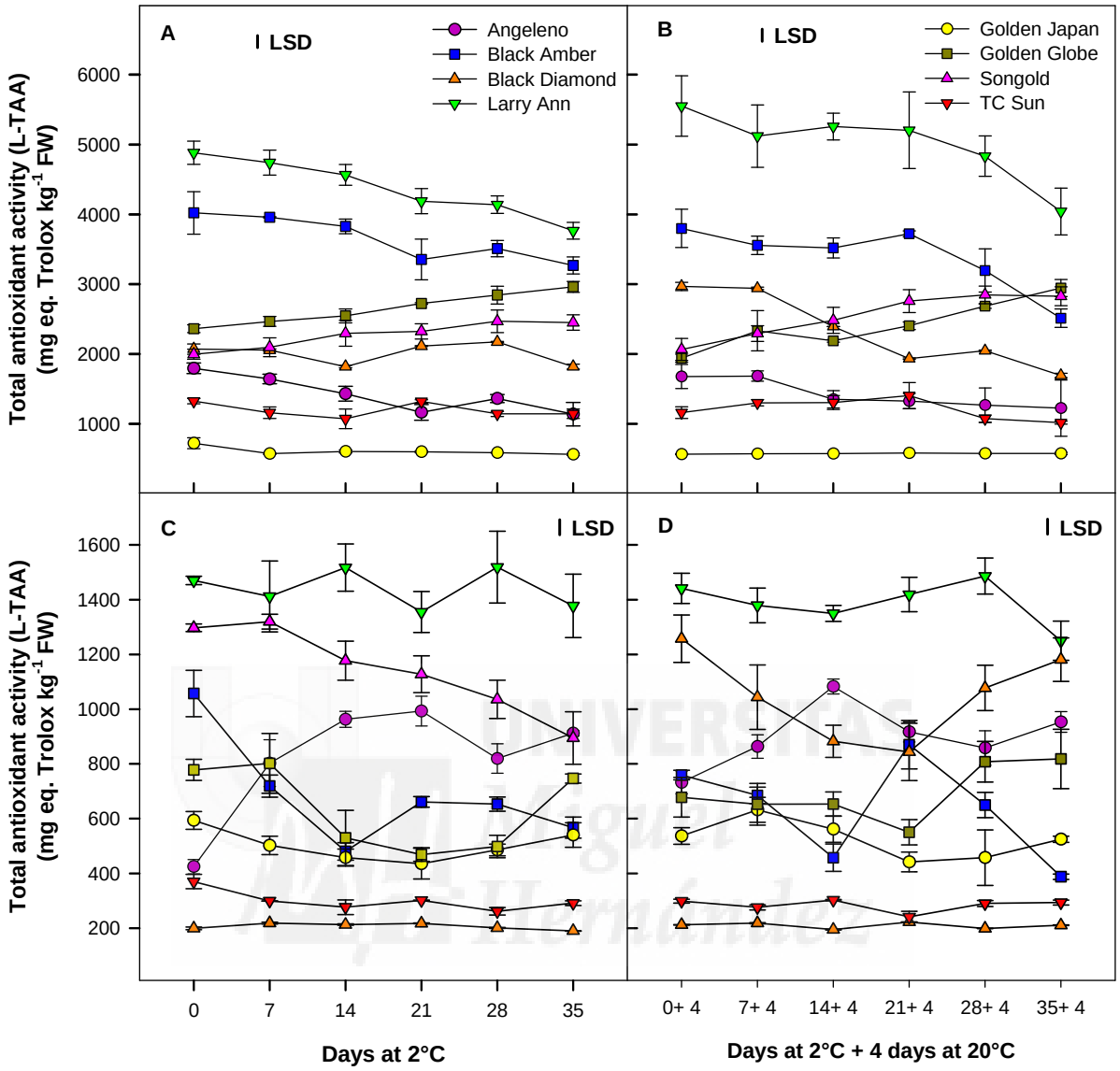

Fig. 6. Changes in total antioxidant activity from the lipophilic extract (L-TAA) in the peel during cold storage (A) and subsequent shelf-life (B) or in the pulp during cold storage (C) and subsequent shelf-life (D) of the eight plum cultivars. Data are the mean ± SE and the LSD ($p < 0.05$) values are shown.

DISCUSSION

The eight plum cultivars were harvested at commercial ripening stage, although the levels in SSC, acidity, firmness and colour showed significant differences among cultivars. The average values are in agreement with those reported in a wide range of plum cultivars (Murray et al., 2005; Menniti et al., 2006; Crisosto et al., 2007; Paz et al., 2008). Although it has been reported that early cultivars have lower SSC than late ones in Californian plums (Crisosto et al., 2007), this did not seem to occur in Spanish cultivars,

since two late plums ('Larry Ann' and 'Songold') showed the minimum and the maximum SSC.

For all cultivars, the typical changes related to ripening (softening, colour changes, decrease in acidity and increase in both SSC and RI) occurred during cold storage and after SL, the magnitude of the change being similar for all plums and within the range of previous reports (Abdi et al., 1997; Martínez-Romero et al., 2003; Serrano et al., 2003; Valero et al., 2003; Murray et al., 2005; Menniti et al., 2006). Although these changes were similar for all cultivars, their ethylene production was clearly different, since after cold storage and subsequent SL, the ethylene production was high in the red cultivars 'Black Amber' and 'Larry Ann' and in the yellow ones 'Golden Globe' and 'Songold', while in 'Angeleno' and 'Black Diamond (red) and in 'Golden Japan' and 'TC Sun' (yellow) the ethylene production was very low. From these results, it can be concluded that the physiological ripening pattern was different depending on cultivar, since 'Angeleno', 'Black Diamond, 'Golden Japan' and 'TC Sun' behaved as suppressed-climacteric phenotypes without any increase in ethylene production during ripening. This behaviour has already been described for 'Golden Japan' (Zuzunaga et al., 2001; Abdi et al., 1997), 'Angeleno' (Khan et al., 2007; Candan et al., 2008) and for 'Black Diamond' (Serrano et al., 2003), while the suppressed-climacteric of 'TC Sun' plum is described for the first time in this paper. In contrast, 'Black Amber', 'Larry Ann', 'Golden Globe' and 'Songold' exhibited the typical climacteric ripening pattern reported for most plum cultivars (Abdi et al., 1997; Zuzunaga et al., 2001; Martínez-Romero et al., 2003; Serrano et al., 2003; Valero et al., 2003).

As commented above, there are numerous reports on the physico-chemical changes during plum storage. In addition, there is some evidence on the bioactive compounds and antioxidant properties of several plum cultivars harvested at commercial ripening stage as will be discussed later. However, as far as we are aware, this is the first paper in which the changes of these bioactive compounds and antioxidant activity during cold storage and subsequent SL are evaluated in a wide range of plums, both dark-purple and yellow ones. Moreover, all the literature on this issue deals with the antioxidant activity of hydrophilic plum extracts (H-TAA), while we have gone a further step with the analysis of antioxidant activity due to lipophilic compounds (L-TAA).

Anthocyanins were present only in the peel and pulp of the dark-purple plums although their levels at harvest were affected by cultivar, with concentrations within the range of previous studies (Tomás-Barberán et al., 2001; Cevallos-Casals et al., 2006; Manganaris et al., 2007; Vizzotto et al., 2007). The main anthocyanin reported for plum is cyanidin 3-glucoside followed by cyanidin 3-rutinoside, while peonidin 3-glucoside is found at low concentrations (Tomás-Barberán et al., 2001; Chun et al., 2003). More recently, another five minor anthocyanins have been identified: cyanidin-3-galactoside, 3-(6"-

acetoyl)glucoside, pelargonidin 3-glucoside, cyanidin 3-xyloside, and cyanidin 3-(6”-acetoyl)glucoside (Wu and Prior, 2005).

The changes in anthocyanin content during cold storage + SL were similar for all dark-purple cultivars, showing a continuous increase in the peel while remaining unchanged for most cultivars. The increase in peel anthocyanin was parallel to the reduction in Hue values, showing that these pigments contribute to the colour changes associated with the postharvest ripening process. Manganaris et al. (2007) reported increases in anthocyanin content in the pulp of ‘Harrow Sun’ plum as a consequence of chilling injury development at 5 ◦C, which was manifested as flesh reddening. However, in our plums no symptoms of chilling injury (internal browning or gel breakdown) were observed during storage. Thus, the enhancement of anthocyanins is attributed to normal ripening, as has been found in other commodities such as blueberry (Zheng et al., 2003), raspberry and strawberry (Kalt et al., 1999), fresh prunes (Hamauzu and Cume, 2005), and cherry (Gonçalves et al., 2004).

The total phenolics results revealed the same behaviour for all dark-purple and yellow plums, that is, a significant increase during storage plus SL, although the phenolic concentration in the peel was always higher in dark-purple than in yellow cultivars, while in the flesh this aspect could not be observed. Plums contain large amounts of phenolic compounds, and among them neochlorogenic acid (3-*O*-caffeoylquinic acid) is considered the major polyphenol followed by anthocyanins in a wide range of cultivars (Chun et al., 2003; Kim et al., 2003; Lombardi-Boccia et al., 2004). The pattern of phenolic changes during postharvest storage seems to be variable among species (and even cultivars) and affected by several postharvest treatments (Brovelli, 2006; Hamauzu, 2006). Low temperature storage has been found to increase total phenolic content in pomegranate arils (Mirdehghan et al., 2007) and apple (Leja et al., 2003), probably due to stimulation of the activity of some enzymes involved in phenolic biosynthesis by cold storage (Hamauzu, 2006). In addition, phenolic changes during storage seem to be dependent on the ripening stage at harvest, since total phenolics decreased in full red strawberry compared to white varieties (Shin et al., 2008).

Carotenoids form a large class of lipophilic molecules synthesised by plants and acting as coloured pigments in fruit. In plums, we have found carotenoids in the eight cultivars for both peel and pulp. Interestingly, the highest concentration of total carotenoids in the peel was found in ‘Black Amber’ and ‘Larry Ann’, which are dark-purple coloured. Thus, although the colour was due to anthocyanins, these cultivars are also enriched with yellow-orange pigments. However, the levels of carotenoids increased only in the yellow cultivars for both peel and flesh tissues during storage, which was correlated to the decrease in Hue colour parameter. This increase in carotenoid content has been also observed in stored kiwifruit (Tavarini et al., 2008), watermelon (Perkins-Veazie and Collins, 2006) and sapote (Alia-Tejacal et al., 2007). For comparative purposes, no

references are available in plums about carotenoid changes during storage, since the levels of these pigments have been only determined at the commercial ripening stage. In these reports, important differences have been found depending on cultivar, although the levels were always higher in the peel than in the flesh, with the main carotenoid being ß-carotene followed by ß-cryptoxanthin (Gil et al., 2002; Lombardi-Boccia et al., 2004).

In recent years, increasing attention has been paid by consumers to the health-beneficial effects of fruit and vegetables intake due to their content in bioactive molecules with antioxidant properties, which can protect the human body against degenerative diseases (Brandt et al., 2004; Hung et al., 2004; Chen et al., 2007). Thus, currently the antioxidant content is becoming an increasingly important measure of quality in relation to fruit and vegetables. Among the substances with antioxidant activity, anthocyanins and other polyphenols (Espín et al., 2007; Stevenson and Hurst, 2007; Dorais et al., 2008), carotenoids (Perera and Yen, 2007), and several vitamins (A, C and E) are included (Hounsome et al., 2008). Most of the assays to measure the antioxidant capacity of fruit and vegetables have been carried out on hydrophilic extracts and only recently the antioxidant activity derived from water-soluble or lipo-soluble molecules separately is being evaluated in a few fruit and vegetables (Wu et al., 2004; Cano and Arnao, 2005; Scalzo et al., 2005; Cho et al., 2007). In plums, we have observed that H-TAA was higher than L-TAA in the peel, while the contrary occurred for the pulp. It is interesting that in plum, L-TAA comprised between 30% and 50% of the total antioxidant activity (sum of H-TAA and L-TAA), while in other fruit the contribution of L-TAA was much lower, as has been reported for strawberry, peach, apricot and apple (Scalzo et al., 2005). Taking into account the results of the linear regressions, the bioactive compounds that are contributing to H-TAA plums were mainly total phenolics, and within this group the anthocyanins were also important antioxidants. We found the results of the relationship between carotenoids and L-TAA very interesting, especially in the dark-purple cultivars, since the carotenoid concentration was much lower than total phenolics (ca. 10-fold) but exhibited an antioxidant activity (L-TAA) just twice as low(H-TAA). In previous studies, the relationship between total phenolics and antioxidant activity in a wide range of plum cultivars harvested at commercial ripening has been reported (Chun et al., 2003; Kim et al., 2003; Cevallos-Casals et al., 2006; Rupasinghe et al., 2006; Vizzotto et al., 2007). However, in these papers just the antioxidant activity of the hydro-soluble extracts was evaluated and the contribution of lipophilic substances, such as carotenoids and tocopherols which are important plant antioxidants (Cho et al., 2007), was not estimated.

In conclusion, with this paper we demonstrate that there are great variations in the content of the bioactive compounds and antioxidant activity depending on plum cultivar, although on a general basis, there are not important losses during prolonged cold storage and subsequent shelf-life. The peel of yellow cultivars had lower H-TAA than for red-purple fruit, which was correlated with phenolic compounds. Moreover, for a better

evaluation of the antioxidant potential of plums, the contribution of carotenoids should be taken into account, especially when comparative studies among fruit and vegetables are carried out, since 'Larry Ann' and 'Black Diamond' had the highest L-TAA, attributable to the higher carotenoid concentration in both peel and pulp tissues.

Acknowledgements

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Chapter 4

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 (HTAA) 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.In this article 11 commercial sweet cherry cultivars were selected to evaluate sensory, nutritive and functional properties over the maturation process on tree.

Key Words: 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 Gonc¸alves 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.

MATERIALS 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 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 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 (cyaniding 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.

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 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*).

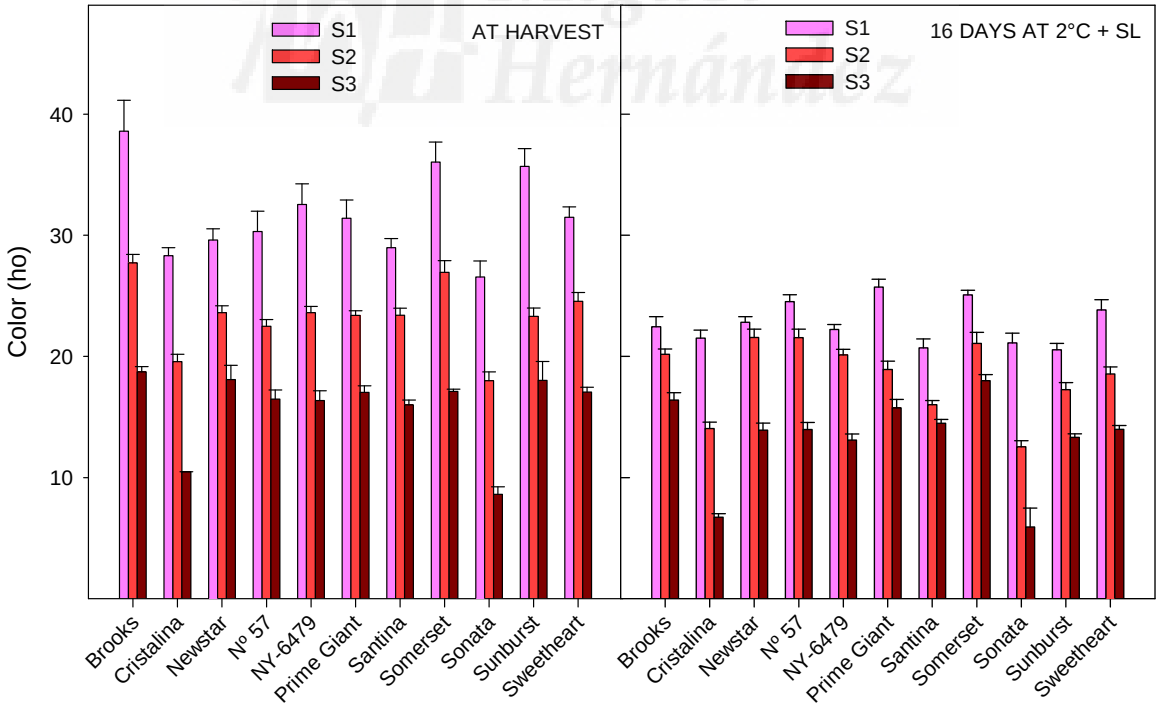

Figure 1: Color hue angle (ho) at harvest and after 16 days at 2 °C + 2 days at 20 °C (SL) of 11 sweet cherry cultivars harvested at three maturity stages (S1, S2, and S3). Data are the mean ± SE.

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*).

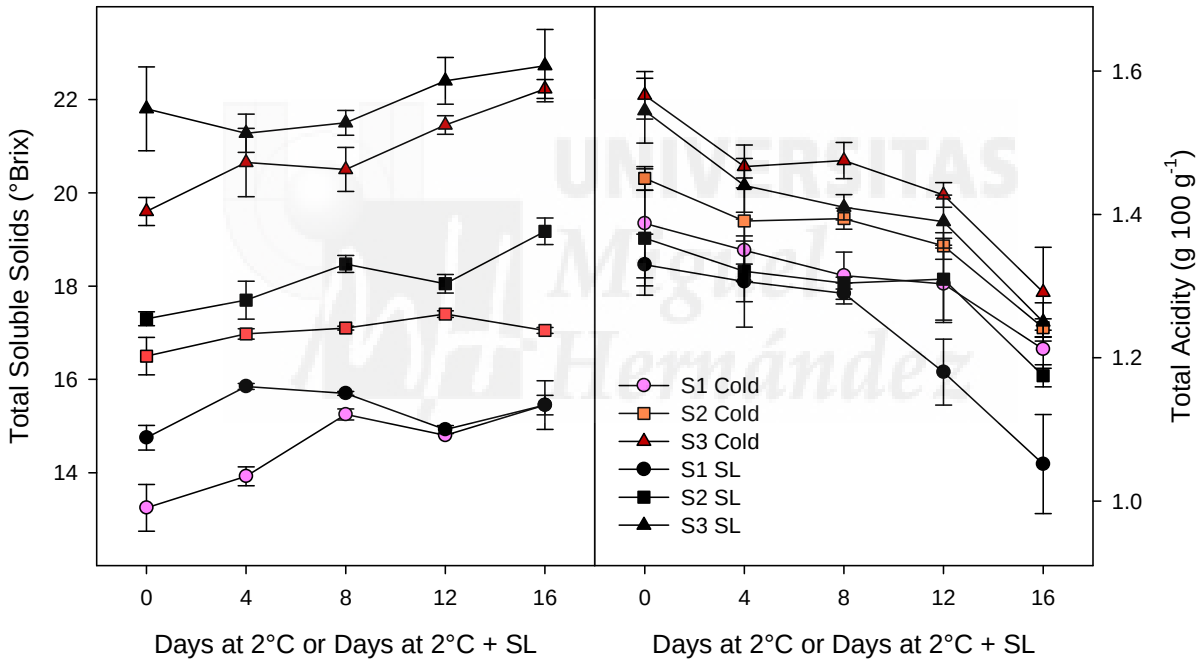

Figure 2: Evolution of total soluble solids and total acidity during storage at 2 ˚C or at 2˚C + 2 days at 20 ˚C (SL) of NY-6479 sweet cherry cultivar harvested at three maturity stages (S1, S2, and S3). Data are the mean ± SE.

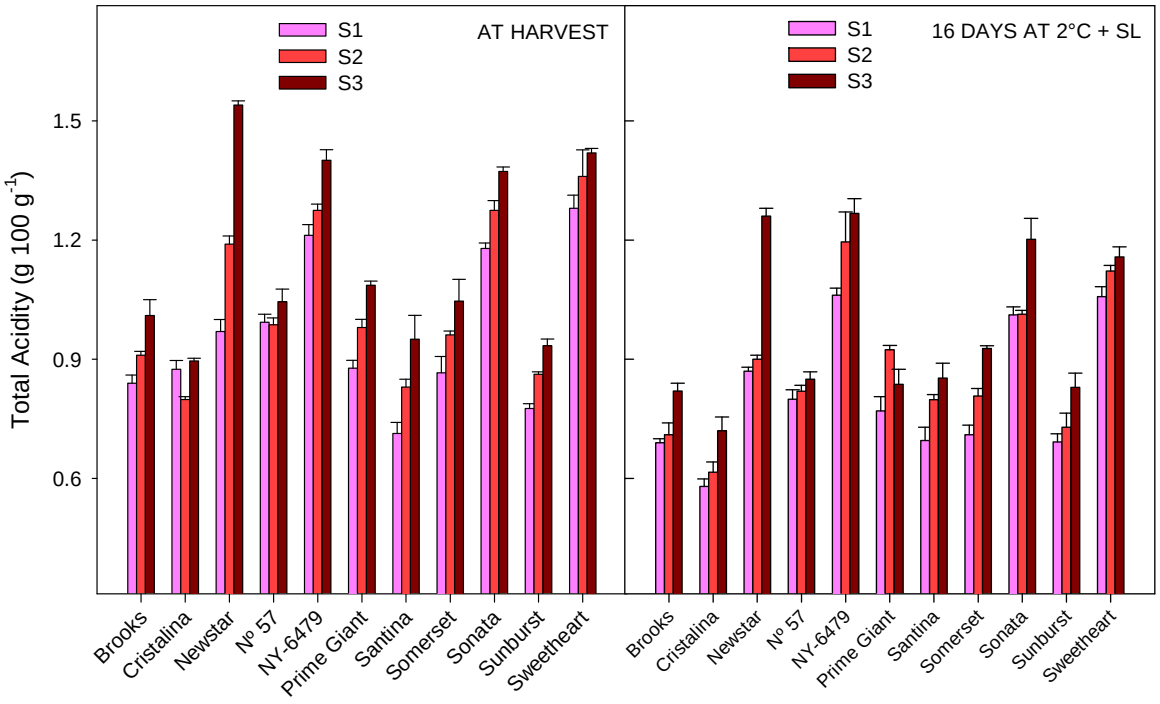

Figure 3: Total acidity at harvest and after 16 days at 2 °C (cold) + 2 days at 20 °C (SL) of 11 sweet cherry cultivars harvested at three maturity stages (S1, S2, and S3). Data are the mean ± SE.

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*).

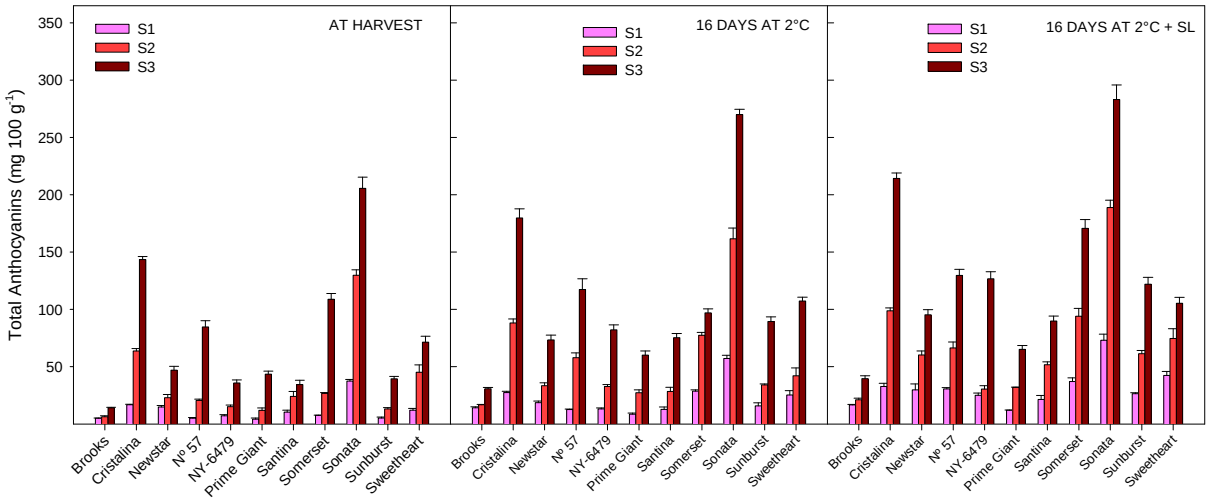

Figure 4. Total anthocyanins at harvest, after 16 days of storage at 2 °C and after 16 days at 2 °C + 2 days at 20 °C (SL) of 11 sweet cherry cultivars harvested at three maturity stages (S1, S2, and S3). Data are the mean ± SE.

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 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.

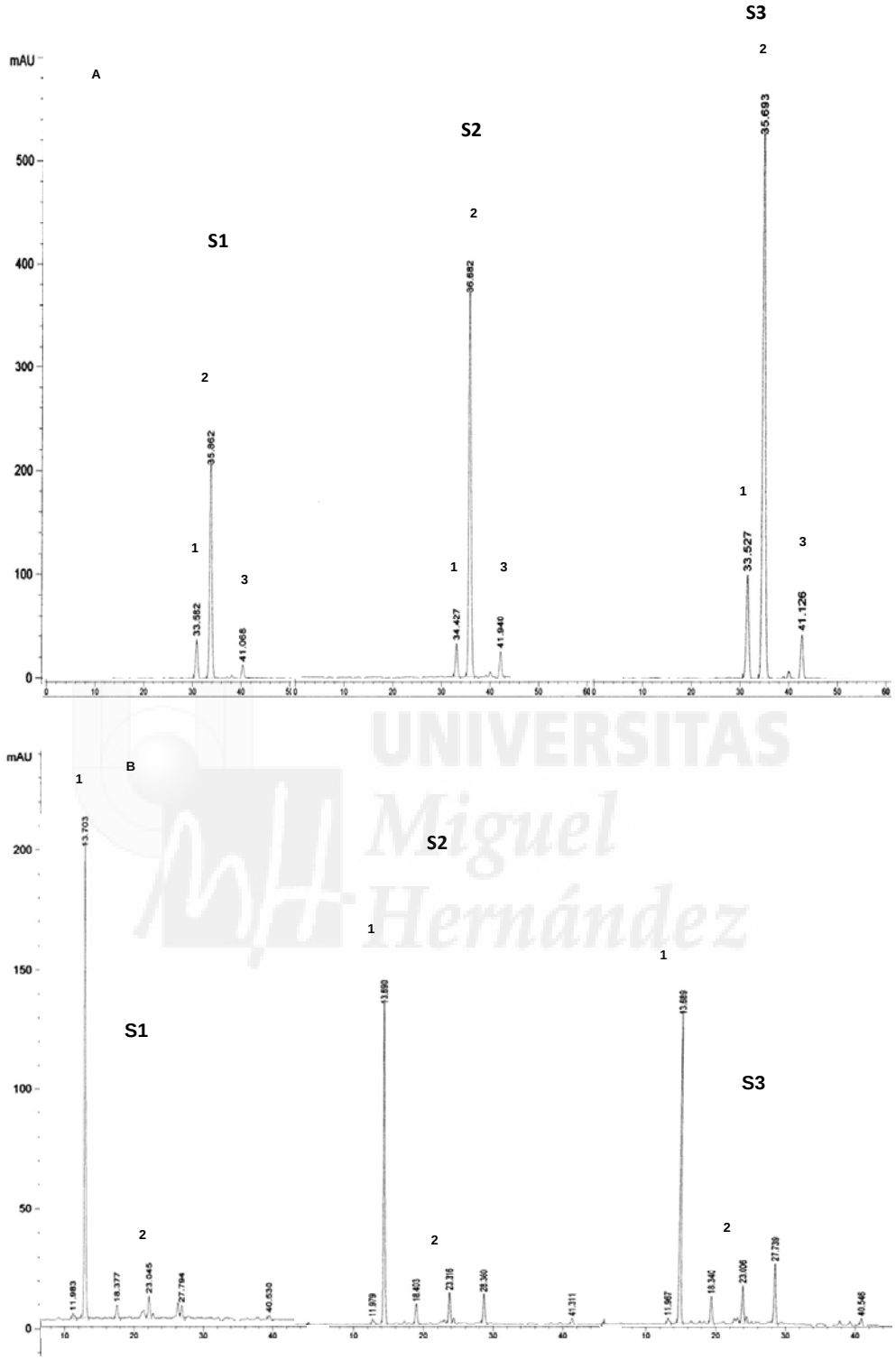

Figure 5: HPLC-DAD chromatograms for anthocyanins (A) and hydroxycinnamic acids (B) in Sonata sweet cherry harvested at 3 maturity stages (S1, S2, and S3). For anthocyanin peaks: (1) cyanidin 3-glucoside, (2) cyanidin 3-rutinoside, and (3) pelargonidin 3-rutinoside. For hidroxycinnamic acids: (1) neochlorogenic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

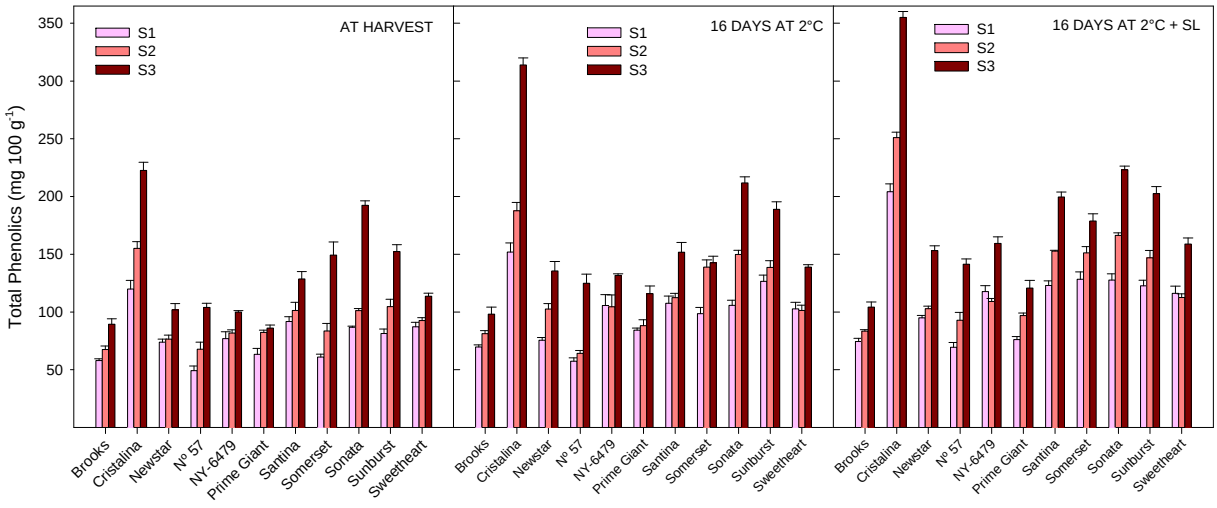

Figure 6: Total phenolics at harvest, after 16 days of storage at 2 °C and after 16 days at 2 °C + 2 days at 20 °C (SL) of 11 sweet cherry cultivars harvested at three maturity stages (S1, S2, and S3). Data are the mean ± SE.

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 (*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.

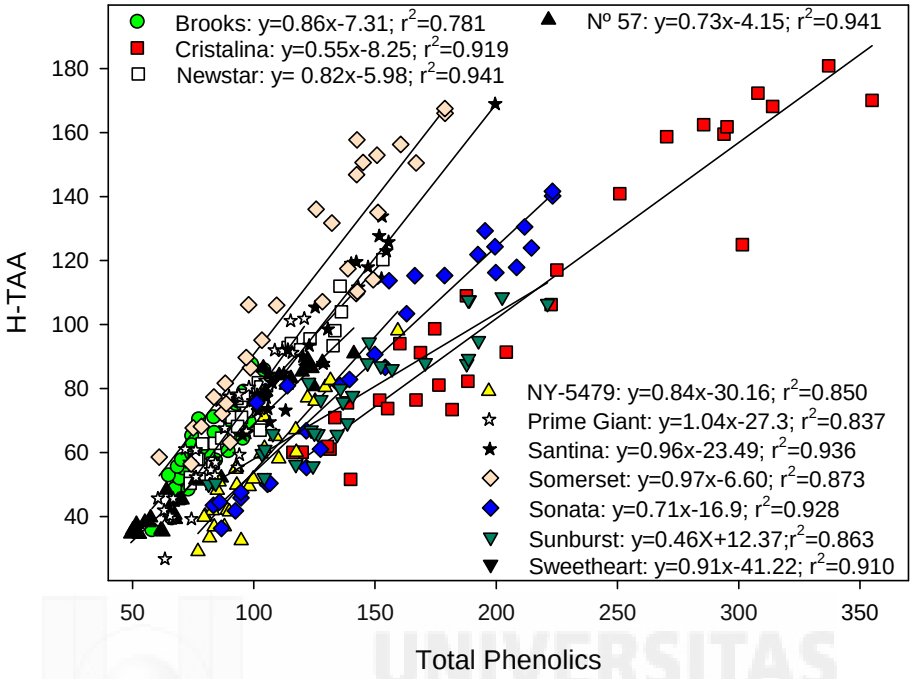

Figure 7: Correlation between hydrophilic total antioxidant activity (HTAA) and total phenolics in 11 sweet cherry cultivars, taking into account data for all maturity stages and sampling data during storage at 2 °C and subsequent shelf life.

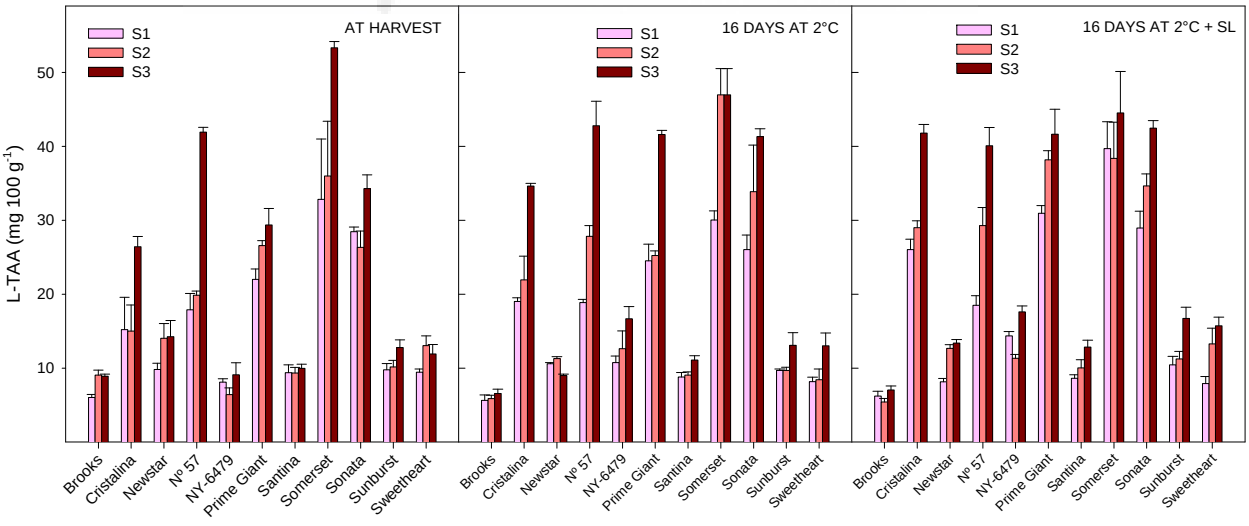

Figure 8: Lipophilic total antioxidant activity (L-TAA) at harvest, after 16 days of storage at 2 °C and after 16 days at 2 °C + 2 days at 20 °C of 11 sweet cherry cultivars harvested at three maturity stages (S1, S2, and S3). Data are the mean ± SE.

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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Chapter 5

Modified atmosphere packaging of yellow and purple plum cultivars. 1. Effect on organoleptic quality

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Modified atmosphere packaging of yellow and purple plum cultivars. 1. Effect on organoleptic quality

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Abstract

The effect of modified atmosphere packaging (MAP) on maintaining plum quality was assayed on 4 plum cultivars (2 with yellow and 2 with purple skin) thermo-sealed in baskets with 2 distinct films (M and H) which differed in permeability. Fruit stored with macroperforated film served as a control and lost their quality attributes very rapidly, manifested by accelerated colour changes, softening, decrease in acidity and increase in total soluble solids. The use of MAP retarded these changes, the efficacy being higher in the fruit packed with film M compared with film H as a result of the delay in postharvest ripening, which could be attributed to the effect of MAP on reducing ethylene production rates. With the use of these packages, the storage time with fruit having high quality attributes could be increased 3–4 weeks more as compared with control plums.

Keywords: *Prunus salicina*; Colour, Firmness; Acidity; Ethylene; Storage

INTRODUCTION

Plums are climacteric fruit with a limited postharvest storage life due to acceleration of quality loss, affecting some properties such as colour, texture, total soluble solids (TSS) and total acidity (TA). The use of cold storage is an appropriate mean to delay ripening by reducing ethylene production, respiration rate, pigment changes, softening, the increase in TSS and the reduction in TA (Crisosto et al., 2007; Guerra and Casquero, 2008; Díaz-Mula et al., 2009). Several attempts have been made to maintain plum quality during storage by combining cold storage and 1-methylcyclopropene (Valero et al., 2003), polyamine (Valero et al., 2002), heat and calcium (Serrano et al., 2004) treatments.

In some commodities, the use of modified atmosphere packaging (MAP) has shown positive effects on maintaining produce quality, such as in table grape (Martínez-Romero et al., 2003), broccoli (Serrano et al., 2006) and sweet cherry (Serrano et al., 2005), among others. MAP consists of sealing a certain amount of fruit or vegetable by using plastic films with selective permeability to CO_2, O_2 and water vapour diffusion. The commodity respiration increases CO_2 and decreases O_2 concentrations inside the packages, and transpiration rate increases water pressure. These modifications lead to reduction of weight loss, respiration rate and ethylene production, as well as to retard changes in properties related to the ripening process, and in turn, postharvest quality can be maintained for longer periods (Alam and Goyal, 2006; Artés et al., 2006; Valero and Serrano, 2010). However, little information exists on the use of MAP to preserve plum fruit quality. Recently, Guan and Dou (2010) reported some effects in reducing chilling injury symptoms in the sensitive plum cultivar 'Friar' using polyvinyl chloride (PVC) film, and Cantín et al. (2008) showed that the use of commercial box liners (LifeSpan) in the same cultivar retarded fruit quality deterioration. On the other hand, MAP exacerbated the effect of 1-MCP in reducing ethylene production and softening during cold storage of 'Tegan Blue' plums (Khan and Sing, 2008).

The aim of this paper was to perform a broad study to evaluate the effect of MAP on preserving fruit quality with 4 plum cultivars, 2 with purple ('Blackamber' and 'Larry Ann') and 2 with yellow skin ('Golden Globe' and 'Songold'). To fulfil this aim, fruit were placed in polypropylene baskets (rigid packages) and then thermo-sealed with two films with different gas permeabilities, or a macroperforated film (control). Gas composition inside the packages, fruit ethylene production and parameters related to quality (firmness, colour, TSS and TA) were analyzed weekly during 35 days of cold storage at 2 ºC.

MATERIALS AND METHODS

Plant material and experimental design

Two purple 'Blackamber' (BA) and 'Larry Ann' (LA) and two yellow skin 'Golden Globe' (GG) and 'Songold' (SG) plum (*Prunus salicina* Lindl.) cultivars were used. Fruit were harvested at the commercial ripening stage according to fruit properties at harvest previously reported (Díaz-Mula et al., 2008). For each plum cultivar, about 650 fruit were manually picked and immediately transferred to the laboratory under cold conditions. Then, 504 fruit were selected based on homogeneous colour and size, and absence of visual defects, and distributed at random into 63 lots of 8 fruit (average weight 750–800 g mass for all cultivars). Three lots from each cultivar were used to determine physicochemical properties at harvest (day 0). The remaining lots were individually deposited in polypropylene baskets and divided into 3 batches for the following modified atmosphere packages (MAP): macro-perforated film as control, film H and film M, which were effective for MAP storage of broccoli and table grapes (Martínez-Romero et al., 2003; Serrano et al., 2006), and appropriate in preliminary studies on plums (data not shown). Baskets were thermo-sealed on top with the different films (total area of 336 cm^2, 14 cm×24 cm). The films properties were: macro-perforated polypropylene film (with 32 holes of 1.5 mm diameter per dm^2, and total perforated area of 0.56%); Film H was composed of polyester 87 (12 µm)-polypropylene (50 µm), and Film M was composed of polyester (12 µm)-polypropylene (60 µm). Macro-perforated polypropylene was purchased from Plásticos del Segura S.L. (Spain), while Films H and M were purchased from Amcor Flexibles (Amcor, Barcelona, Spain) with permeability to O_2 < 100 mL O_2 m^{-2} day^{-1} atm^{-1}. All baskets were stored at 2 ºC and 90% RH for 35 days. For each cultivar and film type, 5 lots were used to follow the gas composition inside the packages, which had silicone septa on the film surfaces, and 3 lots were chosen weekly at random for analytical measurements.

Gas composition

CO_2 and O_2 concentrations were quantified in duplicate in each basket by withdrawal of 1mL of headspace atmosphere using an airtight syringe, and injected into a gas chromatograph GC 14B (Shimadzu, Tokyo, Japan) equipped with a thermal conductivity detector (TCD). CO_2 and O_2 were separated on a molecular sieve 5A column, 80–100 mesh (Carbosieve SII, Supelco Inc., Bellefonte, 105 USA), of 2 m length and 3 mm i.d. Oven and injector temperature were 50 and 110 ºC, respectively. Helium was used as carrier gas at a flow rate of 50 mL min^{-1}. Results (mean ± SE) were expressed as kPa O_2 and kPa CO_2 inside the baskets.

Respiration rate and ethylene production

For each sampling date, packages were opened and after 2 h ethylene production was measured at cold temperature (2 ºC) by placing each replicate of 8 fruit in a 2-L glass jar hermetically sealed with a rubber stopper for 30 min. For ethylene quantification (in duplicate) 1mL of the atmosphere was withdrawn with a gas syringe and then injected into a Shimadzu™ GC-2010 gas chromatograph (Kyoto, Japan), equipped with a flame ionisation detector (FID) and a 3 m stainless steel column with an inner diameter of 3.5 mm containing activated alumina of 80/100 mesh. The carrier gas was helium, at a flow rate of 30 mL min^{-1}, column temperature was 90 ºC, and injector and detector temperatures were 150 ºC. Results were the mean ± SE of determinations for each replicate and expressed as ng kg^{-1} s^{-1}. Similarly, respiration rate at harvest was quantified in duplicate by injecting 1 mL of the atmosphere into the gas chromatograph GC 14B described above. Results were expressed as µg kg^{-1} s^{-1} and were the mean ± SE. For day 0, respiration and ethylene rates were measured at 20 ºC.

Fruit quality parameters

Fruit firmness was measured on the shoulder of each fruit using a flat steel plate coupled with a texturometer (TX-XT2i Texture Analyzer, Stable Microsystems, UK) interfaced to a personal computer. A bevelled holder prevented bruising of the opposite side. For each fruit, the diameter was measured and then a force that achieved a 3% deformation of the fruit diameter was applied. Results were expressed as the force-deformation (N mm^{-1}) and were the mean ± SE (n = 24). Colour was determined in both peel and flesh of each fruit using the CIE Lab System in a Minolta colorimeter CR200 model using D65 illuminant (Minolta Camera Co., Japan). Results were the mean ± SE (n = 48) of 2 determinations for each fruit and expressed as Hue angle. After fruit firmness and colour determination, the fruit were peeled and a flesh tissue portion was taken along the equatorial fruit zone and then combined to obtain a homogenous juice sample for each replicate, in which total soluble solids (TSS) and total acidity (TA) were determined in duplicate. TSS concentration was determined with a digital refractometer Atago PR-101 (Atago Co. Ltd., Tokyo, Japan) at 20 ºC, and expressed as g 100 g^{-1}. TA (g of malic acid equivalent per 100 g fresh weight) was determined by automatic titration (785 DMP Titrino, Metrohm) with 0.1N NaOH up to pH 8.1, using 1mL of diluted juice in 25 mL distilled H_2O. The ratio between soluble solids concentration and titratable acidity was considered as a ripening index (RI).

Statistical analysis

Experimental data were subjected to ANOVA analysis. Sources of variation were MAP packages and storage. The overall least significant differences (Fisher's LSD procedure, $p < 0.05$) were calculated and used to detect significant differences among packages and storage time. Values of LSD are showed in figures. All analyses were performed with SPSS software package v. 11.0 for Windows (SPSS, 2001).

RESULTS AND DISCUSSION

Gas composition and ethylene production

During storage, an increase in CO_2 and decrease in O_2 concentrations occurred inside the MAP packages, reaching a steady-state atmosphere after 7 days at 2 ◦C. Final gas concentrations were affected by the type of film used and the plum cultivar (**Fig. 1**). For all cultivars, the atmosphere modification was higher inside the packages with film M than with film H. For instance, in LA plums CO_2 concentrations at equilibrium were ≈6.5 and 5 kPa, 168 while O_2 concentrations were ≈13 and 15 kPa, for film M and H, respectively. For each particular film (M or H), differences existed among cultivars in the equilibrium atmosphere.

Thus, when film H was used, the highest CO_2 levels were found for LA (≈5 kPa), followed by SG (≈3.2 kPa), BA (≈3 kPa) and GG (≈1.5 kPa). For O_2 levels, in film H, the same order was obtained, with LA (≈15 kPa) and SG (≈16 kPa) being the cultivars with lower O_2 than BA and GG (≈18.5–19.0 kPa). These differences could be attributable to differences in the respiration rate at harvest, since LA showed the highest rate (12.19±0.32 μg kg^{-1} s^{-1}) and GG the lowest (5.10±0.21 μg kg^{-1} s^{-1}), while BA and SG had intermediate respiration rates (≈7 μg kg^{-1} s^{-1}). On the other hand, the steady-state atmospheres achieved could be considered as optimum for plum fruit, according to preliminary experiments and in agreement with previous work (Cantín et al., 2008).

With respect to ethylene production rates (measured under cold condition after opening the packages) significant differences were also found among cultivars and films, especially after the second sampling date (**Fig. 2**). Thus, in control fruit ethylene production was higher than in those stored under MAP conditions, the highest ethylene production being found at the end of the experiment in BA fruit (1.50±0.09 ng kg^{-1} s^{-1}), followed by LA (1.14±0.06 ng kg^{-1} s^{-1}) and the lowest in SG (0.06±0.01 ng kg^{-1} s^{-1}).

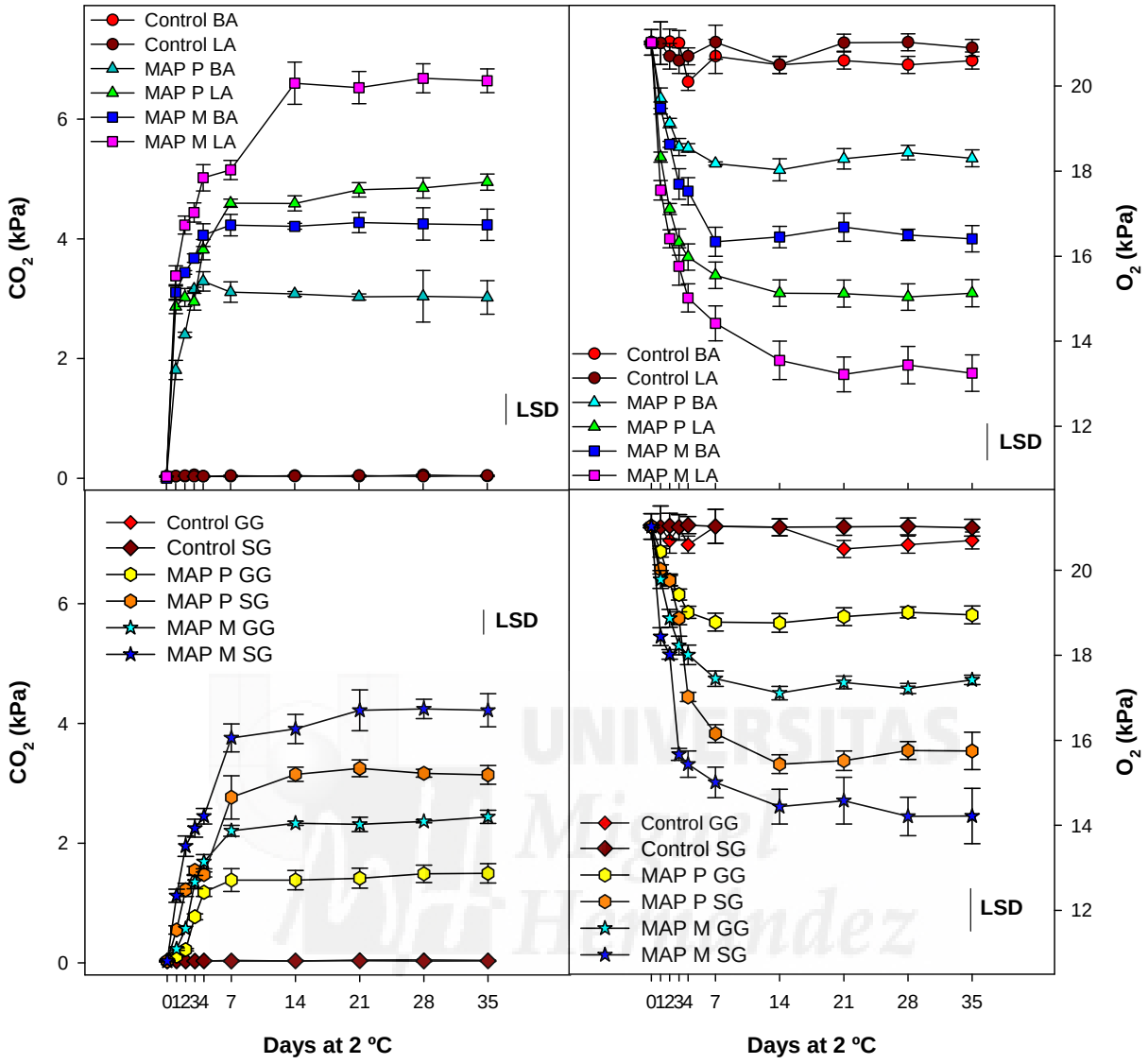

Fig. 1. Changes in CO_2 and O_2 concentrations inside MAP packages (with film H and M) containing the different plum cultivars: 'Blackamber' (BA), 'Larry Ann' (LA), 'Golden Globe' (GG) and 'Sungold' (SG). Data are the mean ± SE (n=5). Least significant differences (LSDs) are shown.

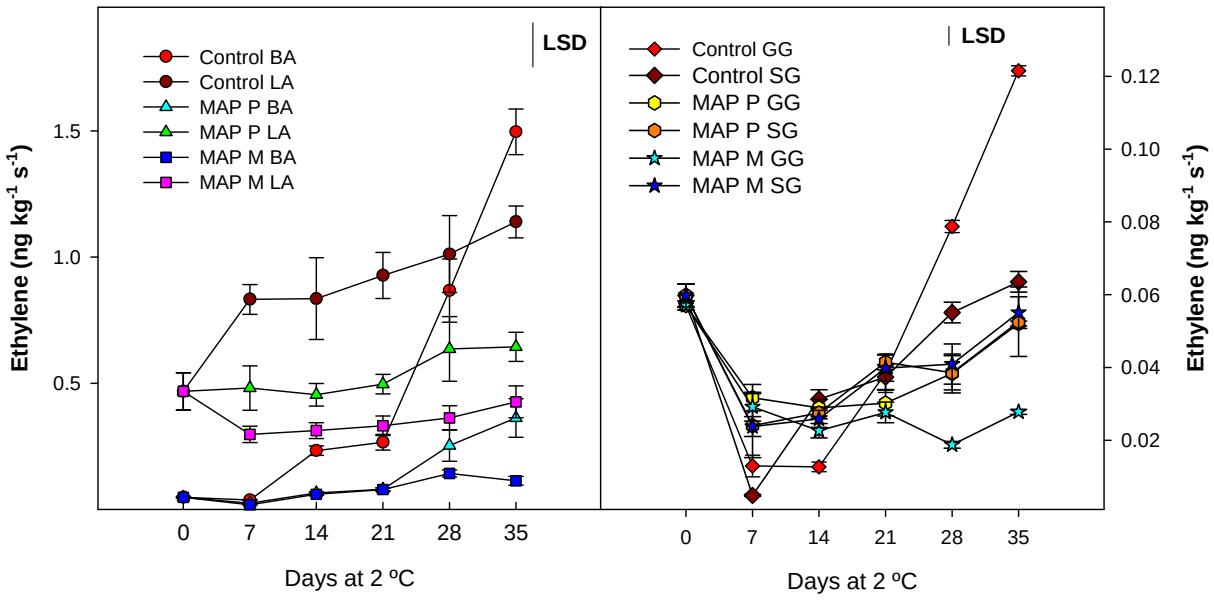

Fig. 2. Ethylene production rates of plums, 'Blackamber' (BA), 'Larry Ann' (LA), 'Golden Globe' (GG) and 'Sungold' (SG), during storage at 2 ºC after removing the fruit from MAP packages (with film H and M). Data are the mean ± SE (n=5). Least significant differences (LSDs) are shown.

In addition, the results showed a significant inhibition in ethylene production rate in all plum cultivars which was higher in those stored under film M than under film H. For instance, in BA fruit, the ethylene production after 35 days of cold storage was 1.50±0.09 ng kg^{-1} s^{-1} in control fruit and 0.36±0.07 and 0.11±0.02 ng kg^{-1} s^{-1} in those stored under MAP conditions with films H and M, respectively. These plum cultivars have the typical climacteric ripening pattern reported for most plum cultivars, with high ethylene production rates associated with plum ripening (Valero et al., 2002; Serrano et al., 2003; Díaz-Mula et al., 2008). For all cultivars, the effect of MAP on inhibiting ethylene production was higher in film M than in film H, which is attributed to both lower O_2 and higher CO_2 concentrations inside film M than in film H. Low O_2 is known to inhibit 1-aminocyclopropane-1-carboxylic acid oxidase (ACO), one of the key enzymes regulating ethylene biosynthesis, while CO_2 is an antagonist of ethylene action and impedes its autocatalytic synthesis when present at concentrations over 1 kPa, these effects being additive to those of low O_2 atmospheres (Artés et al., 2006). In other stone fruit, such as apricot, MAP also resulted in similar inhibition of ethylene biosynthesis (Pretel et al., 1993).

Fruit quality parameters

Colour (expressed as Hue angle) showed changes during storage which were manifested by reduction in colour Hue angle in both peel and flesh for all cultivars (**Fig.**

3), although initial Hue values were lower in the peel of purple (≈16 and ≈25 for BA and LA, respectively) than in yellow cultivars (≈96 and ≈104 for SG and GG, respectively). However, colour differences among cultivars at harvest were lower in the flesh than in the skin, since all were cultivars with yellow flesh, with Hue values of≈100, 95, 90 and 85 for GG, SG, BA and LA, respectively. Colour changes were significantly delayed in those plums stored under MAP conditions, with generally lower changes in both peel and flesh in plums packed with film M than in those with film H.

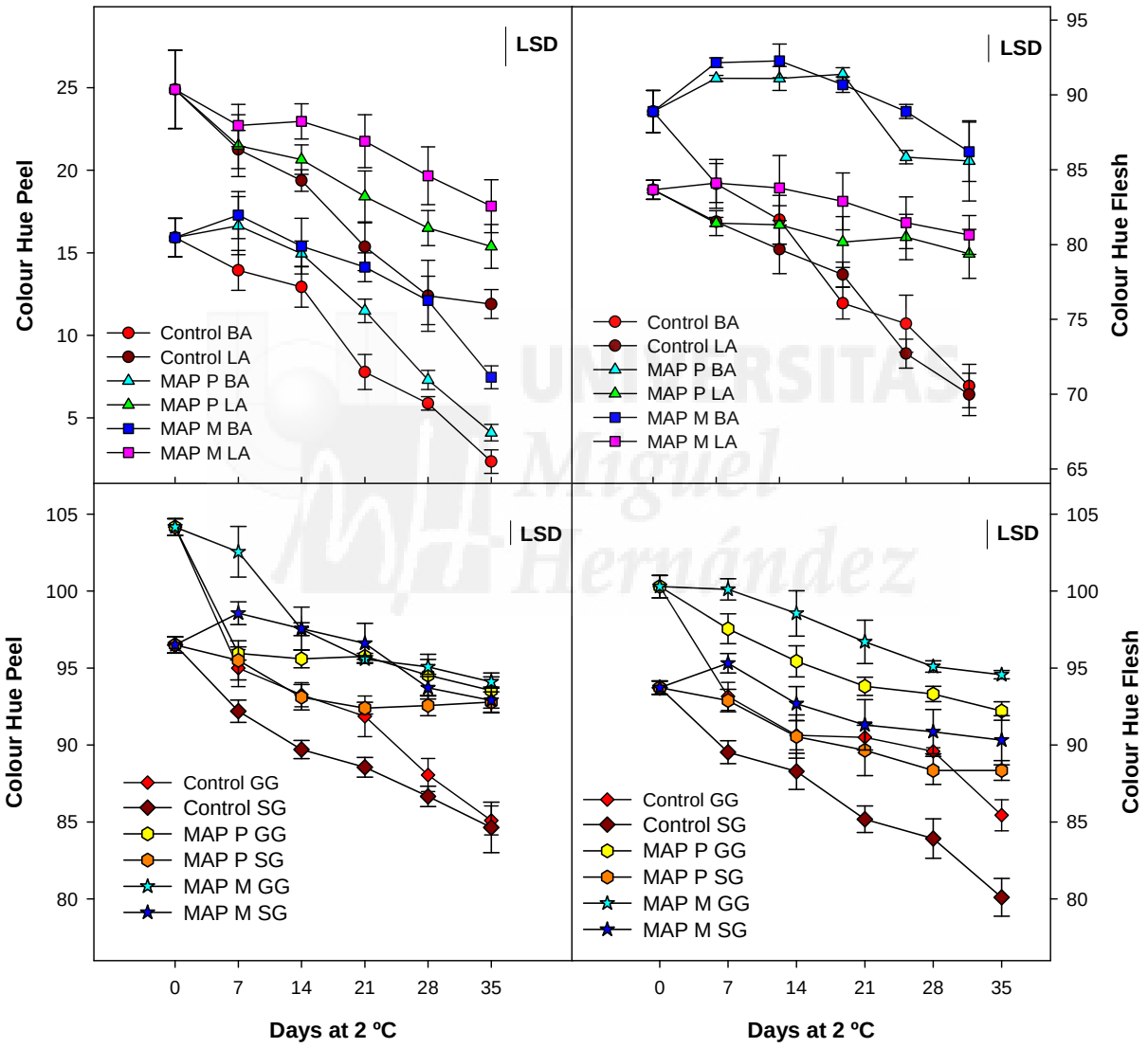

Fig. 3. Colour (Hue angle) changes in peel and flesh during storage of different plum cultivars, 'Blackamber' (BA), 'Larry Ann' (LA), 'Golden Globe' (GG) and 'Sungold' (SG), under MAP conditions (with film H and M). Data are the mean ± SE (n=48). Least significant differences (LSDs) are shown.

The delay in colour change associated with the postharvest ripening process has also been shown in other fruit, such as mango (Pesis et al., 2000), table grape (Martínez-Romero et al., 2003) and loquat (Amorós et al., 2008) under MAP conditions. These effects could be attributed to the delay in anthocyanin and carotenoid biosynthesis induced by MAP, according to a previous report (Artés et al., 2006), which was higher in those plums stored under the more impermeable film.

With respect to fruit firmness, differences existed at harvest among cultivars with GG being the firmest cultivar (12.61±0.40 N mm^{-1}) followed by LA (8.94±0.75 N mm^{-1}), while BA and SG showed the lowest firmness values (≈7.4 N mm^{-1}). For all cultivars, fruit firmness decreased significantly during storage with firmness losses in control fruit being ≈72% for BA, ≈65% for GG and SG, and 51% for LA at the end of the experiment (**Fig. 4**).

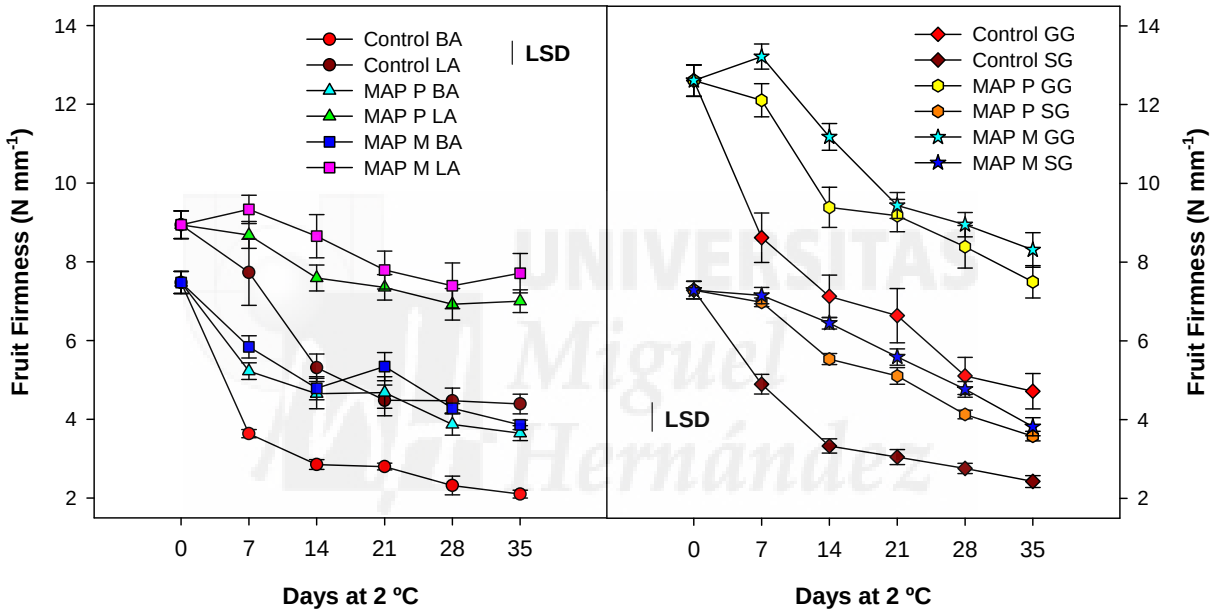

Fig. 4. Fruit firmness changes during storage of different plum cultivars, 'Blackamber' (BA), 'Larry Ann' (LA), 'Golden Globe' (GG) and 'Sungold' (SG), under MAP conditions (with film H and M). Data are the mean ± SE (n=24). Least significant differences (LSDs) are shown.

The softening process was significantly delayed by the use of MAP, with slightly higher fruit firmness retention in those plums packed in film M than in film H. The effect of MAP on delaying softening could be an ethylene-mediated effect, since it was higher in plums under film M, in which ethylene production was inhibited to a greater extent than in those plums under film H. However, a direct effect of high CO_2 and low O_2 on inhibiting cell-wall degrading enzymes could not be discounted, since delay in softening under MAP conditions has been observed in both climacteric fruit, such as kiwifruit, apricot, peach and nectarine (Pretel et al., 1993; Agar et al., 1999; Akbudak and Eris, 2004), and non-climacteric ones, such as strawberry and table grape (García et al., 1998; Martínez-

Romero et al., 2003).Total soluble solids (TSS) increased during storage of control fruit in all plum cultivars, the increases being significantly lower in those plums stored under MAP conditions. No significant differences existed between M and H films, with the exception of SG plums, in which TSS accumulation was lower in plums packaged with film M than with film H (**Fig. 5**). In addition, TSS remained unchanged during storage in BA plums under MAP conditions, irrespective of the film used.

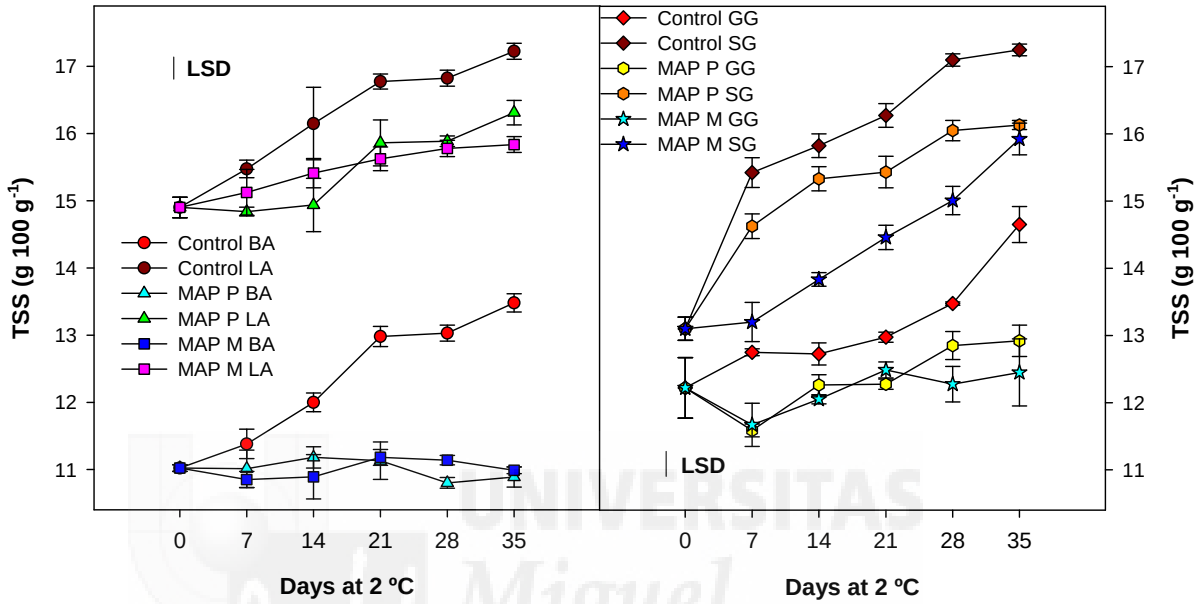

Fig. 5. Total soluble solids (TSS) changes during storage of different plum cultivars, 'Blackamber' (BA), 'Larry Ann' (LA), 'Golden Globe' (GG) and 'Sungold' (SG), under MAP conditions (with film H and M). Data are the mean ± SE (n=24). Least significant differences (LSDs) are shown.

In contrast, total acidity (TA) decreased during storage for all plum cultivars and films assayed, although acidity losses were retarded by the use of MAP with higher acidity retention in the plums packaged with film M than in those with film H (**Fig. 6**). Accordingly, in peach and nectarine, the decrease in total acidity was delayed by the use of MAP, as well as the increase in TSS (Akbudak and Eris, 2004), and in loquat the decrease in individual sugars and malic acid was delayed in MAP packages as compared with fruit stored in open air (Amorós et al., 2008). These results showed a clear effect of MAP on decreasing fruit metabolism, including respiration rate, leading to maintenance of respiration substrates and in turn to a delay of the postharvest ripening process. In fact, the ratio between TSS and TA (TSS/TA) considered as a fruit ripening index, increased sharply during storage in control plums, with initial values of ≈10 (for BA and GG) and ≈16 (for LA and SG) to final values of ≈34 for LA and SG, and 76 ≈29 for BA and GG. However, with the use of both films the values of TSS/TA were lower than in control plums, and even differences were obtained between the 2 film types, with the lowest TSS/TA ratio

for those plums packaged with film M, which were ≈15 for GG and BA, ≈18 for LA and ≈24 for SG.

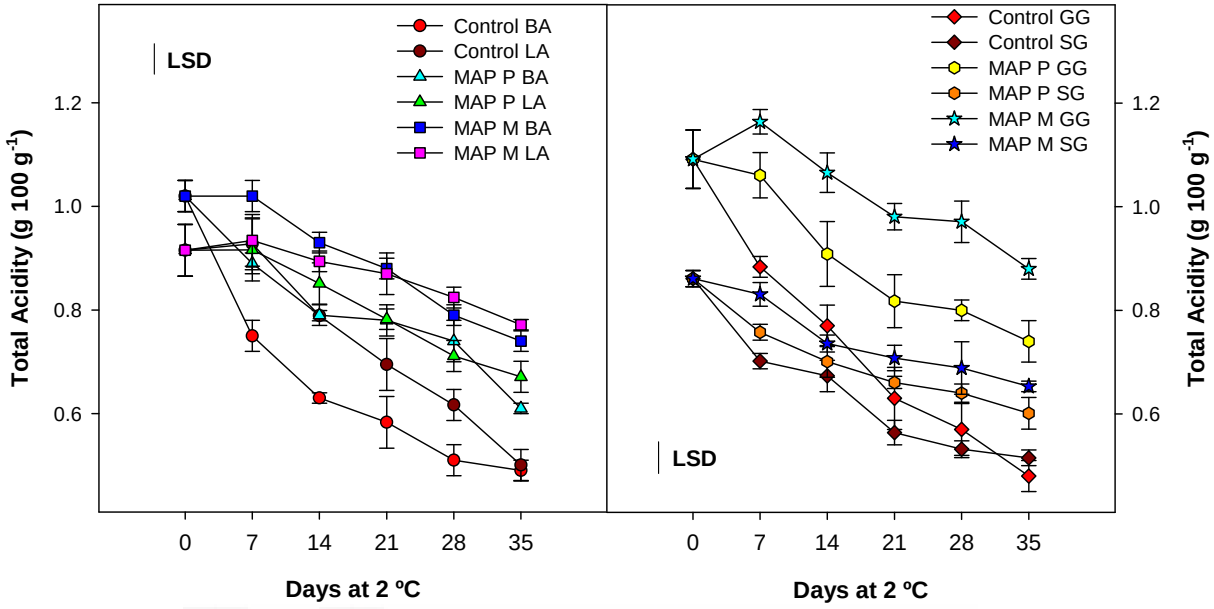

Fig. 6. Total acidity (TA) changes during storage of different plum cultivars, 'Blackamber' (BA), 'Larry Ann' (LA), 'Golden Globe' (GG) and 'Sungold' (SG), under MAP conditions (with film H and M). Data are the mean ± SE (n=24). Least significant differences (LSDs) are shown.

In conclusion, the MAP technique was useful to delay the ripening process of plum cultivars through a delay in the changes in colour, and the losses of firmness and acidity, and in turn an extension of shelf-life could be achieved (3–4 weeks more depending on the evaluated parameter) as compared with controls. In most cases the effect was higher in those plums packaged with film M than film H, probably due to the higher inhibition of ethylene production by the use of film M. However, the effect of these packages on the content of bioactive compounds and antioxidant activity of plum stored under MAP conditions deserves further research, which has not been carried out to date.

Acknowledgements

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Chapter 6

Modified atmosphere packaging of yellow and purple plum cultivars. 2. Effect on bioactive compounds and antioxidant activity

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Modified atmosphere packaging of yellow and purple plum cultivars. 2. Effect on bioactive compounds and antioxidant activity

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Abstract

Changes in bioactive compounds (total phenolics and total carotenoids, and individual anthocyanins) as well as total antioxidant activity (TAA) in separate fractions; hydrophilic (H-TAA) and lipophilic (L-TAA), in the peel and the flesh of 2 purple and 2 yellow skin plum cultivars under modified atmosphere packaging (MAP) conditions, using two films with different gas permeability, were studied. Results revealed that in all cultivars, total phenolics and H-TAA increased in the peel and flesh during storage, as well as the two identified anthocyanins: cyanidin-3-glucoside and cyanidin-3-rutinoside in the purple cultivars. These changes were significantly delayed in fruit stored under MAP conditions. Total carotenoids and L-TAA increased in the yellow cultivars (in both peel and flesh) while decreases were observed in the purple cultivars, these changes also being delayed by the use of MAP. Positive correlations were found between H-TAA and total phenolics and between L-TAA and total carotenoids.

Keywords: *Prunus salicina*; Phenolics, Anthocyanins; Carotenoids; Plum; MAP

INTRODUCTION

Modified atmosphere packaging (MAP) has been poorly studied in plums, although recent papers have shown efficacy of MAP in alleviating chilling injury symptoms in 'Friar' plums through reducing softening and browning (Cantín et al., 2008; Guan and Dou, 2010), and in retarding parameters related with ripening, such as dehydration, respiration rate, colour changes, softening, acidity losses and the increase in total soluble solids (Díaz-Mula et al., 2011).

Plums are known to contain large amounts of phytochemicals and are considered a good source of natural antioxidants in our daily diet. It has been demonstrated that plums have higher total antioxidant capacity (TAA) than many other common fruit such as apple, tomato, and peach, and similar capacity to that of strawberry and blueberry (Wang et al., 1996; Wu et al., 2004; Valero and Serrano, 2010). Phenolic compounds, especially flavonoids, phenolic acids and anthocyanins, have been considered the most important compounds contributing to TAA (Gil et al., 2002; Kim et al., 2003; Cevallos-Casals et al., 2006). The TAA reported in these and other papers are only related to hydrophilic compounds, since extractions were carried out using hydrophilic solvents. However, when extraction is performed with both hydrophilic and lipophilic solvents, it can be shown that lipophilic compounds, such as carotenoids, are also important antioxidant compounds in plums, even in red-purple cultivars (Díaz-Mula et al., 2008; Díaz-Mula et al., 2009).

Currently, there are only a few reports on the effect of MAP conditions on the content and changes in bioactive compounds with antioxidant activity in fruit and vegetables. Broccoli heads stored with polypropylene films showed lower losses in ascorbic acid, total phenolics and antioxidant activity than those stored in air (Serrano et al., 2006). In loquats, MAP was also effective in suppressing ascorbic acid losses that occurred in control fruit (Amorós et al., 2008), and in papaya, the antioxidant potential was maintained under MAP conditions due to retention of both ascorbic acid and lycopene (Singh and Rao, 2005).

In plums, there is only one report in which MAP delayed the increase in anthocyanin and phenolic content in the flesh of 'Friar' plums (Guan and Dou, 2010), and thus more in depth studies are necessary. In this sense, the aim of this paper was to study the effect of MAP on the changes in bioactive compounds (total phenolics and total carotenoids, and individual composition of anthocyanins) as well as the total antioxidant activity (TAA) in separate fractions, hydrophilic (H-TAA) and lipophilic (L-TAA), in the peel and the flesh of 4 plum cultivars. The cultivars 'Blackamber' (BA) and 'Larry Ann' (LA) have purple skin and 'Sungold' (SG) and 'Golden Globe' (GG) have yellow skin, although all of them have yellow coloured flesh.

MATERIALS AND METHODS

Plant material and experimental design

Plum fruit (*Prunus salicina* Lindl.) from 'Blackamber' (BA), 'Larry Ann' (LA), 'Golden Globe' (GG) and 'Songold' (SG) cultivars were picked at the commercial ripening stage according to Díaz-Mula et al. (2008). Once in the laboratory, 240 fruit homogenous in colour, size and with absence of defects, were selected for each cultivar and sorted at random in 30 lots of 8 fruit. Three lots were used to analyze the fruit properties at harvest (Day 0). The remained lots were individually placed in polypropylene baskets and divided into 3 batches at random and then thermo-sealed on top (total area of 336 cm^2, 14 cm × 24 cm) with the following films: macro-perforated film as control, film H and film M, which were effective for preserving plum organoleptic quality during MAP storage (Díaz-Mula et al., 2011). The film characteristics were as follows: macro-perforated polypropylene film (purchased from Plásticos del Segura S.L., Spain), had 32 holes of 1.5 mm diameter per dm^2, and total perforated area of 0.56 %; film H was composed of polyester (12 µm)-polypropylene (50 µm), and film M was composed of polyester (12 µm)-polypropylene (60 µm), and both purchased from Amcor Flexibles (Amcor, Barcelona, Spain) having a permeability to O_2 <100 mL O_2 m^{-2} day^{-1} atm^{-1}. All baskets were stored at 2 °C and 90 % RH for 35 days. For each cultivar and film type, baskets were taken after 7, 21 and 35 days, and analytical determinations were made after removing the fruit from MAP and cold storage. For each sampling date and replicate, the peel and flesh for each fruit were sampled, cut into small pieces and divided into 2 subsamples. Tissues from each subsample were ground under liquid N_2 and stored at −20 °C until the bioactive compounds (phenolics, carotenoids and anthocyanins) and total antioxidant activity (TAA) in both hydrophilic and lipophilic fractions were analyzed.

Bioactive compounds and antioxidant activity determination

The method of Tomás-Barberán et al. (2001) was used for total phenolic extraction by using water:methanol (2:8) containing 2 mM NaF. The phenolic content was quantified as previously described (Díaz-Mula et al., 2008) using the Folin-Ciocalteu reagent and results (mean ± SE) were expressed as mg gallic acid equivalent kg^{-1} fresh weight.

Anthocyanins were extracted from 2 g of peel tissue by homogenization in 4 mL methanol and left 1 h at −18 °C. Extracts were centrifuged at 15,000 × *g* for 15 min at 4 °C, and the anthocyanins in the supernatant was purified onto a C18 Sep-Pak® cartridge (Waters, Madrid, Spain), as described in Serrano et al. (2005). Individual anthocyanins were eluted in a high performance liquid chromatography (HPLC, Hewlett-Packard HPLC series 1100, Agilent, Madrid, Spain) coupled to a diode array detector (DAD). 1 mL from the extracts was filtered through 0.45 µm Millipore filter and then injected into a C18 Supelco column (Supelcogel C-610H, 30 cm × 7.8 mm, Supelco Park, Bellefonte, USA) and

detected by absorbance at 510 nm. The peaks were eluted by the gradient proposed by Tomás-Barberán et al. (2001). The anthocyanin standards cyanidin 3-glucoside and cyanidin 3-rutinoside were kindly provided by Dr. García-Viguera (CSIC, Murcia, Spain).

Total antioxidant activity (TAA) was quantified based on Arnao et al. (2001) and slightly modified by Serrano et al. (2009), which enables determination of TAA due to both hydrophilic (H-TAA) and lipophilic (L-TAA) compounds in the same extraction. In brief, 1 g of peel or 5 g of flesh tissues were homogenized in 5 mL of 50 mM phosphate buffer pH 7.8 and 3 mL of ethyl acetate, and then centrifuged at 15,000 × *g* for 15 min at 4 °C. The upper fraction was used for L-TAA while the lower fraction for H-TAA quantification using the enzymatic system composed of the chromophore 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), horseradish peroxidase enzyme (HRP) and its oxidant substrate (hydrogen peroxide).

Total carotenoids were estimated in the lipophilic extract (Arnao et al., 2001) by reading the absorbance at 450 nm in a UNICAM Helios-α spectrophotometer (Cambridge, UK), and expressed as mg of ß-carotene equivalent kg^{-1} fresh weight, taking into account the $\varepsilon^{1\%}_{cm}$ = 2560 and the results were the mean ± SE.

Statistical analysis

Experimental data were subjected to ANOVA analysis. Sources of variation were MAP packages and storage. The overall least significant differences (Fisher's LSD procedure, $p < 0.05$) were calculated and used to detect significant differences among packages and storage time. All analyses were performed with SPSS software package v. 11.0 for Windows (SPSS, 2001, VERSION 12.0 for Windows, Inc., Chicago, IL). Linear regressions were performed between total antioxidant activity (either hydrophilic or lipophilic) and the bioactive compounds taking into account all sampling data (either peel or flesh).

RESULTS AND DISCUSSION

During storage, CO_2 concentrations increased and O_2 concentrations decreased inside the MAP packages and the steady-state atmosphere was reached after 7 days at 2 °C, the composition being dependent on the film and the plum respiration rate (Díaz-Mula et al., 2011). The highest atmosphere modification was for LA plums with film M, with CO_2 and O_2 concentrations at equilibrium of ≈6.5 and ≈13 kPa, respectively, while the lowest atmosphere modification was reached in GG under film H, ≈1.5 and 19 kPa for CO_2 and O_2, respectively.

Bioactive compounds

Total phenolic concentration at harvest was different depending on cultivar in both, peel and flesh tissues. In peel tissue total phenolic concentration ranged from 1267±19 to 4584±87 mg kg^{-1}, for GG and BA plum, respectively, while for flesh tissue this range was smaller, from 846±54 to 1562±129 mg kg^{-1}, for GG and SG, respectively. During storage a similar trend was observed for both peel and flesh, that is an increase between 40-50% for all cultivars in control fruit (**Fig. 1**).

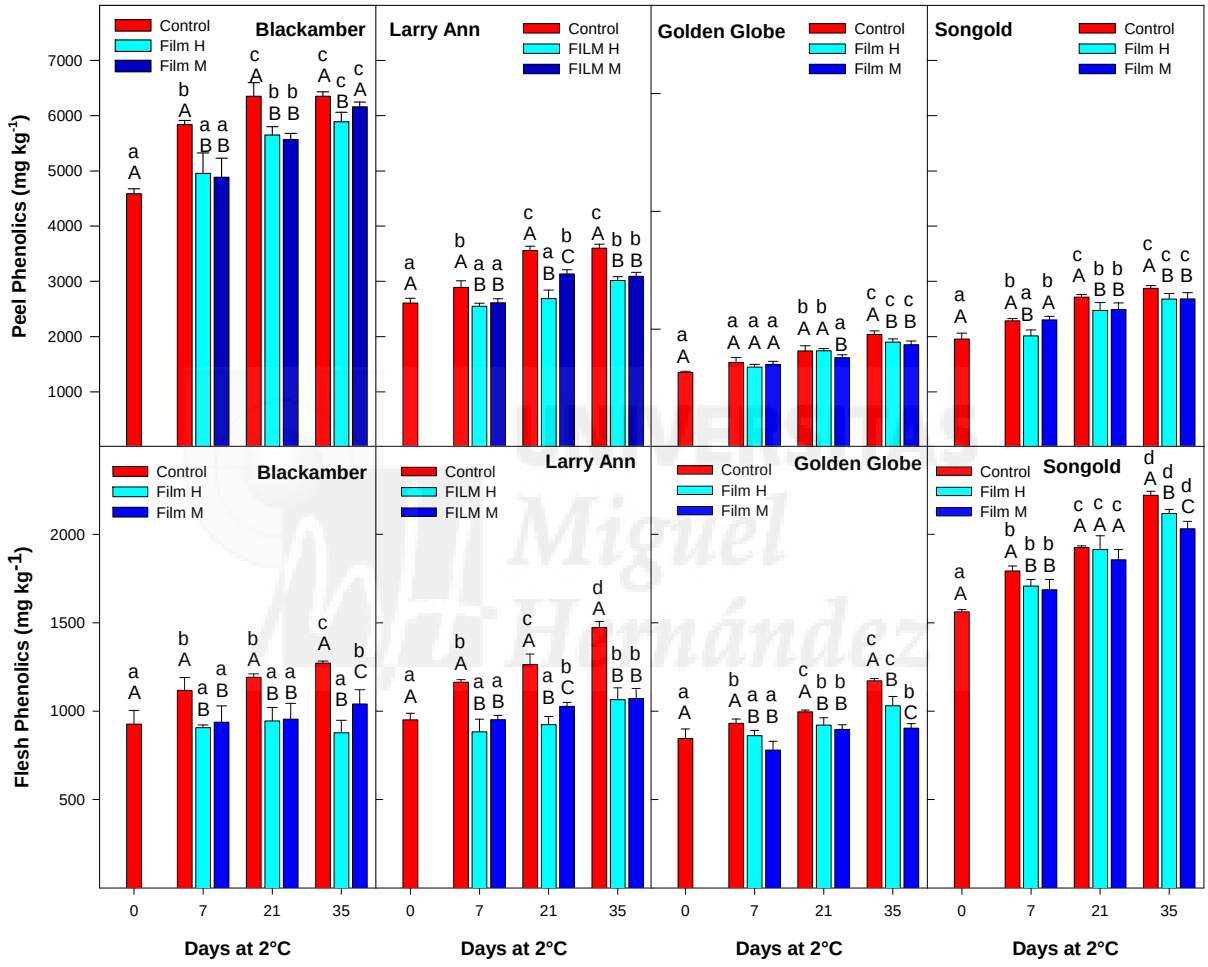

Fig. 1. Changes in total phenolics (peel and flesh) during storage at 2 °C of 4 plum cultivars under MAP conditions with 2 different films (M and H) or with a macroperforated film (control). Results are the mean ± SE (n=4). Minor and capital letters show significant differences (p < 0.05) during storage for each treatment and among treatments for each storage time, respectively.

The increases in total phenolics were delayed by the use of MAP packages, without significant differences between H and M films. Polyphenols, which are the most abundant secondary metabolites in fruit, showed a similar trend during storage of the 4 plum cultivars. These results are in agreement with previous reports on these and other plum cultivars ('Black Diamond', 'Golden Japan', 'TC Sun' and 'Angeleno) as well as other stone fruit such as sweet cherry, peach and nectarine (Di Vaio et al., 2008; Díaz-Mula et al.,

2009; Serrano et al., 2009). When low temperature storage was combined with MAP, there was a delay in the phenolic accumulation, which might be due to the effect of MAP (low O_2 and high CO_2) in retarding the postharvest ripening, as can be inferred by the reduced ethylene production, fruit softening, colour evolution and acidity loss (Díaz-Mula et al., 2011). In addition, the possible effect of low O_2 and high CO_2 on the delay in the phenylalanine ammonia lyase (PAL), chalcone synthase or anthocyanidin synthase, the key enzymes in the biosynthesis pathway of phenolic compounds (Desjardins, 2008), or the reduced polyphenol oxidase (PPO) or peroxidase activities (Pourcel et al., 2007), the main enzymes responsible of polyphenol degradation, should not be discharged.

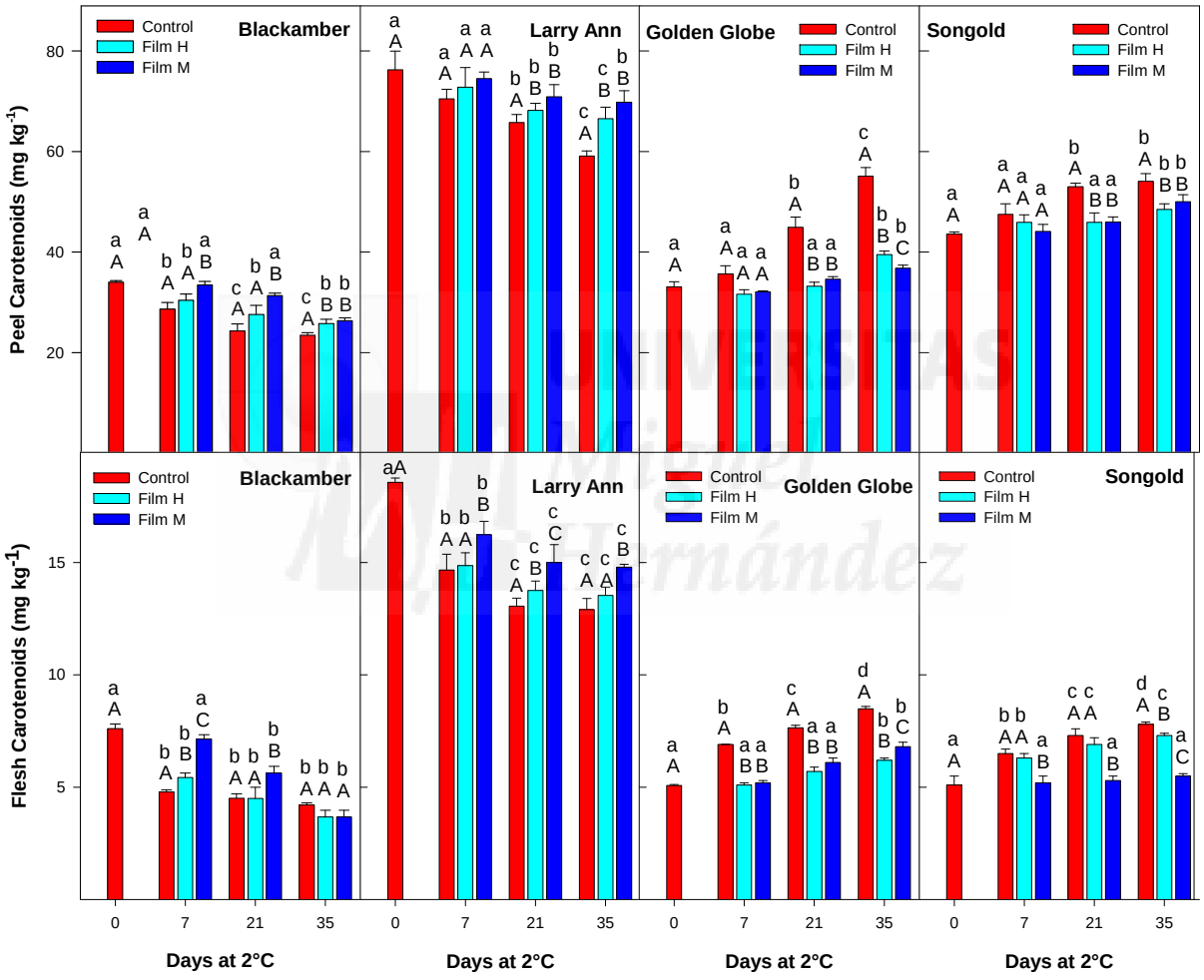

Fig. 2. Changes in total carotenoids (peel and flesh) during storage at 2 °C of 4 plum cultivars under MAP conditions with 2 different films (M and H) or with a macroperforated film (control). Results are the mean ± SE (n=4). Minor and capital letters show significant differences ($p < 0.05$) during storage for each treatment and among treatments for each storage time, respectively.

As occurred for total phenolics, differences in carotenoid concentrations existed among cultivars and types of tissue (**Fig. 2**). In this regard, the peel always had 5-7-fold more carotenoids than flesh tissues, LA being the cultivar with the highest carotenoid concentrations in both peel and flesh (76.28±3.17 and 18.56±0.49 mg kg^{-1}, respectively), while in BA plum the lowest total carotenoid concentrations was found for both tissues (34.01±0.30 and 7.62±0.21 mg kg^{-1}, respectively). During postharvest storage a different behaviour was observed between yellow and purple cultivars, since increases were found for both peel and flesh of plum cultivars with yellow skin, while the contrary occurred for BA and LA (plums with purple skin). For all cultivars, MAP packages induced a delay in the change of total carotenoids over storage.

Anthocyanins were analysed by HPLC–DAD and were detected in the peel and flesh of the purple plum cultivars (BA and LA), with two individual anthocyanins identified, cyanidin-3-glucoside and cyanidin-3-rutinoside (**Fig. 3**), the latter found at significant lower concentrations, according to previous reports in other purple plum cultivars (Tomás-Barberán et al., 2001; Chun et al., 2003; Wu and Prior, 2005; Díaz-Mula et al., 2008). In addition, differences existed in anthocyanin concentrations between the 2 cultivars.

Thus, concentrations in the peel at harvest of cyanidin-3-glucoside were 1456±71 and 744±24 mg kg^{-1}, for BA and LA, respectively, and 644±42 and 416±17 mg kg^{-1} of cyanidin-3-rutinoside. In control fruit of both cultivars, significant increases in the concentration of both anthocyanins in the peel were found during storage, while these increases were retarded in those plums stored under MAP conditions with generally no significant differences attributable to film type. However, in the flesh, concentrations at harvest were much lower (≈30 and 60 mg kg^{-1} of cyanidin-3-glucoside, for BA and LA, respectively, and ≈10 and 20 mg kg^{-1} of cyanidin-3-rutinoside) and no significant changes were observed during storage, irrespectively of the treatment (data not shown).

Increases in carotenoids and anthocyanins concentrations in yellow and purple plums, respectively, are responsible for the colour changes associated with the ripening process on the tree (Díaz-Mula et al., 2008) or after cold storage (Díaz-Mula et al., 2009). However, these increases were delayed in plums stored under MAP conditions, leading to a reduction in the change of colour as shown in our previous paper (Díaz-Mula et al., 2011). Accordingly, increases in anthocyanins in sweet cherry, strawberry, blueberry and raspberry, and in lycopene in tomato and watermelon, were lower during storage under MAP conditions than in control fruit stored in open air, due to the effect of MAP on delaying the development of the postharvest ripening process (Jones et al., 2007).

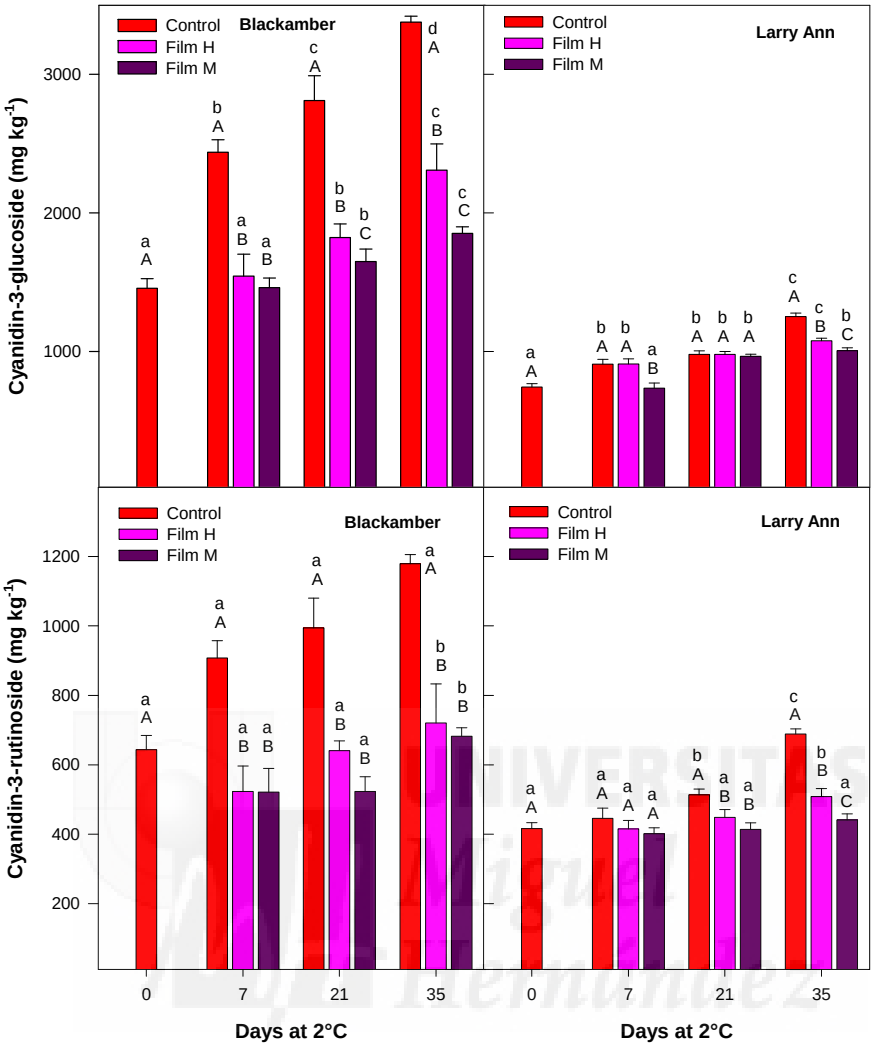

Fig. 3. Changes in anthocyanins (cyanidin-3-glucoside and cyanidin-3-rutinoside) in the peel of purple plum cultivars during storage at 2 °C under MAP conditions with 2 different films (M and H) or with a macroperforated film (control). Results are the mean ± SE (n= 4). Minor and capital letters show significant differences (p < 0.05) during storage for each treatment and among treatments for each storage time, respectively.

Total antioxidant activity

TAA was measured in both hydrophilic (H-TAA) and lipophilic (L-TAA) fractions for both peel and flesh tissues, for which H-TAA and L-TAA were always higher in the peel than in the flesh although important differences existed among cultivars. Thus, H-TAA was 3–25 fold higher in the peel than in the flesh, while L-TAA was 5-fold in all cultivars (**Figs. 4 and 5**). In addition, H-TAA and L-TAA were also different at harvest, the BA plums having the highest H-TAA (8308±428 mg kg^{-1}) and SG the lowest (1733±89 mg kg^{-1}) in the peel, while in the flesh the highest H-TAA was found in SG (462± 37mg kg^{-1}) and the lowest in GG (213±26 mg kg^{-1}). With respect to L-TAA, lower variations were found among

cultivars at harvest, with levels of ≈2500 and ≈550 mg kg^{-1} in the peel and flesh, respectively, for BA, GG and SG plum cultivars. The exception was LA plums, in which L-TAA levels were 4606±83 and 874±17 mg kg^{-1} in peel and flesh, respectively (**Fig. 5**). During storage, control fruit exhibited significant increases in H-TAA in both peel and flesh of all cultivars although these increases were retarded by the use of plastic films, but without significant differences attributable to film type. However, changes in L-TAA during storage were dependent on cultivar, since decreases were observed in the peel and flesh of purple cultivars (BA and LA) while increases occurred in the yellow ones. For all cases, a significant delay in those changes of L-TAA was observed in the plums stored under MAP conditions with both film types. It is interesting to highlight that to measure the antioxidant capacity of plums, the contribution of L-TAA should be taking into account, since the values obtained for L-TAA were as high as those of H-TAA, in both peel and flesh of these plum cultivars.

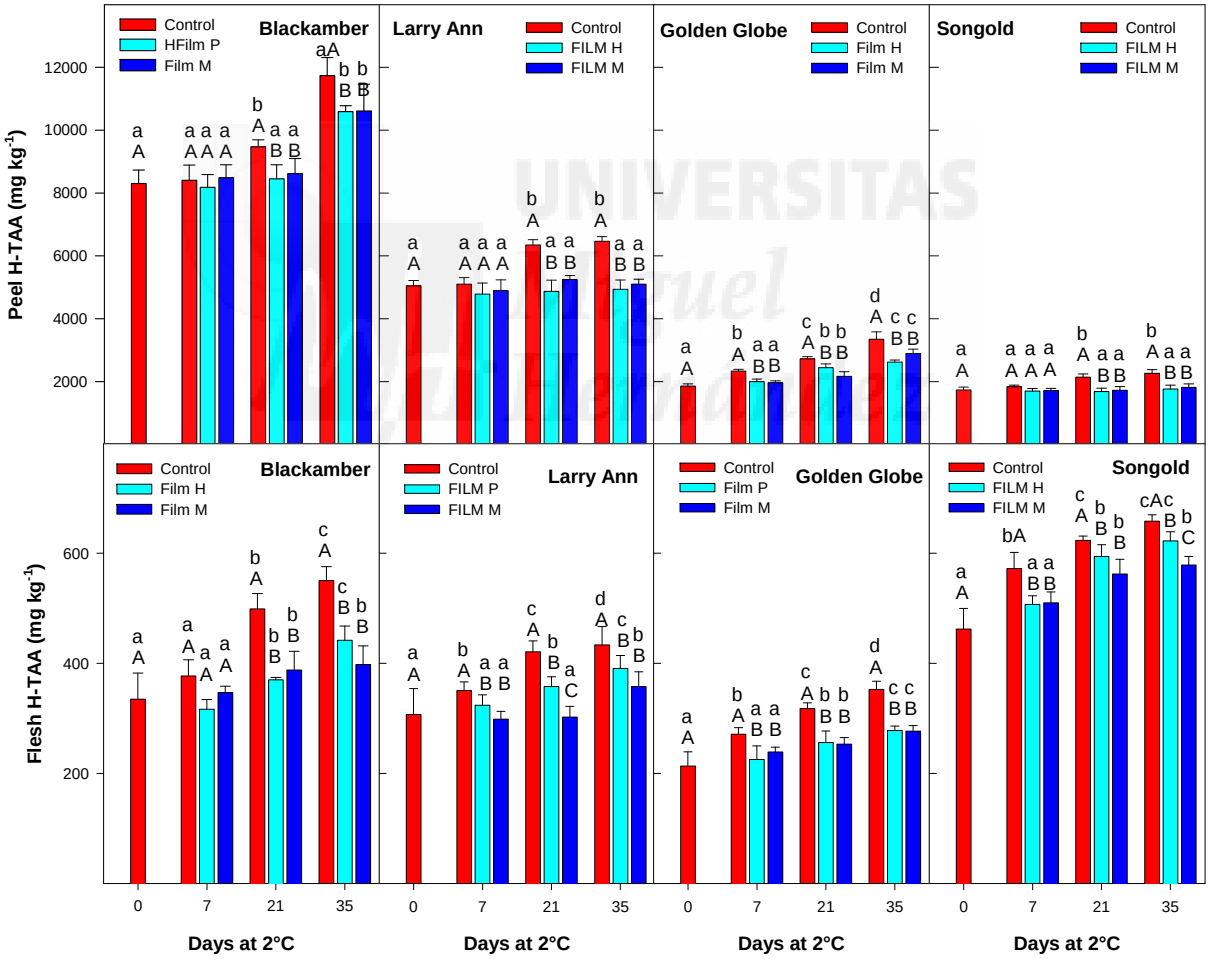

Fig. 4. Changes in total hydrophilic antioxidant activity (H-TAA) in the peel and flesh of 4 plum cultivars stored at 2 °C with 2 different films (M and H) or with a macroperforated film (control). Results are the mean ± SE (n=4). Minor and capital letters show significant differences ($p < 0.05$) during storage for each treatment and among treatments for each storage time, respectively.

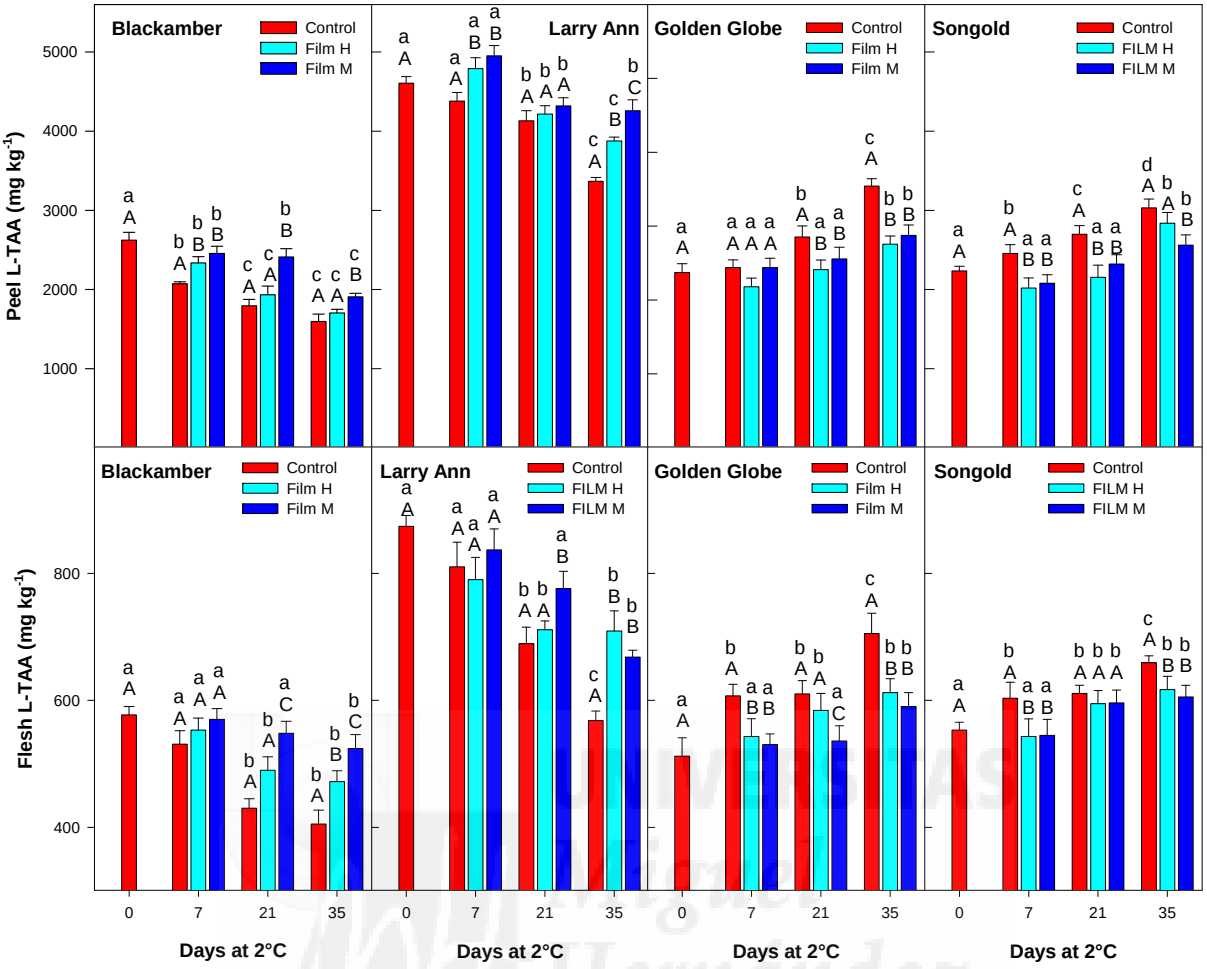

Fig. 5. Changes in total lipophilic antioxidant activity (L-TAA) in the peel and flesh of 4 plum cultivars stored at 2 °C with 2 different films (M and H) or with a macroperforated film (control). Results are the mean ± SE (n=4). Minor and capital letters show significant differences ($p < 0.05$) during storage for each treatment and among treatments for each storage time, respectively.

Taking into account data for all cultivars, sampling dates and storage conditions, a positive correlation was found between H-TAA and total phenolics content (y = 1.82x − 1021; R^2 = 0.874, for peel and y = 0.27x + 67; R^2 = 0.826, for flesh), and between L-TAA and total carotenoids (y = 53.32x + 420; R^2 = 0.855, for peel and y = 22.83x + 422; R^2 = 0.790, for flesh). Correlations between total phenolics and H-TAA levels at harvest have been found in other plum cultivars and stone fruit, such as peach, nectarine and sweet cherry (Gil et al., 2002; Cevallos-Casals et al., 2006; Rupasinghe et al., 2006; Vizzotto et al., 2007; Díaz-Mula et al., 2008), although during storage these correlations have only been found in our previous work (Díaz-Mula et al., 2009; Serrano et al., 2009). On the other hand, L-TAA has been also been correlated with total carotenoids in a wide range of plum cultivars during on-tree ripening as well as in tomatoes, and some vegetables and legumes (Wu et al., 2004; Lenucci et al., 2006; Cho et al., 2007; Díaz-Mula et al., 2008).

As previously stated, almost no information exists on the effect of MAP on antioxidant capacity of fruit in general, and in plums particularly. Our results suggest that MAP does not impart any negative effects on TAA and just reflects the delay of the ripening process occurring in the plums stored under MAP conditions. Additionally, it is interesting to consider that H-TAA might be underestimated, since an increase in TAA has been recently reported when the extraction residues were subjected to two different acidic treatments to release hydrolysable tannins and non-extractable proanthocyanidins (Kristl et al., 2011).

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Chapter 7

Postharvest Treatments with Salicylic Acid, Acetyl Salicylic Acid or Oxalic Acid Delayed Ripening and Enhanced Bioactive Compounds and Antioxidant Capacity in Sweet Cherry

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ABSTRACT

Sweet cherry cultivars ('Cristalina' and 'Prime Giant') harvested at commercial ripening stage were treated with 9 salicylic acid (SA), acetylsalicylic acid (ASA) or oxalic acid (OA) at 1 mM and then stored for 20 days under cold temperature. Results showed that all treatments delayed the postharvest ripening process, manifested by lower acidity, color changes and firmness losses, and maintained quality attributes for longer periods than controls. In addition, total phenolics, anthocyanins and antioxidant activity increased in untreated fruit during the first 10 days of storage and then decreased, while in fruits of all treatments, these parameters increased continuously during storage without significant differences among treatments. Thus, postharvest treatments with natural compounds, such as SA, ASA or OA, could be innovative tools to extend the storability of sweet cherry with higher content of bioactive compounds and antioxidant activity as compared with control fruits.

KEYWORDS: phenolics, anthocyanins, carotenoids, total antioxidant activity, salicylic acid, acetylsalicylic acid, oxalic acid, postharvest

INTRODUCTION

Sweet cherry quality is determined by attributes affecting fruit marketing appeal and consumer satisfaction. Among the attributes related to consumer purchase decision are visual appearance, fruit size and color, firmness, and especially sweetness and flavor. However, these parameters change with cultivar[1] and are associated with the ripening process.[2] On the other hand, sweet cherry has been reported to contain several phenolic compounds and anthocyanins which contribute to the antioxidant capacity.[3,4] The two dominant polyphenols in cherries are caffeoyltartaric acid and 3-*p*-coumaroylquinic acid, while the main anthocyanins are cyanidin-3-rutinoside and cyanidin-3-glucoside followed by pelargonidin-3-rutinoside, peonidin-3-rutinoside and peonidin- 3-glucoside.[5,6]

Cherry fruits deteriorate rapidly during postharvest storage, and in some cases do not reach consumers with optimal quality after transport and marketing. The main causes of sweet cherry deterioration are weight loss, color changes, softening, surface pitting, stem browning, loss of acidity and slight increases in TSS.[6-8] The storage period also affects the content of bioactive compounds, with general increases in phenolic and anthocyanin concentrations associated with the postharvest ripening process.[5,6]

Consumers demand food preservation systems with absence of chemicals or pesticide residues, and thus there is increasing interest in the use of natural compounds. In the case of sweet cherry the combined use of modified atmosphere packaging and essential oils[7] or *Aloe vera* as edible coating[8] resulted in a reduction of spoilage microorganisms, maintenance of fruit quality and extension of shelf life. Other natural compounds, such as salicylic acid (SA), acetylsalicylic acid (ASA) and oxalic acid (OA), are present in fruits and vegetables, and have shown important roles in delaying the ripening process when applied as postharvest treatment.

SA and ASA retarded the ripening process in banana,[9] sugar apple fruit,[10] kiwifruit,[11] mango[12] and peach,[13] through an induction of antioxidant enzymes, such as peroxidase, catalase and superoxidodismutase and reduction of lipoxygenase activity. In pomegranate, ASA treatment reduced occurrence of chilling injury (CI) and maintained higher content in nutritive and bioactive compounds.[14] OA has been involved in controlling litchi browning,[15] and delaying the ripening process in some climacteric fruits such as mango,[16] peach,[17] and jujube fruit,[18] through an inhibition of the ethylene biosynthesis. more recently, Sayyari et al.[19] reported that application of oxalic acid alleviated CI symptoms of pomegranate, a nonclimacteric fruit.

As far as we know, few reports exist on the role of SA in increasing resistance against cherry decay,[20,21] but there is no literature about the effect of postharvest treatments with SA, ASA or OA on the sweet cherry ripening process and parameters related to fruit quality. Thus, the objective of this research was to analyze the effect of

these treatments on the organoleptic parameters related to sweet cherry quality and the content of bioactive compounds and antioxidant activity during storage.

MATERIALS AND METHODS

Plant Material and Experimental Design

Sweet cherry cultivars ('Cristalina' and 'Prime Giant') were harvested at commercial ripening stage, S2 according to Serrano et al.,[6] from a commercial plot (Finca Los Frutales, Villena Alicante, Spain), with scores of 5 and 4 for 'Cristalina' and 'Prime Giant', respectively, according to the color chart from Centre Technique Interprofessionel de Fruits et L egumes (CTIFL, Paris). For each cultivar, about 12 kg of cherries were picked and immediately transferred to the laboratory. Then, 1020 homogeneous fruits in color and size and without visual defects were selected for each cultivar and randomly grouped in 51 lots of 20 fruits. Three lots were used to analyze the properties at harvest and 48 lots for the following treatments in triplicate: control (distilled water), 1 mM salicylic acid (SA), 1 mM acetylsalicylic acid (ASA), and 1 mM oxalic acid (OA). These concentrations were chosen based on a preliminary experiment, in which higher concentrations did not show delays in the ripening process additional to the effect found with 1 mM (data not shown). Treatments were performed by dipping fruits in 10 L of solution for 10 min, and then they were left to dry at room temperature before cold storage at 2 ºC and RH of 85% in darkness for up to 20 days. After 5, 10, 15, and 20 days, 3 lots from each cultivar and treatment were sampled at random from cold chambers for analytical determinations. In these fruits, color and firmness were individually measured and then the edible portion of each lot was cut in small pieces to obtain a homogeneous sample. For each sample 5 g was used to determine in duplicate total soluble solids (TSS) and total acidity (TA) and the remaining sample was frozen in liquid N_2, mixed and stored at -20 ºC until total anthocyanins, total phenolics, total carotenoids, and antioxidant activity in both hydrophilic and lipophilic fractions were determined.

Ripening Parameters

Color was determined in a Minolta colorimeter (CRC200, Minolta Camera Co., Japan), using the CIELab coordinates and expressed as chroma $[(a^2 + b^2)^{1/2}]$. Fruit firmness was determined using a TX-XT2i Texture Analyzer (Stable Microsystems, Godalming, U.K.) interfaced to a personal computer, with a flat steel plate mounted on the machine. For each fruit, the cheek diameter was measured and then a force that achieved a 3% deformation of the fruit diameter was applied. Results were expressed as the mean ± SE of the force deformation ratio (N mm^{-1}). TSS was determined from the juice obtained for each subsample with a digital refractometer (model PR-101, Atago Co. Ltd., Tokyo, Japan)

at 20 ºC, and results (mean ± SE) were expressed as % (Brix). Total acidity (TA) was determined by automatic titration (785 DMP Titrino,Metrohm, Herisau, Switzerland) 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 (mean ± SE) were expressed as g of malic acid equivalent per 100 g fresh weight.

Total Antioxidant Activity Determination

Total antioxidant activity (TAA) of hydrophilic and lipophilic compounds was quantified according to Arnao et al.[22] which enables determination of TAA due to both hydrophilic and lipophilic compounds in the same extraction. Briefly, for each subsample, five grams of tissue was homogenized in 5 mL of 50 mM Na-phosphate buffer (pH = 7.8) and 3 mL of ethyl acetate, and then the mixture was centrifuged at 10000*g* for 15 min at 4 ºC. 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 mM ABTS, 15 μMH_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 results are expressed as mg of Trolox equivalent per 100 g.

Bioactive Compound Determination

Total phenolics were extracted according to Tomás-Barberán et al.[23] 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, and results (mean ± SE) were expressed as mg of gallic acid equivalent per 100 g fresh weight. Total anthocyanins were determined according to García-Viguera et al.[24] 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 results were expressed as mg per 100 g fresh weight and were the mean ± SE. Total carotenoids were extracted according to Mínguez-Mosquera and Hornero-Méndez.[25] Briefly, 2 g of sweet cherry fruit was extracted with acetone and shaken with diethyl ether and 10% NaCl for separation of the two phases. The lipophilic phase was washed with Na_2SO_4 (2%) and saponified with 10% KOH in methanol, and the pigments were subsequently extracted with diethyl ether, evaporated and then made up to 25 mL with acetone. Total carotenoids

were estimated by reading the absorbance at 450 nm according to Díaz-Mula et al. 2008[26] and expressed as mg of ß-carotene equivalent per 100 g, taking into account the $\varepsilon^{1\%}_{cm}$ = 2560. Analytical reagents were purchased from Sigma-Aldrich (Madrid, Spain).

Statistical Analysis

Data for the analytical determinations were subjected to analysis of variance (ANOVA). Sources of variation were storage time and treatment. Mean comparisons were performed using HSD Tukey's test to examine if differences were significant at $P < 0.05$. Linear regressions were performed between the color chroma parameter and anthocyanin concentration, as well as among the bioactive compounds and H-TAA or L-TAA taking into account data from both for cultivars and all sampling dates. All analyses were performed with SPSS software package v. 12.0 for Windows.

RESULTS AND DISCUSSION

Sweet Cherry Ripening Parameters

During storage of control fruits, color (chroma index), fruit firmness and total acidity (TA) decreased in both cultivars while total soluble solids (TSS) increased. These changes were significantly delayed in 'Cristalina' and 'Prime Giant' treated with SA, ASA and OA (**Figure 1**). Interestingly, the observed values after 20 days of storage in treated fruits were similar to those of control fruit just after 10 days. Thus, for both cultivars shelf life of control cherries was established at 10 days while it was extended up to 20 days in treated fruits.

The loss of firmness and TA and the increase of color to dark-red have been associated with ripening of sweet cherry and loss of quality attributes.[4-6] OA and SA delayed the ripening process in climacteric fruit, such as mango,[16] peach[17] and jujube fruit,[18] as well as SA and ASA on kiwifruit[11] due to the inhibition of ethylene production. In addition, retention of fruit firmness after SA treatment has been reported in several crops due to inhibition of cell-wall degrading enzymes, such as polygalacturonase, cellulase, and pectinmethylesterase.[27] Although sweet cherry is a nonclimacteric fruit, the results show a clear effect of these treatments on delaying the ripening process and maintaining fruit quality in this fruit, as has been reported for other nonclimacteric fruit such as pomegranate treated with SA,[28] OA[19] and ASA.[14]

Bioactive Compounds and Total Antioxidant Activity

At harvest, the anthocyanin concentration differed between cultivars with values of 81.89 ± 1.62 and 18.56 ± 0.62 mg per 100 g, for 'Cristalina' and 'Prime Giant',

respectively (**Figure 2**). For both cultivars a similar pattern was found over storage in control fruits, that is, increases until day 10 of storage followed by a decrease. The application of SA, ASA and OA led to a continuous increase in anthocyanin concentration until the end of the experiment, the values being higher for OA-treated cherries followed by ASA and SA treated fruit.

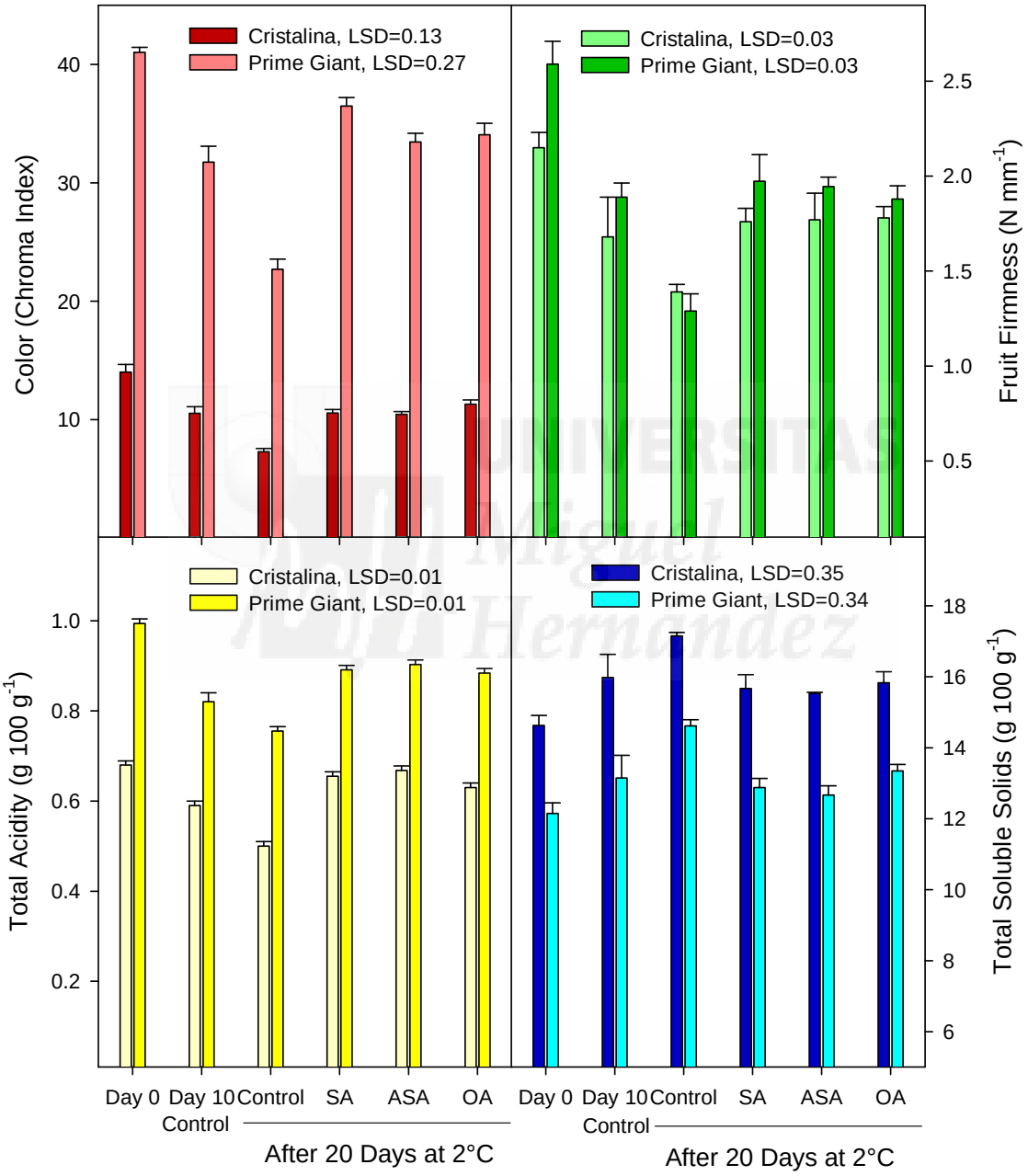

Figure 1: Color, firmness, total acidity and total soluble solids at harvest (day 0) and after 10 or 20 days of storage at 2 ºC in control fruit, and after 20 days of storage in sweet cherry 'Cristalina' and 'Prime Giant' treated with salicylic acid (SA), acetylsalicylic acid (ASA) or oxalic acid (OA). Data are the mean ± SE. LSD value for each cultivar is shown inside figures.

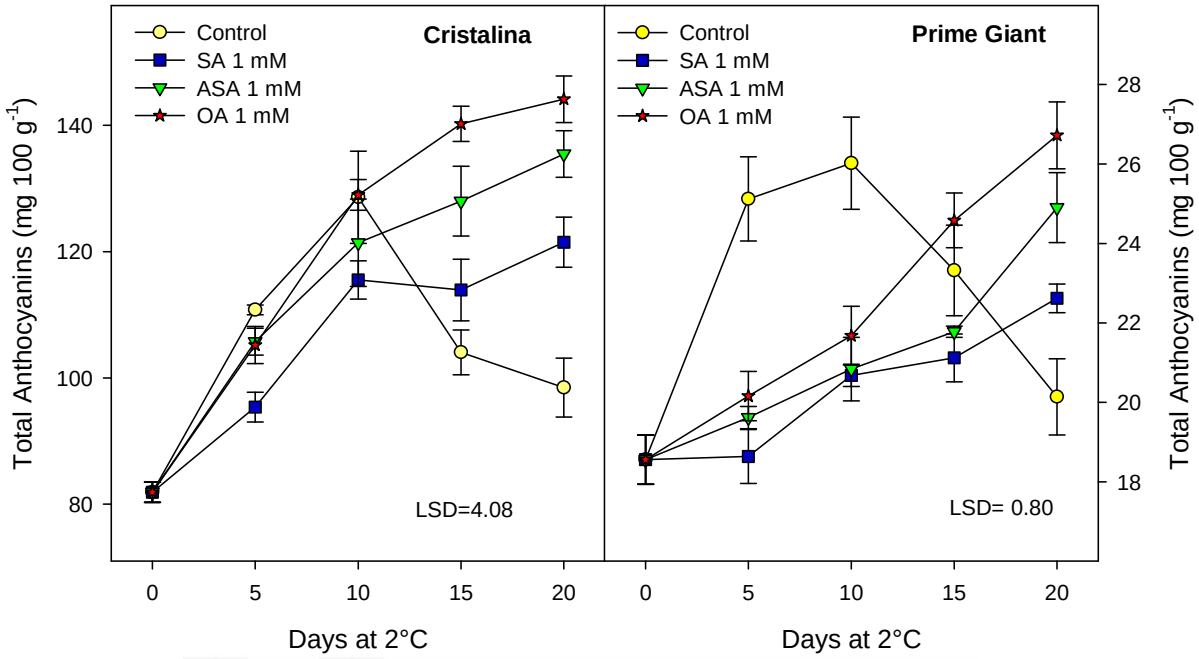

Figure 2: Total anthocyanin concentration in sweet cherry 'Cristalina' and 'Prime Giant' during storage at 2 ºC in control fruits and fruits treated with salicylic acid (SA), acetylsalicylic acid (ASA) or oxalic acid (OA). Data are the mean ± SE.

The main anthocyanins in 'Cristalina' and 'Prime Giant' cultivars are cyanidin-3-rutinoside followed by cyanidin-3-glucoside and pelargonidin-3-rutinoside, and their changes have been related to the advancement of the ripening process, either on-tree[1,2] or during storage,[6] and negatively correlated with color parameters.[3,6] Thus, control fruits exhibited the lowest chroma index, acquiring a dark-red color after 10 days, at which the highest anthocyanin concentration was found. After this period, control cherries became even darker and however loss of anthocyanins was detected. Discrepancies exist on the changes of anthocyanins during postharvest storage. Thus, in 'Sciazza' and 'Ferrovia' cherry cultivars, over 50% of the anthocyanin concentration was lost after 15 days at 1 ºC, while in 'Burlat', 'Saco', 'Summit' and 'Van' total anthocyanin concentration increased during 14 days and no changes have been found in 'Lambert Compact' cherries.[5,29] In our work, the storage period was extended up to 20 days and the fruit could be considered as over-ripe and in senescence phase, at which a diminution of total anthocyanins occurred. Accordingly, in 'Bing' cultivar the cyanidin-3-rutinoside, which is the major anthocyanin in cherries, decreased from day 15 to day 30 of storage, and the increase in color intensity was attributed to minor anthocyanins that impart dark-red color.[30] On the contrary, the clear effect of SA, ASA and OA on delaying the ripening process was reflected in the increase in anthocyanins over storage.

A similar pattern to that of total anthocyanins was found for total phenolic compounds, that, is increases in control fruits up to day 10 of storage and further decreases, while in all treated fruits total phenolics increased throughout the experiment (**Figure 3**). Among treatments, the highest levels of total phenolics were found in those cherries treated with OA and ASA followed by SA. In our and other sweet cherry cultivars nechlorogenic acid was the predominant hydroxycinnamic acid followed by 3-*p*-coumaroylquinic acid.[5,6,29]

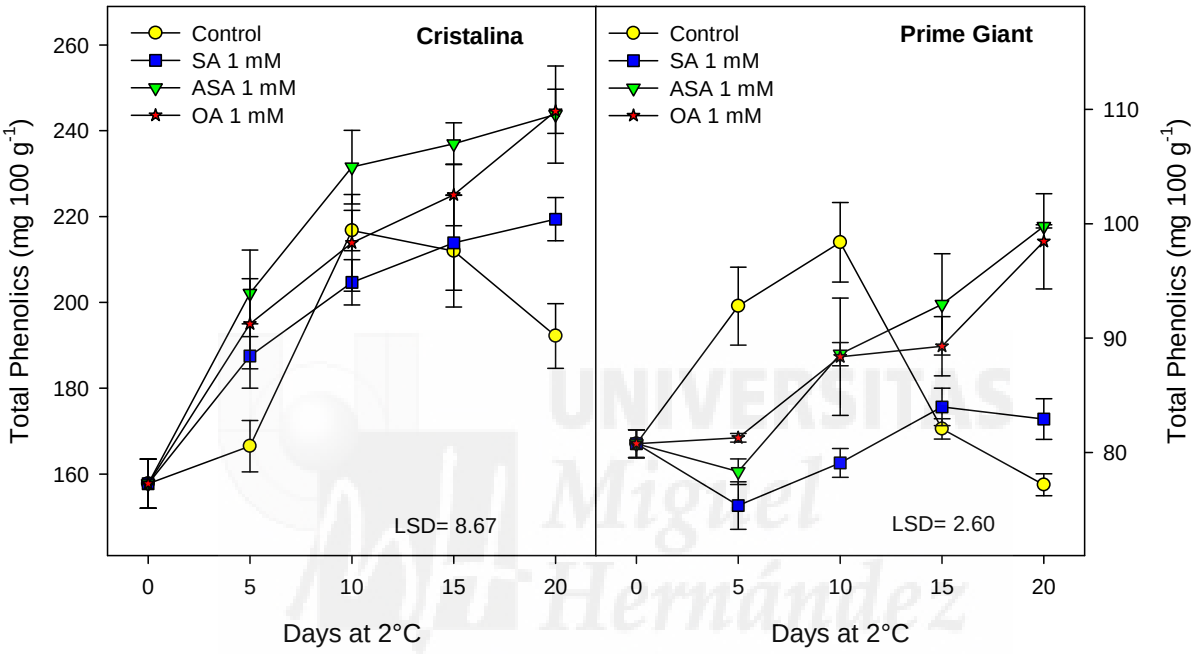

Figure 3: Total phenolics concentration in sweet cherry 'Cristalina' and 'Prime Giant' during storage at 2 ºC in control and treated fruits with salicylic acid (SA), acetylsalicylic acid (ASA) or oxalic acid (OA). Data are the mean ± SE. LSD is shown inside figures.

Phenolic compounds increased their concentration (40 60%) during cold storage and also were related to the advancement of the ripening process. However, the change of total phenolics during postharvest storage could be affected by several factors including ripening stage at harvest, cultivar, season growth and duration of storage time, since a general increase occurred in a short period of storage for a wide range of cherry cultivars,[6] although decreases or increases were reported for 'Summit' and 'Van' cherries depending on year and season growth.[5] The data reported herein suggest that increases occurred during the first days of storage and the prolongation of storage led to significant decreases in phenolic content. Linear regression was performed between total polyphenols and anthocyanins taking into account all data (cultivar, treatment and storage), and a high correlation was found (y =1.30x + 57.23; R^2 = 0.978), which suggests that

anthocyanins are the main phenolic compounds as has been shown in other cherry cultivars.[3,4,6] No literature exists on the effect of SA, ASA or OA on the content of total phenolics in fruits for comparative purposes, apart from our previous papers on pomegranate treated with OA or ASA, which alleviated CI and reduced the phenolic losses found in control fruits.[14,19]

Although carotenoids are other important bioactive constituents in fruits,[31] no evidence exist on their occurrence in sweet cherry. In both cultivars carotenoids were present but at different concentrations, with 'Prime Giant' having significantly higher total carotenoids (1.06 ± 0.07 mg per 100 g) than 'Cristalina' (0.61 ± 0.04 mg per 100 g). Along storage, an accumulation of total carotenoids was observed in both control and treated cherries for both cultivars (**Figure 4**). This increase was retarded in treated fruits with respect to controls, although at the end of the experiment the carotenoid concentration was similar for all cases, with final values ≈1.3 and ≈2.2 mg per 100 g, for 'Cristalina' and 'Prime Giant', respectively. In other stone fruits, such as plums, an increase of carotenoid levels was also observed along storage and related to the advancement of the ripening process in both yellow and red-purple cultivars.[26,32]

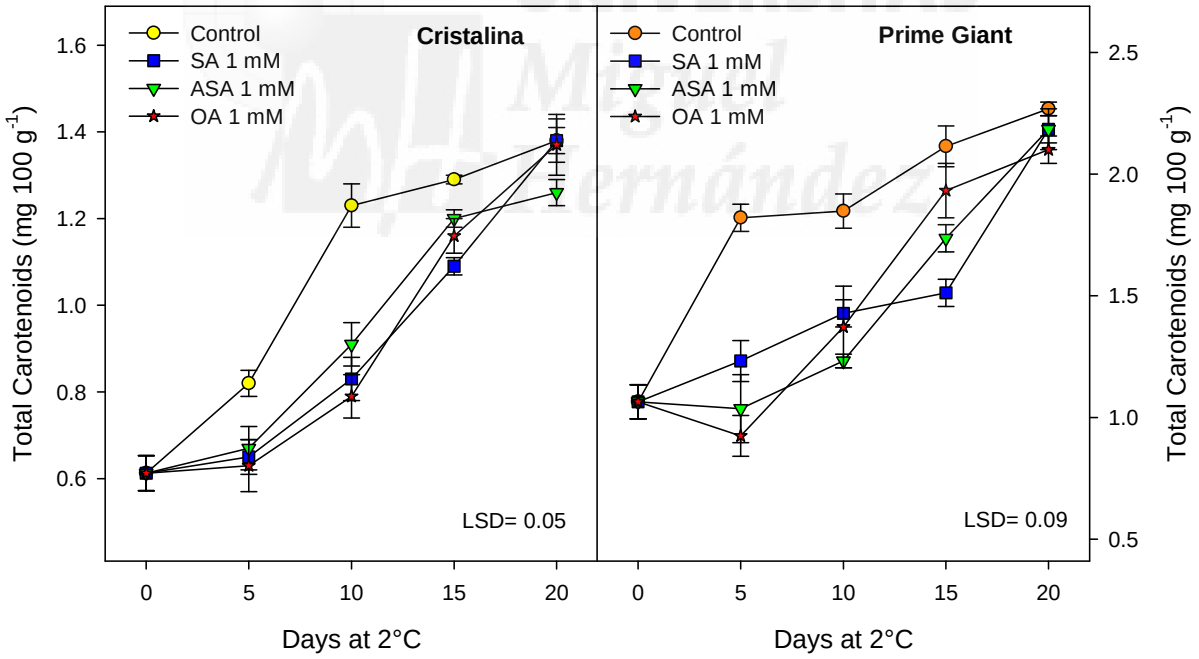

Figure 4: Total carotenoid concentration in sweet cherry 'Cristalina' and 'Prime Giant' during storage at 2 ºC in control and treated fruits with salicylic acid (SA), acetylsalicylic acid (ASA) or oxalic acid (OA). Data are the mean ± SE. LSD is shown inside figures.

Total antioxidant activity (TAA) was measured in hydrophilic (H-TAA) and lipophilic (L-TAA) fractions separately, since early reports demonstrated that the contribution of L-

TAA supposed about 20-30% of the TAA in a wide range of sweet cherry cultivars.[1,6] The change of L-TAA was similar to that obtained for carotenoids, that is, a continuous increase alongside the storage, although the application of SA, ASA or OA induced a significant delay in the increase of L-TAA (**Figure 5**). In fact, a high correlation was found between L-TAA and carotenoids ($y = 29.63x - 6.71$; $R^2 = 0.824$), which would indicate that carotenoids are the main lipophilic bioactive compounds contributing to L-TAA, although other lipophilic compounds such as tocopherols could also be present in sweet cherry and having a role as antioxidant moieties.[33]

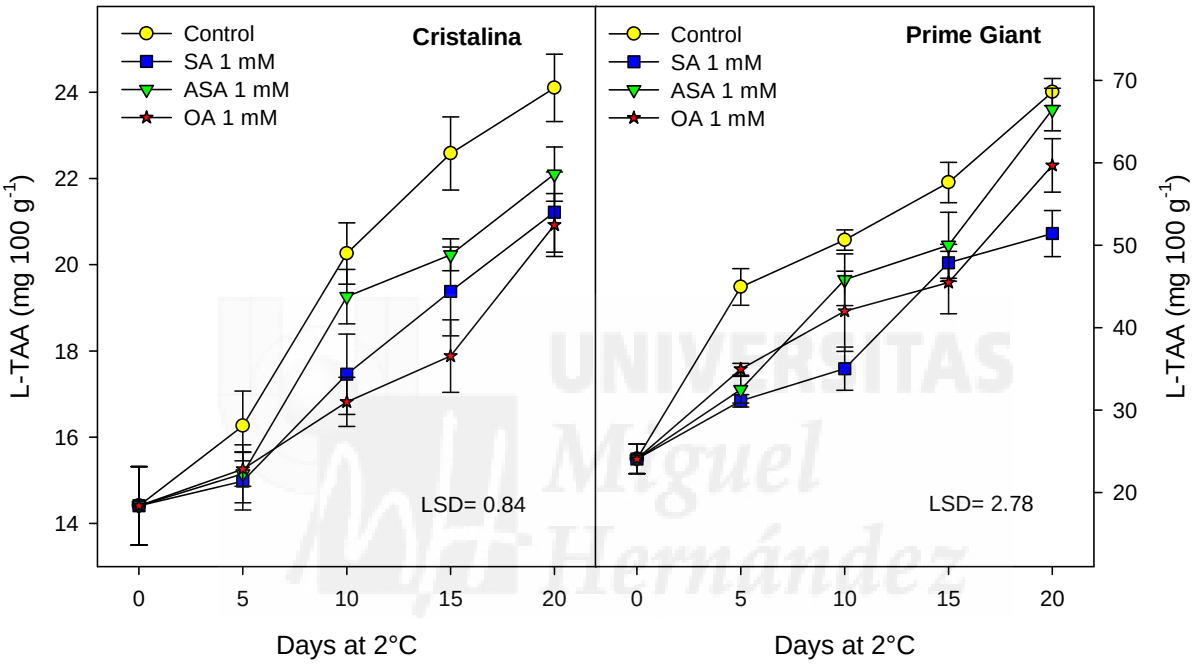

Figure 5: Lipophilic total antioxidant activity (L-TAA) in sweet cherry 'Cristalina' and 'Prime Giant' during storage at 2 ºC in control and treated fruits with salicylic acid (SA), acetylsalicylic acid (ASA) or oxalic acid (OA). Data are the mean ± SE. LSD is shown inside figures.

During storage, continuous increases occurred in H-TAA for those cherries treated with SA, ASA or OA, while in control fruits H-TAA peaked at day 10 and showed decreases after that (**Figure 6**). The pattern of H-TAA was similar to that observed for total phenolics, and therefore high correlation was found between total phenolic and H-TAA ($y = 0.51x + 16.70$; $R^2 = 0.975$), which is in agreement with previous papers reporting that phenolic compounds are the main compounds responsible for antioxidant capacity of sweet cherry,[2,4] although ascorbic acid can also contribute to this activity.[2] Early reports have demonstrated that these treatments have also effects on antioxidant enzymes. For example, increases in catalase, peroxidase, superoxide dismutase and ascorbate peroxidase have been found in fruits such as banana, sugar apple fruit and mango after

SA, ASA or OA treatment.[9,10,12] Moreover, in kiwifruit and loquat, ASA application delayed the increase in lipoxygenase associated with senescence leading to a lower production of superoxide free radicals.[11,34] During storage of these fruits, a decline in the content of free SA occurred, and thus exogenous treatments with SA or ASA led to increase its endogenous concentration and delaying of the ripening process.

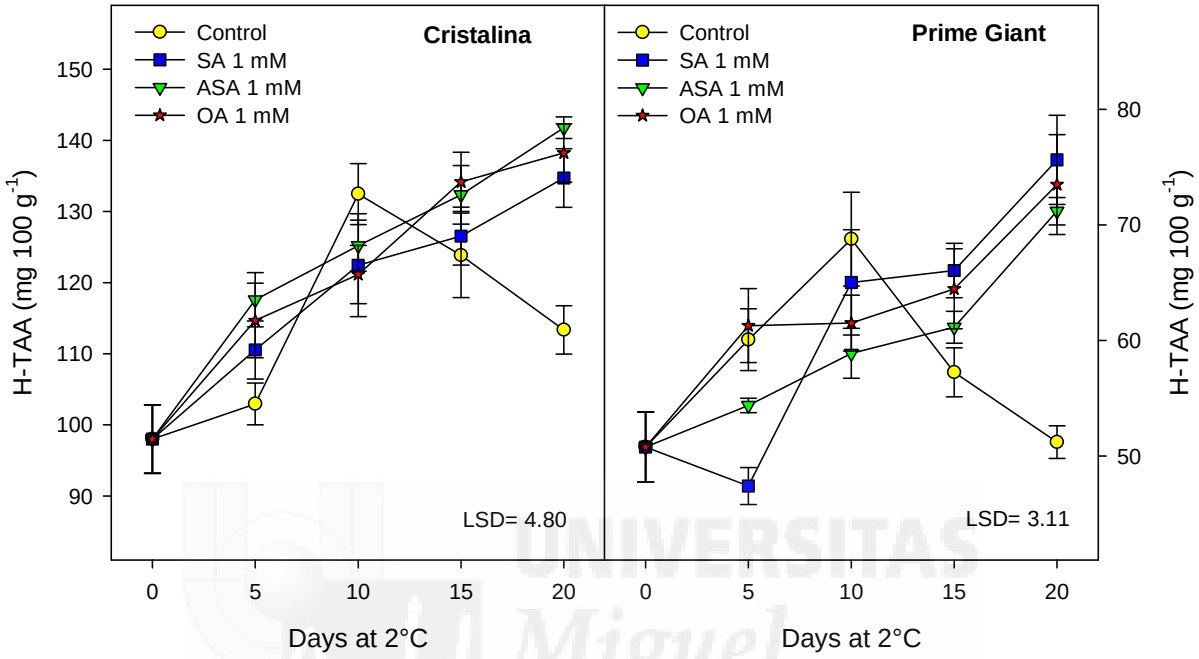

Figure 6: Hydrophilic total antioxidant activity (H-TAA) in sweet cherry 'Cristalina' and 'Prime Giant' during storage at 2 ºC in control and treated fruits with salicylic acid (SA), acetylsalicylic acid (ASA) or oxalic acid (OA). Data are the mean ± SE. LSD is shown inside figures.

Given the relationship between L-TAA-carotenoids and H-TAA-phenolics, it could be concluded that phenolic compounds, including anthocyanins, and carotenoids could be responsible for the health-beneficial effects after sweet cherry consumption in relation to its reported effect on reducing the risk of several diseases, such as cancer, diabetes, Alzheimer's and cardiovascular diseases.[35] The application of natural compounds as postharvest tools, such as SA, ASA or OA, resulted in delayed ripening rates of sweet cherry during storage, and maintained higher contents of bioactive compounds and antioxidant activity as compared with control fruit. Thus, control fruit could be stored for 10 days while this period was extended up to 20 days in treated cherries, without significant differences among treatments.

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Chapter 8

Alginate Coatings Preserve Fruit Quality and Bioactive Compounds During Storage of Sweet Cherry Fruit

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Alginate Coatings Preserve Fruit Quality and Bioactive Compounds During Storage of Sweet Cherry Fruit

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Abstract

Sweet cherry fruits harvested at commercial maturity stage were treated with an edible coating based on sodium alginate at several concentrations (1, 3 or 5% w/v). The coatings were effective on delaying the evolution of the parameters related to postharvest ripening, such as colour, softening and loss of acidity, and reducing respiration rate. In addition, the edible coatings showed a positive effect on maintaining higher concentration of total phenolics and total antioxidant activity, which decreased in control fruits associated with the over-ripening and senescence processes. Results from quality parameters and antioxidant activity suggested that the maximum storability period for control fruits was 8 days at 2°C plus 2 days at 20°C, while alginate-coated cherries could be stored with optimal quality and enhanced antioxidant activity up to 16 days at 2°C plus 2 days at 20°C.

Keywords: *Prunus avium* L. • Edible coating • Quality • Anthocyanins • Phenolics • Antioxidant activity

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Running head: Sweet cherry coated with alginate

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INTRODUCTION

Sweet cherry is one of the most appreciated fruits by consumer due to its precocity and excellent quality. Among the factors determining the consumer's acceptability total soluble solids (TSS), acidity and colour are the most important with great differences among cultivars (Crisosto et al., 2003; Díaz-Mula et al., 2009a). Red colour intensity in sweet cherry is used as indicator of quality and ripening, which is related to both accumulation and anthocyanin profile (Serrano, et al., 2005; Serrano et al., 2009).

Nowadays, inverse associations between fruit and vegetable intake and chronic diseases, such as different types of cancer and cardiovascular disease, have been demonstrated in numerous epidemiological studies, in which phytochemicals have been indicated to be responsible for this observed protective effect (Schreiner and Huyskens-Keil, 2006). Among these compounds, special interest has been focused on anthocyanins and other polyphenolics, carotenoids and vitamins C and E. In cherry, antioxidant potential has been associated with ascorbic acid and phenolics, the two dominant polyphenols being caffeoyltartaric acid and 3-*p*-coumaroyl-quinic acid, while the major anthocyanins are cyanidin 3-rutinoside and cyanidin 3-glucoside followed by pelargonidin-3-rutinoside, peonidin-3-rutinoside, and peonidin-3-glucoside which are found at lower concentrations (Chaovanalikit and Wrolstad, 2004; Serrano et al., 2005; 2009).

Sweet cherry fruits deteriorate rapidly after harvest and in some cases do not reach consumers at optimal quality after transport and marketing. Thus, adequate postharvest technologies to be combined with cold storage are needed. In this sense, in recent years, there is an increasing interest in the use of edible coating to maintain fruit quality with the additional benefit of reducing the volume of non-biodegradable packaging materials (Campos et al., 2010). Such edible coatings act as physical barriers on fruit surface and decrease its permeability to O_2, CO_2 and water vapour, leading to reductions in respiration rate and transpiration and to a retard in the natural physiological ripening process. Thus, maintenance of fruit quality has been achieved by using some edible coatings such as chitosan in peach (Li and Yu, 2001; Ruoyi et al., 2005), methylcellulose in avocado (Maftoonazad and Ramaswany, 2005) and in apricot (Ayranci and Tunc, 2004), hydroxypropylmethylcellulose in plum (Navarro-Tarazaga et al., 2008) and whey protein in plum (Reinoso et al., 2008).

Alginate is a natural polysaccharide extracted from brown sea algae (*Phaeophyceae*) and it is composed of two uronic acids: ß-D-mannuronic acid and α-L-guluronic acid. Sodium alginate is composed of block polymers of sodium poly(L-guluronate), sodium poly(D-mannuronate), and alternating sequences of both sugars. Alginate is known as a hydrophilic biopolymer that has a coating function because of its well-studied unique colloidal properties, which include its use for thickening, suspension forming, gel forming, and emulsion stabilizing (Acevedo et al., 2010). Sodium-alginate has

been effective on maintaining postharvest quality of tomato (Zapata et al., 2008) and peach (Maftoonazad et al., 2008).

In sweet cherry some beneficial effects in terms of maintaining fruit quality have been obtained with some edible coating based on chitosan (Romanazzi et al., 2003) and *Aloe vera* gel (Martínez-Romero et al., 2006) as well as with Semperfresh™, an edible coating composed of sucrose esters of fatty acids, sodium carboxy-methylcellulose and mono-diglycerides of fatty acids (Yaman and Bayindırlı, 2001; 2002). However, these authors showed an increase in fungal spoilage in Semperfresh coated cherries. In addition, sweet cherries coated with Food Coat®, an edible coating composed of derivatives of fatty acids an polysaccharides in alcohol solution (Domca, Granada, Spain) were shiner, more turgid and more attractive than controls after storage, although no significant effects were observed on the evolution of parameters related to fruit quality such as colour and firmness (Alonso and Alique, 2004).

Although literature exists about the effect of several edible coatings on fruit organoleptic quality, as far as we know there is no information on the impact of coatings on the changes in bioactive compounds and antioxidant activity during postharvest storage of fruits. In addition, the use of alginate, as edible coating to preserve fruit quality during postharvest storage of sweet cherry is investigated for the first time in this paper. Thus, the aim of this study was to analyse the effect of sodium alginate applied as edible coating at three concentrations (1, 3, 5 % w/v) on quality, bioactive compounds and antioxidant activity on 'Sweetheart' cherry cultivar during storage.

MATERIAL AND METHODS

Plant material and experimental design

Sweet cherry (*Prunus avium* L. Cv. 'Sweetheart') fruits were harvested on year 2009 from a commercial plot (Finca Los Frutales, Villena, Alicante, Spain). Fruits were picked at commercial maturity stage, with a score of 4 of the colour chart from Centre Technique Interprofessionel de Fruits et Légumes (CTIFL, Paris), as described in previous paper (Díaz-Mula et al., 2009a), Fruits were transported immediately at laboratory and then 111 homogenous lots (based on colour and size) of 10 fruits each were performed at random. Three lots were used to determine the fruit properties at harvest (Day 0) and the 108 remained were split into 4 groups for the following treatments in triplicate: 0 (control), 1, 3 and 5 % (w/v) alginate coating. Alginate (alginic acid sodium salt from brown algae purchased from Sigma, Madrid, Spain) was prepared according to previous paper (Zapata et al., 2008) at different concentrations (1, 3, and 5% w/v) dissolved in hot water (45°C) with continuous shaking until solution became clear. After cooling to 20°C, glycerol at 20% v/v was added as plasticiser, and treatments were performed by dipping the fruits twice in fresh coating solution for 1 min to assure the uniformity of the coating

of the whole surface. After dipping, fruits were dried for 30 min under air-flow heater at 25°C. Control fruits were dipped in distilled water. After drying the lots were weighed and then 1 lot from each replicate and treatment was kept at 20°C for 2 days, and the remained in a controlled chamber at 2°C and relative humidity of 90%. After 4, 8, 12 and 16 days of cold storage at 2°C, 2 lots from each replicate and treatment were taken at random; 1 was analysed immediately and the remained lot was analysed after 2 days at 20°C (shelf life, SL).

Respiration rate was measured in each lot and colour and firmness individually in each fruit. Then, the pulp of each lot was cut in small pieces and combined to obtain a homogeneous sample from each lot, from which 5 g were used to quantify total acidity (TA) and total soluble solids (TSS) in duplicate, and the remained frozen in liquid N_2, milled and stored at -20°C for total phenolics, total anthocyanins and total antioxidant activity determinations, all of them made also in duplicate for each of the 3 replicates.

Fruit quality parameters

Weight loss was determined in each lot by percentage of weight loss with respect to day 0. Texture was determined in each fruit using a TX-XT2i Texture Analyser (Stable Microsystems, Godalming, UK) interfaced to a PC. Force-deformation ratio (whole fruit firmness) was measured using a flat steel plate mounted on the machine. For each fruit, the diameter was measured and then a force that achieved a 3% deformation of the fruit diameter was applied. Results were expressed as the ratio between the force (Newton) that provoked the 3% deformation of the fruit diameter and the fruit diameter (N mm^{-1}). A bevelled holder prevented bruising of the opposite side. Colour was determined in the equatorial perimeter at two opposite fruit zones by using a Minolta colorimeter (CRC200, Minolta Camera Co., Japan) and expressed as Hue angle.

For each sample the juice was obtained by choking with a mortar and pestle and filtrated with cheese cloth. Total soluble solids (TSS) were measured in duplicate with a digital refractometer Atago PR-101 (Atago Co. Ltd., Japan) at 20°C, and results are the means ± SE expressed in percentage (°Brix). The pH of the juice was recorded and then total acidity (TA) was determined in duplicate by potentiometric titration with 0.1 N NaOH up to pH 8.1, using 1 mL of diluted juice in 25 mL distilled H_2O and results are the means ± SE expressed as g of malic acid equivalent per 100 g^{-1} fresh weight (FW).

Respiration rate

Respiration rate was measured by placing each lot in a 0.5 L glass jar hermetically sealed with a rubber stopper for 1 h. One mL of the holder atmosphere was withdrawn with a gas syringe, and the CO_2 quantified using a ShimadzuTM 14A gas chromatograph

(Kyoto, Japan), with a thermal conductivity detector. Results were the mean of 2 determinations for each one of the replicates used and expressed as mg CO_2 kg^{-1} h^{-1}.

Total phenolics, total anthocyanins, and total antioxidant activity determination

Phenolic extraction for each sample was performed with a Polytron® homogeniser at maximum rpm using water:methanol (2:8) containing 2 mM NaF (to inactivate polyphenol oxidase activity and prevent phenolic degradation) as described by Tomás-Barberán et al. (2001) and quantified using the Folin-Ciocalteu reagent and results were expressed as mg pyrogallol equivalent 100 g^{-1} FW, both purchased from Sigma, Sigma-Aldrich, Madrid, Spain. A relative calibration procedure was performed using pyrogallol at 5-20 µg in the reaction medium, which showed linearity with the absorbance at 760 nm ($y=0.035\ x + 0.0078$; $R^2=0.995$).

Total anthocyanins were determined in duplicate according to previously reported (Serrano et al., 2005) 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 results expressed as mg 100 g^{-1} FW, and were the mean ± SE.

Total antioxidant activity (TAA) was quantified as described by Serrano et al. (2009), which enables to determine TAA due to both hydrophilic and lipophilic compounds in the same extraction. Briefly, for each sample, five grams of tissue were homogenised with a Polytron® at maximum rpm in 5 ml of 50 mM phosphate buffer pH=7.8 and 3 ml of ethyl acetate, and then centrifuged at 10,000 x *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), in which $ABTS^{\bullet+}$ radicals are generated and monitored at 730 nm. The decrease in absorbance after adding the sweet cherry 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) which showed linearity with the absorbance at 730 nm ($y=0.138\ x + 0.033$; $R^2=0.999$; for H-TAA, and $y=0.093\ x + 0.068$; $R^2=0.998$; for L-TAA) and results are expressed as mg of Trolox equivalent 100 g^{-1}. All reagents were purchased from Sigma, Sigma-Aldrich, Madrid, Spain.

Statistical analysis

Analytical data were subjected to analysis of variance (ANOVA). Sources of variation were storage time and treatment. Mean comparisons were performed using HSD

the Duncan's multiple test to examine if differences were significant at $p<0.05$. All analyses were performed with SPSS software package v. 12.0 for Windows. A summary of the statistical results is shown in Table 1.

Table 1. ANOVA for dependent variables for alginate treatments, storage time and their interactions for sweet cherry cv. Sweetheart [a]

	Time	Treatment	Time x Treatment
Colour (Hue angle)	* *	* *	* *
Fruit Firmness	* *	*	*
Total Soluble Solids	NS	NS	NS
Total Acidity	* *	* *	* *
Total Polyphenols	**	* *	*
Total Anthocyanins	**	**	*
H-TAA	**	*	*
L-TAA	NS	NS	NS

[a] **, and * represent significance at the 0.01, and 0.05 levels, respectively, and NS represents non-significance at $P< 0.05$.

RESULTS AND DISCUSSION

Sweet cherry quality parameters

It is widely accepted that the most important quality parameters determining sweet cherry acceptability by consumers are bright red colour, firmness and flavour which is mainly due to the ratio between TSS and TA, and they show important differences among cultivars and maturity stages (Crisosto et al., 2003; Usenik, et al., 2008; Díaz-Mula et al., 2009a). 'Sweetheart' cherry at harvest had a red bright colour with a Hue angle of 27.98 ± 1.26. During storage, a reduction in Hue angle was observed in control fruits, which was greater during the SL periods, with final value of 14.72 ± 1.21 in control fruits (**Figure 1**), showing an advance of the ripening process and occurrence of dark-red colour, similarly to that found in other sweet cherry cultivars (Serrano et al., 2009). These colour changes were significantly delayed in all alginate-coated fruits, since the final values after the last sampling date were significantly higher (≈20) for all coated fruits than in controls. In addition, no significant changes were observed in those fruits treated with 3 and 5% respect to colour evolution during cold storage. A sharp decrease in Hue angle would indicate an over-ripe and senescence process of control sweet cherry, which is considered as detrimental. On the contrary, the coated fruits (with higher Hue angle) maintained the typical red bright colour of recently harvested fruits even after 16 days of cold storage, especially with alginate at 3 or 5 %.

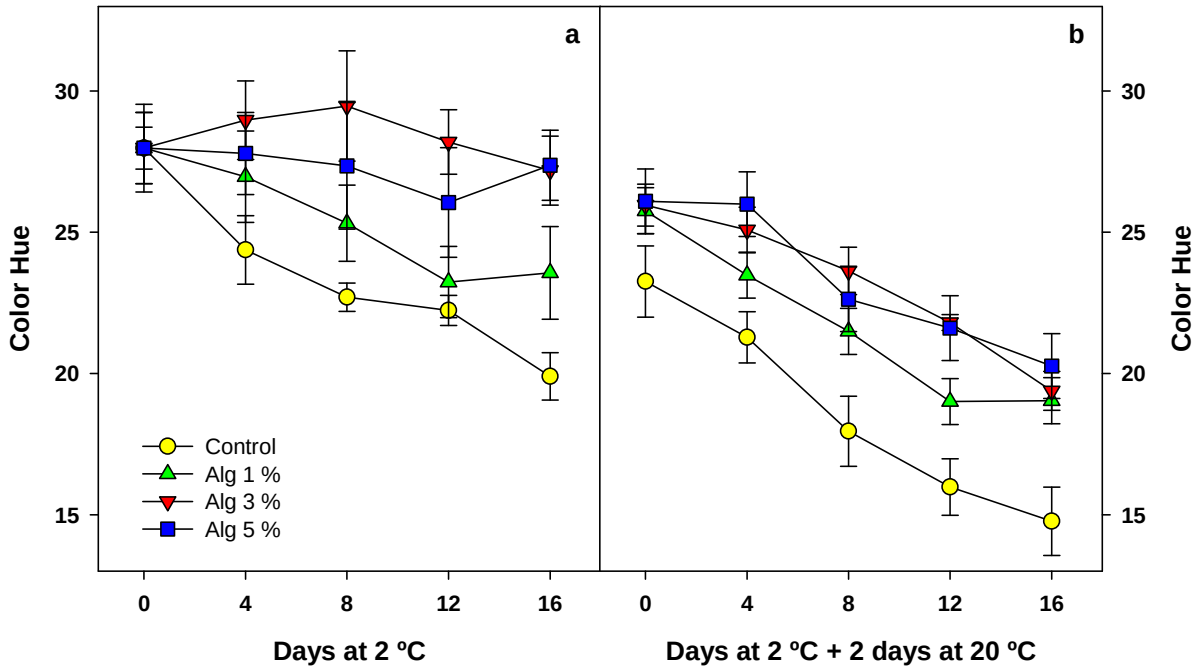

Figure 1: Evolution of colour (Hue angle) during cold storage (A) or after cold storage + 2 days at 20°C (SL) (B) on sweet cherry fruits coated with 1, 3 or 5% alginate (Alg). Data are the mean ± SE (n=30).

Any symptom of physiological disorders were observed for both control an treated fruits at any of the alginate concentration applied during the 16 days of cold storage or after further shelf life at 20°C. In addition, no decay symptoms were visually observed in any fruit (either in control or treated). On the contrary, it has been reported occurrence of decay during storage in other sweet cherry cultivars, which could be attributed to the different assayed varieties or environmental conditions during the growth cycle. Moreover, different effects of the edible coatings on cherry decay development were shown, since Semperfresh™ increased spoilage while chitosan positively reduced the incidence of this disorder (Yaman and Bayindırlı, 2001; Romanazzi et al., 2004).

Weight loss increased during storage, reaching values or 6.81 ± 0.08% in control fruits after 16 days of cold storage and significantly lower, 5.93 ± 0.12, 4.88 ± 0.15 and 3.71 ± 0.09 % in those coated with alginate at 1, 3 and 5 %, respectively, these values increasing up to 13.77 ± 0.30 in control fruits after two days more at 20 ºC and up to 11.22 ± 0.31, 10.41 ± 0.30 and 8.80 ± 0.12 % in those treated with alginate at 1, 3 and 5 %, respectively. Weight loss of fruits is due to the gradient of water vapour pressure between the fruit and the surrounding air, which is usually reduced by both epidermal cell layer and cuticle. However, edible coating acts as an extra layer which also coats the stomata leading to a decrease in transpiration and in turn, to a reduction in weight loss, this being the primary beneficial effect of edible coatings, as has been demonstrated in a wide range

of fruits including apricot, pepper, peach, sweet cherry and avocado, among others (Yaman and Bayindırlı 2002; Ayranci and Tunc, 2004; Maftoonazad and Ramaswamy, 2005; Maftoonazad et al., 2008). Moreover, differences in the ability of reducing weight loss are attributed to the different water vapour permeability of the compounds used in the formulation of the edible coating (Vargas et al., 2008). These barrier properties also reduce the selective permeability to O_2 and CO_2 of the fruit surface leading to an increase in CO_2 concentration in the fruit tissues and a decrease in O_2 concentration, which could be responsible for the reduced respiration rate in the alginate-coated fruits. Thus, respiration rate, as measured by CO_2 production, after different periods of cold plus 2 days at 20°C was lower in alginate-coated fruits with respect to control ones, especially with the highest alginate concentration (**Figure 2a**), in agreement with previous reports in peaches coated with chitosan, methylcellulose or alginate (Li and Yu, 2001; Maftoonazad et al., 2008). However, during cold storage respiration rate was very low, between 10 and 15 mg CO_2 kg^{-1} h^{-1}, and no differences were found among control and coated cherries (data not shown).

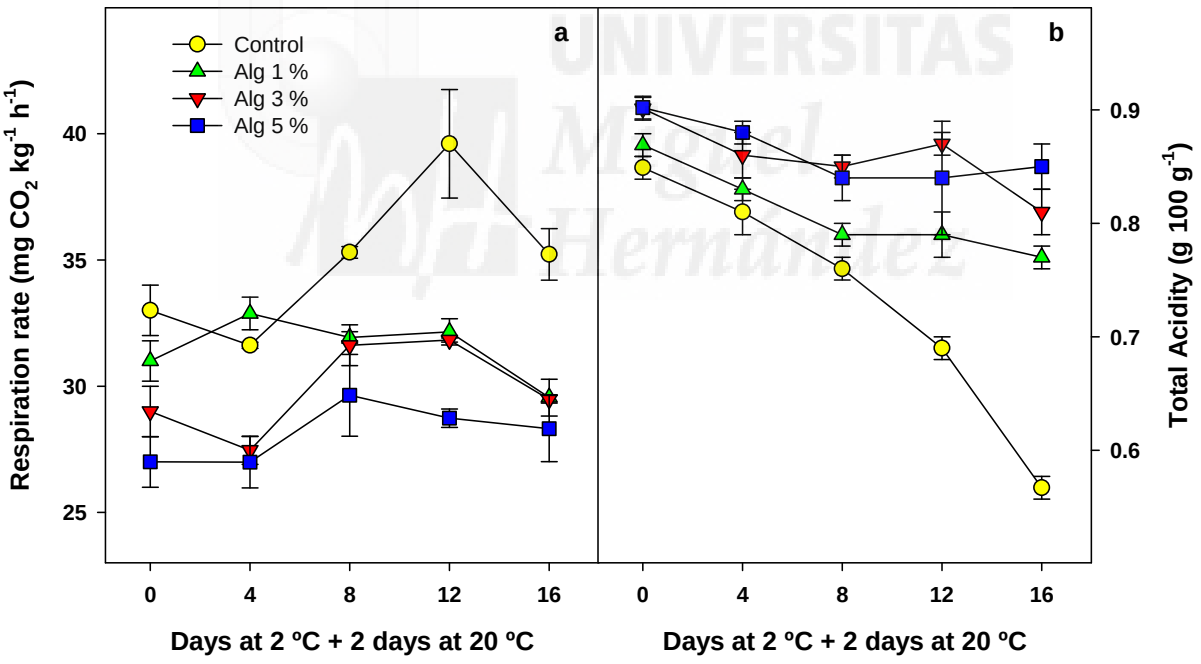

Figure 2: Respiration rate (A) and total acidity (B) evolution during several cold storage periods + 2 days at 20°C on sweet cherry fruits coated with 1, 3 or 5% alginate (Alg). Data are the mean ± SE (n=3).

TSS at harvest was 19.43 ± 0.31 °Brix and slightly increased over storage with final concentration of ≈21 °Brix without significant effect of alginate-treatments (data not shown), which could related to the loss of weight during storage. On the contrary, the

alginate coatings were effective in delaying the loss of TA, which occurred either during cold storage (data not shown) or after 20°C in control fruits, with values decreasing from 0.91 ± 0.01 g 100 g^{-1} at harvest to 0.57 ± 0.01 g 100 g^{-1} in control fruits after 16 days at 2°C + 2 days SL at 20°C, while they were significantly higher, 0.77 ± 0.01, 0.81 ± 0.02 and 0.85 ± 0.02 g 100 g^{-1} in cherries coated with 1, 3 and 5 % alginate, respectively (**Figure 2b**). Similarly, fruit softening was retarded in alginate-treated cherries while control fruits exhibited a significantly higher reduction in firmness (**Figure 3**). For this parameter, the alginate concentrations of 3 and 5% were more effective than 1% in reducing softening, especially at the last sampling date, either during cold storage or after transferring the fruits at 20°C.

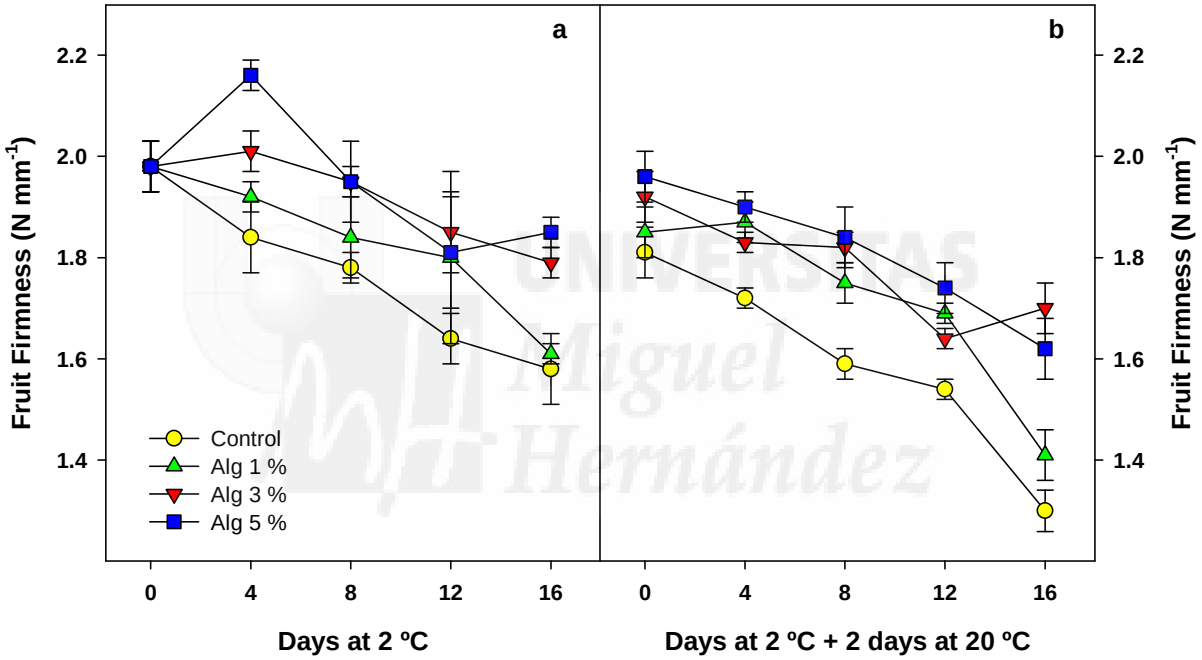

Figure 3: Evolution of fruit firmness during cold storage (A) or after cold storage + 2 days at 20°C (B) on sweet cherry fruits coated with 1, 3 or 5% alginate (Alg). Data are the mean ± SE (n=30).

The reduction of both TA and firmness during postharvest storage has been found in other sweet cherry cultivars, and associated with acceleration of the ripening process, being responsible for significant quality loses (Esti et al., 2002; Usenik et al., 2008; Serrano et al., 2009). The effects of edible coating decreasing acidity losses and softening have been also found in chitosan and alginate coated peaches (Li and Yu, 2001; Maftoonazad et al., 2008), in avocado coated with methylcellulose (Maftoonazad and Ramaswany, 2005) and in sweet cherry coated with Senperfresh (Yaman and Bayindırlı, 2002) or with *Aloe vera* gel (Martínez-Romero et al., 2006). The effect of coating on acidity retention could be a result of the lower respiration rate found in coated fruits,

since organic acids are substrates for many reactions during aerobic respiration in plant cell. By other hand, retention of firmness in coated fruits could be explained by delayed degradation of cell wall components, especially water insoluble pectin and NaOH insoluble pectins, due to the effect of the internal fruit atmosphere with high CO_2 and low O_2 on decreasing the activity of the cell wall hydrolases responsible for fruit softening (Valero and Serrano, 2010). Thus, the overall results show a delay in the postharvest ripening/maturation process in alginate-coated cherries, leading to maintenance of organoleptic and nutritive quality parameters.

Bioactive compounds and antioxidant activity

It has been reported that bioactive compounds and antioxidant activity show changes during cold storage of sweet cherry cultivars (Usenik et al., 2008; Serrano et al., 2009). In this work, 'Sweetheart' cherry also showed changes in the content of bioactive compounds and antioxidant activity but affected by treatments. Thus, total anthocyanin concentration increased either during cold storage or after 2 days at 20°C, this increase being delayed in coated fruits in a concentration dependent manner (**Figure 4**). Anthocyanin concentration at harvest was 23.53 ± 2.13 mg 100 g^{-1} and increased in control fruits until 40.39 ± 2.03 mg 100 g^{-1} after 16 days of cold storage plus SL, while these values were 37.24 ± 1.05, 32.04 ± 1.27, and 26.34 ± 1.21 mg 100 g^{-1}, for fruits coated with alginate at 1, 3, and 5%, respectively.

The anthocyanin accumulation during storage is attributed to normal sweet cherry ripening, as has been shown in other sweet cherry cultivars and commodities, such as berries and plums (Valverde et al., 2005; Mozetiĉ et al., 2006; Gonçalves et al., 2007; Díaz-Mula et al., 2009b; Serrano et al., 2009). The results show that alginate treatments delayed the ripening process, as discussed above with respect to colour results (**Figure 1**).

Uncoated fruits showed accumulation of phenolic compounds from values at harvest (49.50 ± 1.50 mg 100 g^{-1}) until day 8 of storage, either under cold conditions (74.66 ± 3.14 mg 100 g^{-1}) or 2 days further at 20°C (83.85 ± 4.10 mg 100 g^{-1}), followed by significant reduction until the end of storage. On the contrary, in coated cherries total phenolics increased continuously throughout both storage conditions but affected by alginate concentration, since alginate at 5% showed the lowest accumulation with final concentration of 73.27 ± 2.37 mg 100 g^{-1} after 16 days of cold storage plus SL (**Figure 5**).

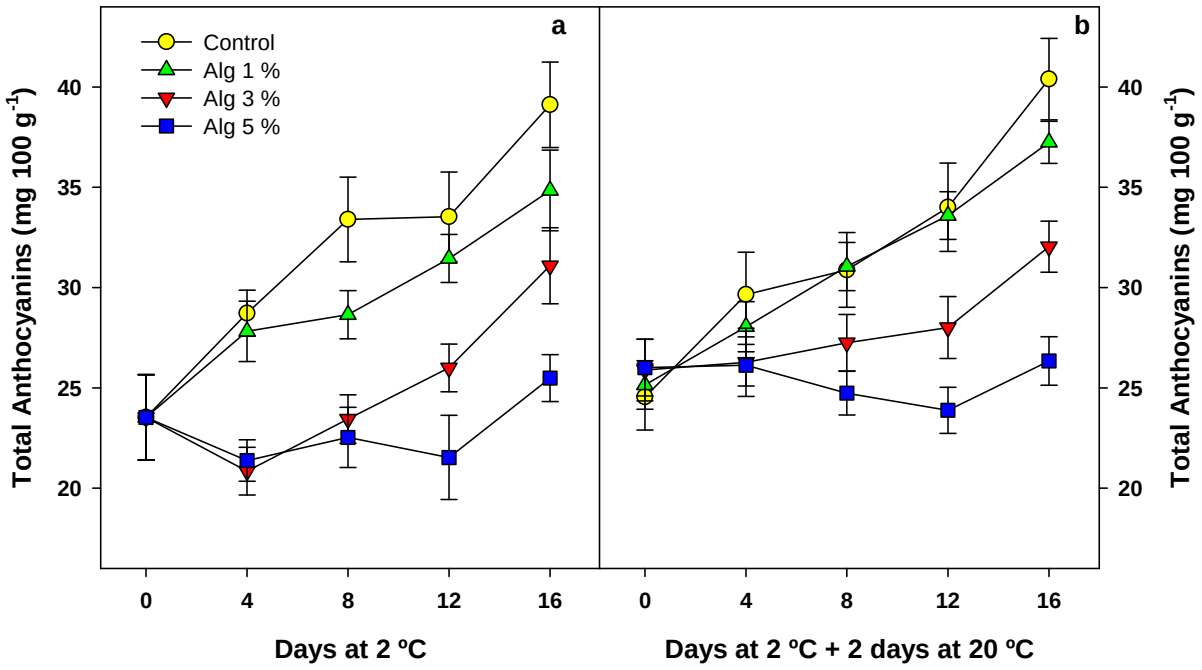

Figure 4: Evolution of total anthocyanins during cold storage (A) or after cold storage + 2 days at 20°C (B) on sweet cherry fruits coated with 1, 3 or 5% alginate (Alg). Data are the mean ± SE (n=6).

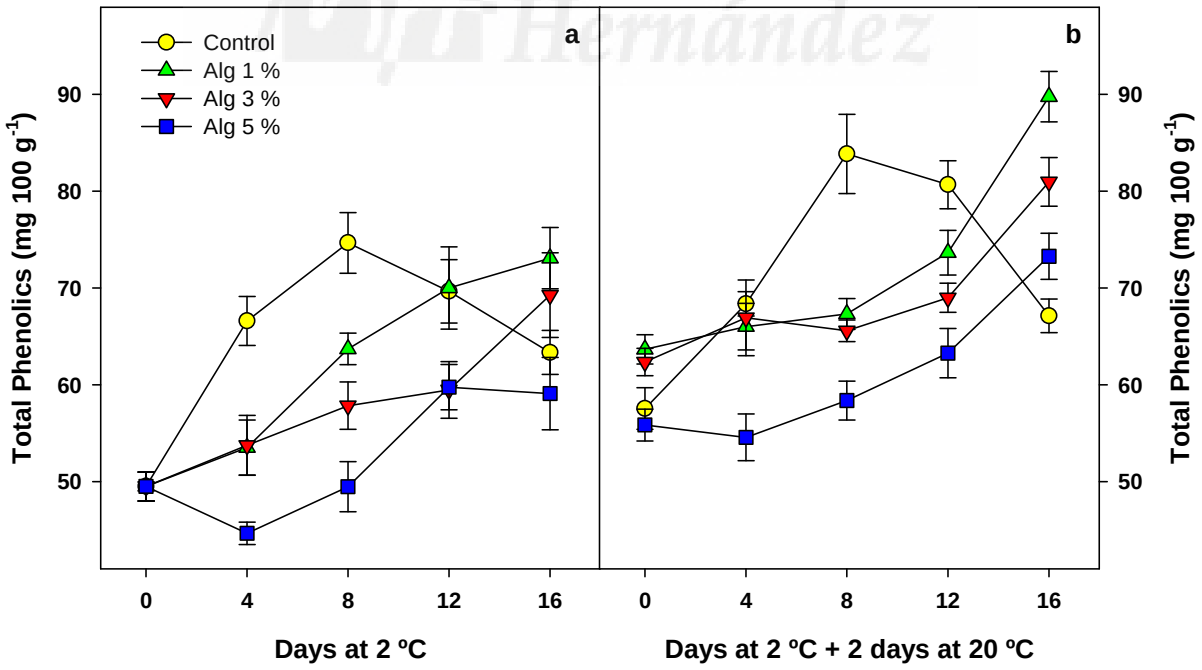

Figure 5: Evolution of total phenolics during cold storage (A) or after cold storage + 2 days at 20°C (B) on sweet cherry fruits coated with 1, 3 or 5% alginate (Alg). Data are the mean ± SE (n=6).

Since phenolic compounds contribute to fruit quality in terms of colour, taste, aroma and flavour (Tomás-Barberán and Espín, 2001) those coated fruits with higher phenolics content would have higher quality than controls. Although there are some phenolic compounds, especially tannins, that impart bitter and astringent taste and thus adversely affect the sensory properties, in 'Sweetheart' cherry the main polyphenols are the hydroxy-cinnamic acids caffeoyltartaric acid and 3-*p*-coumaroyl-quinic acid (Serrano et al., 2009), which contribute to the appreciated flavour of these fruits.

Total antioxidant activity (TAA) was quantified in hydrophilic (H-TAA) and lipophilic (L-TAA) extracts separately, the values at harvest time being 25.50 ± 1.05 mg 100 g^{-1} for H-TAA, which evolved in a similar way to that of total phenolics during cold storage and shelf life, that is progressive increases in coated fruits and decreases after 8 days of storage in control ones (**Figure 6**).

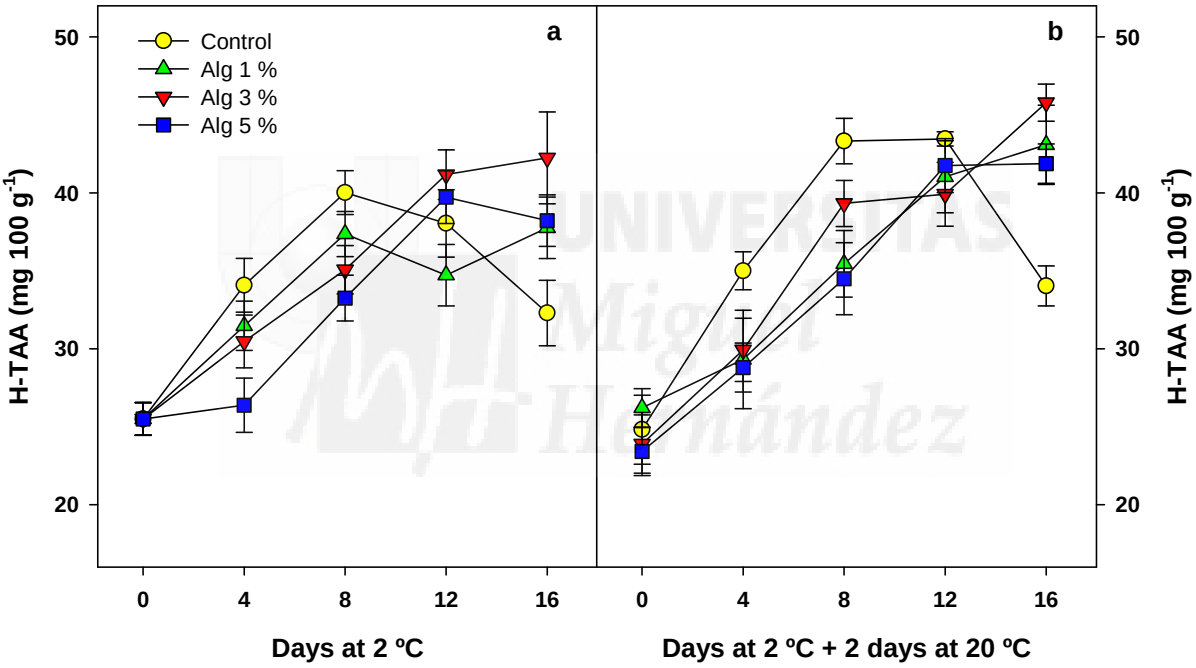

Figure 6: Evolution of total antioxidant activity (hydrophilic fraction H-TAA) during cold storage (A) or after cold storage + 2 days at 20°C (B) on sweet cherry fruits coated with 1, 3 or 5% alginate (Alg). Data are the mean ± SE (n=6).

L-TAA at harvest was 15.93 ± 1.32 mg 100 g^{-1}, and did not show significant differences attributable either storage or treatments applied (data not shown). The values of H-TAA and L-TAA show that the major contributors to antioxidant activity are hydrophilic compounds according to previous reports in other sweet cherry cultivars (Díaz-Mula et al. 2009a; Serrano et al., 2009). From these results it can be inferred that bioactive compounds and TAA increase during the ripening process, similarly that occurred during on-tree maturation, although when storage period is prolonged over-ripe and

senescence processes took place and in turn a decrease in both total phenolics and TAA occurred, as has been observed in control fruits after 12 days of storage. On the contrary, coated cherries showed an increase of these parameters during the overall storage period as consequence of the delayed ripening process by the application of alginate coating. Since the ingestion of fruits and vegetables with higher amounts of phenolics has antioxidant activity "in vivo" by increasing the plasmatic antioxidants (Fernández-Panchón et al., 2008), the use of alginate as edible coating led to fruits with higher proportion of functional properties than control ones. However, no data exist on the bioavailability and bioconversion of phenolic compounds after the intake of sweet cherry and thus more research is needed about this issue.

CONCLUSIONS

Alginate treatment is an effective tool to delay the postharvest ripening process of sweet cherry manifested by reduced colour changes, acidity and firmness losses and respiration rate, as well as a positive effect in maintaining higher concentration of total phenolics and TAA. Overall, results suggest that the maximum storability period of control fruits could be established in 8 days at 2°C plus 2 days at 20°C, while coated cherries could be stored with optimal quality and enhanced antioxidant activity up to 16 days at 2°C plus 2 days at 20°C.

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Conclusions

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In the present Thesis the evolution of the changes in organoleptic and nutritive parameters as well as bioactive compounds with antioxidant activity on plum and sweet cherry during ripening on-tree and postharvest cold storage were analysed. In addition, the effect of several postharvest treatments such as modified atmosphere packaging (MAP), dips in salicylic acid, acetylsalicylic acid or oxalic acid, and edible coatings based on alginate on the content and evolution of the above parameters was evaluated.

Plum and sweet cherry ripening on-tree

1

Plum and sweet cherry fruits exhibited a double-sigmoid growth pattern, which is typical of stone fruits, which can be followed by fruit weight determination. The main changes related to ripening started at the early stages of fruit development with important differences among cultivars.

2

Firmness increased during the first developmental stage (S1) and reached a maximum peak coinciding with the pit hardening (S2), and showing after that a softening process. At stage 3 (S3) of growth started to increase the TSS and occurred colour changes due to an increase in anthocyanin concentration in sweet cherry and red purple plum cultivars ('Angeleno', 'Black Amber', 'Black Diamond' and 'Larry Ann') or an increase in carotenoids in the yellow plum cultivars ('Golden Globe', 'Golden Japan', 'Songold' and 'TC Sun'). TA evolved in a different way in plum and sweet cherry, since a decrease was observed in all plum cultivars while an increase was shown for sweet cherry cultivars.

3

In plum cultivars ethylene production was very low (< 0.5 nl g^{-1} h^{-1}) along the growth cycle, although significant peaks were detected at S2 and S3 stages coinciding with pit lignifications and the beginning of colour changes, respectively. Finally, ethylene production increased at S4 (ripening phase) in all plum cultivars with the exception of 'Golden Japan', confirming the suppressed-climacteric pattern of this cultivar.

4

TAA in hydrophilic and lipophilic fractions increased during on-tree ripening in both plum and sweet cherry although important differences existed among cultivars, H-TAA being correlated with total phenolics and total anthocyanins while L-TAA was correlated with total carotenoids.

5

Plums or sweet cherries harvested at the commercial maturity stage do not reach their maximum concentration of bioactive compounds, since keeping them 7 days (plums) or 4 days (cherries) longer on tree led to significant increases in these compounds and the corresponding total antioxidant activity (by 10–20% on average). In order to achieve the maximum health-beneficial effects of plum and sweet cherry consumption with optimal organoleptic and nutritive properties, it would be advisable to harvest the fruits at the fully ripe stage.

Postharvest cold storage of plum and sweet cherry

6

During postharvest storage, 4 plum cultivars ('Blackamber', 'Larry Ann', 'Golden Globe' and 'Songold') showed the typical climacteric ripening pattern as did during on-tree ripening. However, 'Angeleno', 'Black Diamond' and 'TC Sun' behaved as suppressed-climacteric during postharvest storage but showed increases in ethylene production during ripening on-tree. Finally, Golden Japan' plum exhibited a suppressed-climacteric behaviour either of tree ripening or during postharvest storage. 'TC Sun' plum is described for the first time as suppressed-climacteric ripening type during storage.

7

All plum and sweet cherry cultivars showed losses in both firmness and acidity during postharvest storage, while colour was intensified (measured by decreases in Hue angle) through dark-yellow and dark-purple, for yellow and purple plum cultivars, and dark-red colour for cherry cultivars. TSS slightly increased in all fruits, with the exception of 'Golden Japan' and 'Golden Globe' plums.

8

Large variations were found among plum and sweet cherry cultivars in phytochemical concentrations and antioxidant activity. In plums, the levels of bioactive compounds and H-TAA and L-TAA were always higher in the peel than in the flesh. However, their evolution was similar, that is a continuous increase of H-TAA and of total phenolics for all plum and sweet cherry cultivars, of total anthocyanins in cherries and purple plum cultivars, and total carotenoids in the yellow plum cultivars. In sweet cherry 3 anthocyanins were detected, the major being cyanidin-3-rutinoside followed by cyanidin-

3-glucoside and pelargonidin 3-rutinoside, while the hydroxycinnamic acids derivatives neochlorogenic acid and 3'-*p*-coumaroylquinic acid were the main phenolic compounds.

9

TAA was measured for the first time in separate fractions (H-TAA and L-TAA) in both plum and sweet cherry cultivars. On a general basis, H-TAA was correlated with total phenolics and total anthocyanins, while L-TAA was correlated with total carotenoids in yellow plums. Interestingly, L-TAA contributed to 20-30% of the total capacity of sweet cherry and while accounted 30-50% in plums. Then, for a better evaluation of the antioxidant potential of plums and sweet cherry the contribution of lipophilic compounds should be taken into account, especially when comparative studies among fruits and vegetables are carried out.

Modified atmosphere packaging (MAP) in plums

10

The use of MAP maintained the quality attributes of 'Blackamber', 'Larry Ann', 'Golden Globe' and 'Songold plum cultivars by delaying the colour changes, softening, acidity loss and the increase in TSS, the effect being attributed to the reduced ethylene production in these climacteric cultivars by the increase in the CO_2 and decrease of O_2 concentrations.

11

With the use of MAP packages, the storage time with fruit having high quality attributes could be increased 3-4 weeks more as compared with control plums, especially with the film M (which had lower permeability than film H) probably due the higher inhibition of ethylene production by the use of this film.

12

This is the first time in which the effect of MAP on the changes in bioactive compounds and antioxidant activity has been studied in fruits, and results permit to conclude that MAP does not impart any negative effects on TAA and bioactive compounds, and just reflects a delay in the accumulation in total phenolics, total anthocyanins (purple plums) and total carotenoids (yellow plums) due to the above commented effect of MAP on retarding the postharvest ripening process of plums.

Salicylic acid, acetyl salicylic acid and oxalic acid in sweet cherry

13

In this Thesis the postharvest treatments with SA, ASA and OA on quality attributes and bioactive compounds with antioxidant activity have been studied for the first time. The application of these natural compounds was effective on delaying the postharvest ripening process of 'Cristalina' and 'Prime Giant' sweet cherry cultivars by reducing colour changes, softening, acidity loss and the increase in TSS.

14

Total phenolics, total carotenoids, H-TAA and L-TAA continuously increased during the 20 days of storage at 2 °C in the cherry cultivars treated with SA, ASA and OA and showing significant higher concentrations than control fruits at the end of storage, the effect of these natural compounds being related to a delay of the sweet cherry ripening process.

15

Taking into account all the parameters related to organoleptic, nutritive and antioxidant properties the cherries treated with SA, ASA or OA could be stored up to 20 days with high quality attributes for consumption, while this time was only 10 days for control fruits.

Alginate as edible coating in sweet cherry

16

The use of alginate as edible coating to preserve fruit quality (organoleptic, nutritive and bioactive compounds with antioxidant activity) is studied for the first time, and results revealed that this treatment was an effective tool to delay the postharvest ripening process of 'Sweet Heart' cherry cultivar manifested by reduced colour changes, acidity and firmness losses and respiration rate.

17

Edible coating based on alginate had also positive effects on maintaining higher concentration of total phenolics and total antioxidant activity at the end of storage.

18

Overall results suggest that the maximum storability period of sweet cherry with optimal quality could be increased two-fold in coated-fruit with respect to control ones, especially with the use of alginate at 3% concentration.

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Changes in physicochemical and nutritive parameters and bioactive compounds during development and on-tree ripening of eight plum cultivars: a comparative study

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Abstract

BACKGROUND: There is no literature on changes in organoleptic, nutritive and functional properties during plum development and on-tree ripening. In this work a comparative study on the evolution of physical, chemical and nutritive parameters and bioactive compounds of eight plum cultivars (yellow and dark-purple) was performed.

RESULTS: The main changes related to ripening (colour, total soluble solids, acidity, firmness and bioactive compounds) started at the early stages of fruit development, with significant differences among cultivars. Colour hue angle was highly correlated with increase in anthocyanins or carotenoids (in both skin and flesh). Total antioxidant activity (TAA) was determined in the hydrophilic (H-TAA) and lipophilic (L-TAA) fractions separately, and values were always higher in the skin than in the flesh. A continuous increase in both H-TAA and L-TAA during the process of ripening occurred. H-TAA was about twofold higher than L-TAA in the dark-purple cultivars, while the opposite was found in the yellow cultivars. In addition, H-TAA was correlated with total phenolics and total anthocyanins, while L-TAA was positively correlated with total carotenoids.

CONCLUSION: In order to achieve the optimal organoleptic, nutritive and health-beneficial properties of plum consumption, it would be advisable to harvest the fruits at the fully ripe stage.

Keywords: fruit growth; harvest; anthocyanins; carotenoids; polyphenols; antioxidant activity

INTRODUCTION

Plum fruit is one of the most important commodities consumed worldwide owing to its degree of acceptance by consumers. For plums and other related *Prunus* species the degree of acceptance depends on organoleptic properties such as colour, texture, flavour and aroma,[1,2] which vary among cultivars and production areas and from season to season. In this regard it has been established that the ratio between total soluble solids and titratable acidity is more closely related to quality than total soluble solids or acidity alone.[3] There are a few papers describing changes related to plum ripening on tree, such as colour changes, softening, accumulation of soluble solids and decrease in acidity.[4–6] However, nowadays, other properties of fruits are gaining in importance, such as their antioxidant potential due to a wide range of bioactive compounds with health-beneficial effects via a putative role in decreasing risk of cancer development and cardiovascular, atherogenic and neurological diseases, among others. Some of these compounds are polyphenols (including flavonoids and anthocyanins), carotenoids, vitamin C and tocopherols, which are present at various concentrations in fruits and vegetables.[7–11]

Literature on the concentration of phytochemicals in a wide range of plum cultivars harvested at the commercial ripening stage shows that cultivar is a key factor determining antioxidant potential because of large differences in vitamin C, carotenoid, polyphenol and anthocyanin contents.[7,12–17] However, as far as we know, there are no studies on the evolution of bioactive compounds during the development and on-tree ripening of plums. Thus the objective of this research was to analyse the changes in organoleptic and functional properties occurring in eight plum cultivars,

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in order to establish the optimal harvest date to reach a high content of phytochemicals related to antioxidant activity, as well as their organoleptic quality.

MATERIALS AND METHODS

Plant material and experimental design

The experiment was carried out during the developmental cycle of the 2007 spring–summer period. In a commercial plot located at 'Finca Los Frutales' (Villena, Alicante, Spain), eight different plum cultivars from 15-year-old trees were selected: four yellow skinned ('Golden Japan', 'Golden Globe', 'Songold' and 'TC Sun') and four dark-purple skinned ('Angeleno', 'Black Amber', 'Black Diamond' and 'Larry Ann'). Immediately after fruit set, for each cultivar, three trees were selected and ten fruits were labelled around the equatorial perimeter of each tree. These marked fruits served to evaluate growth by measuring three linear dimensions of each fruit: polar (P, mm), suture (S, mm) and cheek (C, mm) diameters. The harvest date (H) for each cultivar (commercial harvest) was determined according to the Company's Technician and based on size, colour and total soluble solids. In addition, fruits were also kept 1 week longer on tree (H + 1).

Sampling schedule

For each sampling date, 30 similar fruits to those labelled on tree were collected at 7 day intervals and immediately transported to the laboratory for further analyses. Fruit weight, firmness and colour (skin and flesh) were determined individually for each fruit. Then, for each sampling date and cultivar, five subsamples of six fruits were taken at random for ethylene and respiration rate determination. In addition, when fruits were of an appropriate size (last six sampling dates), the skin and flesh were separated manually using a knife and cut into small pieces to obtain five homogeneous subsamples of both skin and flesh. A 5 g portion of the flesh was used for the determination of total soluble solids and titratable acidity. Finally, the skin and flesh of each subsample were immediately frozen in liquid nitrogen and milled for bioactive compound determination.

Fruit weight, firmness and colour

Fruit weight was determined using an ST-360 Gram Precision digital balance (Barcelona, Spain). Fruit firmness was measured using a TX-XT2i texture analyser (Stable Microsystems, Godalming, UK) interfaced to a personal computer, with a flat steel plate mounted on the machine. For each fruit the diameter was measured and then a force that achieved 3% deformation of the fruit diameter was applied. Results were expressed as the ratio between this force and the covered distance (N mm^{-1}). Three colour determinations were made on each fruit using the Hunter L^*, a^*, b^* system in a Minolta CR200 colorimeter (Minolta Camera Co., Osaka, Japan). Following the recording of L^*, a^* and b^* values, colour was expressed as hue angle (hue $= \tan^{-1}(b/a)$).

Ethylene production

Ethylene production was measured by placing each subsample in a glass jar and sealing the jar hermetically with a rubber stopper. After 1 h, 1 mL of the jar atmosphere was withdrawn with a gas syringe and its ethylene content was quantified using an HP 5890A gas chromatograph (Hewlett-Packard, Wilmington, DE, USA) equipped with a flame ionsation detector and a 3 m stainless steel column with an inner diameter of 3.5 mm containing activated alumina of 80/100 mesh. The column temperature was 90 °C and the injector and detector temperatures were both 150 °C. Results were expressed as nL g^{-1} h^{-1}.

Total soluble solids (TSS) and titratable acidity (TA)

TSS content in the juice obtained from each subsample of flesh tissue was determined in duplicate using an Atago PR-101 digital refractometer (Atago Co. Ltd, Tokyo, Japan) at 20 °C. Results were expressed as g kg^{-1}. The pH of the juice was recorded and its TA was then determined by potentiometric titration with 0.1 mol L^{-1} NaOH up to pH 8.1, using 1 mL of diluted juice in 25 mL of distilled water. Results were expressed as g malic acid equivalent kg^{-1}.

Bioactive compounds

Total anthocyanins were determined according to the method of García-Viguera *et al.*,[18] adapted as previously reported.[19] Total anthocyanin concentration was calculated using cyanidin-3-glucoside (molar absorption coefficient 23 900 L cm^{-1} mol^{-1}, molecular weight 449.2 g mol^{-1}) and results were expressed as mmol kg^{-1} fresh weight (FW).

Total carotenoids were extracted according to Mínguez-Mosquera and Hornero-Méndez.[20] Briefly, 1 g of skin or 2 g of flesh tissue was extracted with acetone and shaken with diethyl ether and 100 g L^{-1} NaCl to separate the two phases. The lipophilic phase was washed with 20 g L^{-1} Na_2SO_4 and saponified with 100 g L^{-1} KOH in methanol. The pigments were subsequently extracted with diethyl ether, evaporated and then made up to 25 mL with acetone. Total carotenoids were estimated by reading the absorbance at 450 nm in a Helios-α spectrophotometer (Unicam, Cambridge, UK) and expressed as μmol β-carotene equivalent kg^{-1} FW, taking $\varepsilon_{cm}^{1\%} = 2560$. Total phenolics were extracted according to Tomás-Barberán and Espín[7] using water/methanol (2:8 v/v) containing 2 mmol L^{-1} NaF and quantified using Folin–Ciocalteu reagent.[21] Results were expressed as mmol gallic acid equivalent kg^{-1} FW. For both total carotenoids and total phenolics, five replications and two technical replications per sample were conducted.

Total antioxidant activity (TAA) was also quantified in duplicate for each subsample according to the

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method of Arnao *et al.*,[22] which allows the determination of TAA due to both hydrophilic (H-TAA) and lipophilic (L-TAA) compounds in the same extract. Briefly, 1 g of skin or 2 g of flesh tissue was homogenised in 5 mL of 50 mmol L^{-1} phosphate buffer (pH 7.8) and 3 mL of ethyl acetate and then centrifuged at 10 000 × *g* for 15 min at 4 °C. The upper fraction was used for L-TAA and the lower fraction for H-TAA quantification. In both cases, TAA was determined using an enzymatic system composed of the chromophore 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), the horseradish peroxidase enzyme (HRP) and its oxidant substrate (hydrogen peroxide), in which $ABTS^{\bullet+}$ radicals are generated and monitored at 730 nm. The decrease in absorbance after adding the skin or flesh extract is proportional to TAA of the sample. Calibration curves (0–20 nmol) were constructed with Trolox ((*R*)-(+)-6-hydroxy-2,5,7,8-tetramethyl-croman-2-carboxylic acid; Sigma, Madrid, Spain) in aqueous and methanolic media for H-TAA and L-TAA quantification respectively. Results were expressed as mmol Trolox equivalent kg^{-1} FW.

Statistical analysis

Data from the analytical determinations were subjected to analysis of variance (ANOVA). Sources of variation were cultivar and developmental sampling date. Mean comparisons were performed using Tukey's highest significant difference (HSD) test to examine if differences were significant at $P < 0.05$, $P < 0.01$ or $P < 0.001$. Linear regressions (skin or flesh) were performed between colour hue angle and anthocyanin or carotenoid concentration as well as between functional compounds and H-TAA or L-TAA. The regressions were carried out for each cultivar individually and taking into account data for all cultivars and sampling dates. All analyses were performed with the SPSS Version 12.0 (SPSS Inc., Chicago IL, USA) for Windows software package.

RESULTS AND DISCUSSION

Changes in parameters related to fruit growth and ripening

Important changes occurred in the physiological and physicochemical parameters of all plum cultivars during development and on-tree ripening (Tables 1 and 2). As an example, Fig. 1 shows the results for 'Golden Globe'. The parameters related to fruit growth (volume, polar, suture and cheek diameters and fruit weight) followed a double-sigmoid pattern (data not shown), which is typical for *Prunus* species, in which four distinct stages (S1–S4) could be established according to fruit weight evolution. S1 is the first exponential growth phase and characterised by cell division and elongation. S2 shows little or no fruit growth, but the endocarp hardens to form a solid stone. S3 is the second exponential growth phase due to cell enlargement, while in S4 the fruit ripening or climacteric stage occurs.[5,23,24] However, fruit weight at commercial harvest was cultivar-dependent and no significant differences were found when fruits were kept 1 week longer on tree (H + 1) (Table 1). In addition, although the full blossom dates were very close among cultivars (from 2 to 14 March), the harvest date ranged from 4 July for 'Golden Japan' to 6 September for 'Angeleno', and, in turn, the necessary days to achieve the commercial ripening stage differed greatly among cultivars, from 112 to 185 days for 'Golden Japan' and 'Angeleno' respectively (data not shown).

At the beginning of each phase the occurrence of ethylene peaks was detected, although its physiological role is markedly different. Thus at S1 ethylene is associated with cell division, at S2 with pit hardening due to stimulation of lignin synthesis by increasing phenylalanine ammonia lyase (PAL) activity, at S3 with colour changes and at S4 with climacteric ethylene production. This confirms the climacteric ripening pattern for most plum cultivars and the role of ethylene as the plant hormone responsible for the acceleration of physicochemical changes during fruit ripening.[25–27] However, no

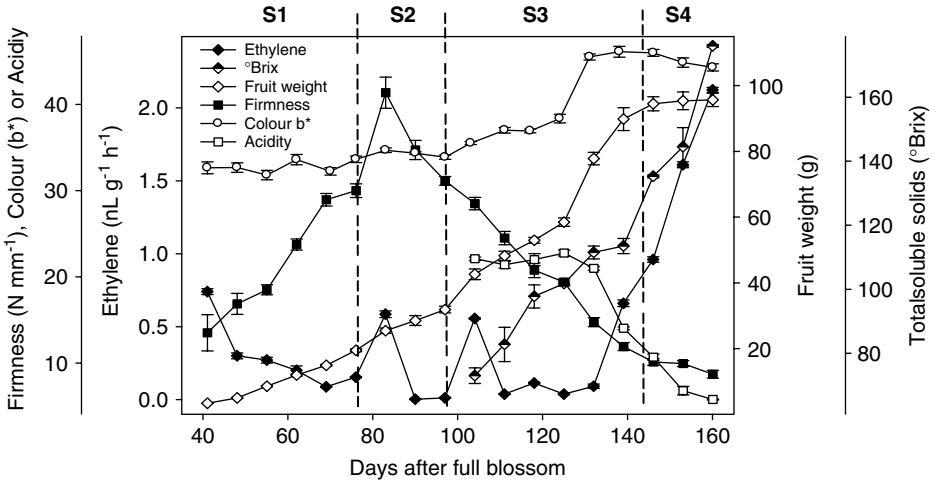

Figure 1. Evolution of some physical, chemical and physiological parameters during fruit development and ripening on tree of 'Golden Globe' plum cultivar. Data are mean ± standard error. S1–S4 denote developmental stages according to fruit weight evolution.

Table 1. Fruit properties at commercial harvest date (H) and after 1 week of further ripening on tree (H + 1) for eight plum cultivars

	Fruit weight (g)		Fruit firmness (N mm^{-1})		TSS (g kg^{-1})		TA (g kg^{-1})	
Cultivar	H	H + 1	H	H + 1	H	H + 1	H	H + 1
Golden Japan	59.2 ± 1.7aA	59.9 ± 2.9aA	7.2 ± 0.5aA	5.7 ± 0.3bA	106 ± 1aA	109 ± 1aA	16.0 ± 0.1aA	13.9 ± 0.4bA
Black Amber	117.4 ± 1.3aB	120.0 ± 2.3aB	6.7 ± 0.4aA	5.6 ± 0.2bA	131 ± 1aB	150 ± 1bB	12.5 ± 0.2aB	8.6 ± 0.1bB
Black Diamond	119.5 ± 2.5aB	120.0 ± 1.8aB	9.9 ± 0.3aB	7.8 ± 0.5bB	124 ± 1aC	140 ± 1bC	7.4 ± 0.1aC	5.7 ± 0.1bC
Golden Globe	95.4 ± 2.7aC	95.7 ± 2.0aC	9.9 ± 0.3aB	8.7 ± 0.4bB	145 ± 2aD	176 ± 1bD	6.8 ± 0.5aC	5.8 ± 0.1bC
TC Sun	113.8 ± 3.1aB	113.0 ± 3.1aB	8.9 ± 0.2aC	8.1 ± 0.2bB	158 ± 1aE	167 ± 2bE	7.5 ± 0.1aC	7.3 ± 0.1aD
Larry Ann	97.1 ± 4.3aC	98.0 ± 2.5aC	8.3 ± 0.2aC	5.4 ± 0.5bA	146 ± 1aD	149 ± 2aB	10.5 ± 0.1aD	7.2 ± 0.1bD
Songold	148.4 ± 4.9aD	148.6 ± 4.0aD	6.2 ± 0.2aA	5.7 ± 0.1bA	134 ± 2aB	140 ± 2bC	8.8 ± 0.4aE	7.2 ± 0.3bD
Angeleno	89.0 ± 2.0aC	88.9 ± 2.9aC	9.5 ± 0.2aB	8.7 ± 0.2bB	137 ± 1aB	139 ± 1aC	10.2 ± 0.6aD	8.4 ± 0.2bB

For each parameter and cultivar, different lowercase letters indicate significant differences at $P < 0.05$ between harvest dates. For each parameter and harvest date, different capital letters indicate significant differences at $P < 0.05$ among cultivars.

increase in climacteric ethylene production was found in 'Golden Japan' during S4 (data not shown), confirming other reports that this cultivar is of the suppressed climacteric type during both on-tree development and postharvest storage,[5,28] as also are 'Shiro' and 'Rubyred' cultivars.[4] At transcriptomic level, such differences within plum cultivars have been attributed to differences in the accumulation patterns of mRNAs implicated in ethylene perception and signal transduction components.[24]

Differences in fruit firmness, TSS and TA were found among cultivars (Table 1), with values being within the ranges reported for other plums.[1,3,6,29] However, no relationship between TSS or TA and harvest season can be established, although there is a tendency for early season plums to have lower TSS than late season plums.[3] For consumers the levels of TSS in plums have special importance for their acceptability, since TSS has been correlated with perception of sweetness, flavour and aroma. In this regard, plums harvested at H + 1 would be more appreciated by consumers, since higher levels of TSS and lower TA are achieved for all cultivars (Table 1).

For comparative purposes, colour hue angle was chosen to show the changes in both external and flesh colour during fruit development and on-tree ripening. For all cultivars, external hue angle remained high (120–115°) between S1 and the beginning of S3, which means that the plums had a dark-green colour. From this moment, hue angle decreased sharply in the purple/black-pigmented cultivars, while in the yellow-pigmented cultivars the decrease was slower (Fig. 2). For flesh colour the same behaviour was detected, although the decrease in hue angle occurred at the later developmental stages (Fig. 3). Intriguingly, flesh hue angle in 'Black Diamond' reached values of ~20° at the ripe stage, which indicates a purple flesh, while the other dark-purple cultivars had higher hue angle values owing to their orange/yellow flesh.

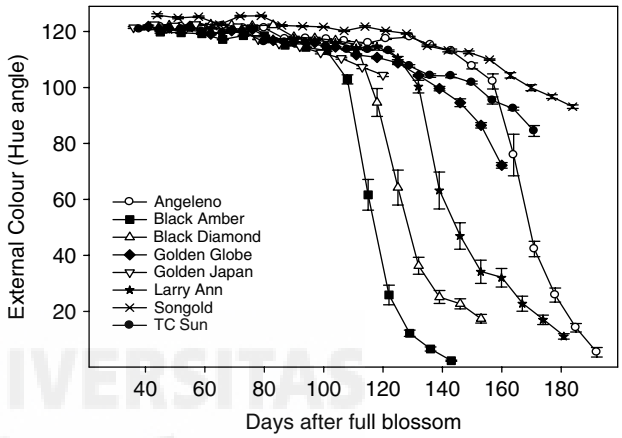

Figure 2. Evolution of external colour (hue angle) during fruit development and ripening on tree of eight plum cultivars. Data are mean ± standard error.

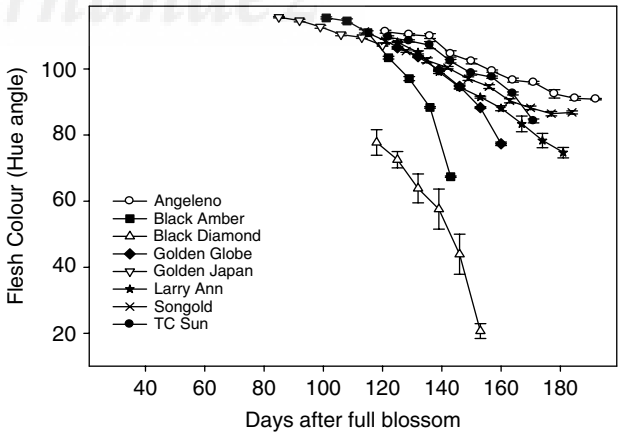

Figure 3. Evolution of flesh colour (hue angle) during fruit development and ripening on tree of eight plum cultivars. Data are mean ± standard error.

Changes in bioactive compounds

In the dark-purple cultivars the levels of anthocyanins were analysed in both skin and flesh. The results revealed that anthocyanins started to accumulate first in the skin, then in the flesh, and showed a continuous increase until the last sampling date (H + 1), although significant differences ($P < 0.001$) were found among cultivars. For all cultivars the anthocyanin concentration was significantly higher (~50-fold) in the skin than in the flesh. Among cultivars, 'Black Amber' and 'Black Diamond' had the highest (18.89 ± 0.67 mmol kg^{-1}) and lowest (5.93 ±

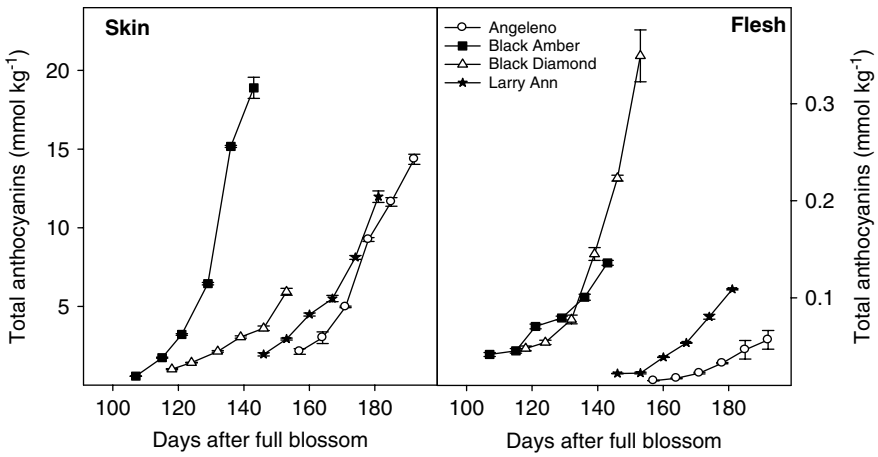

Figure 4. Evolution of total anthocyanin content in skin and flesh during last 6 weeks of fruit development and ripening on tree of four dark-purple plum cultivars. Data are mean ± standard error.

0.22 mmol kg^{-1}) skin anthocyanin concentrations respectively. In the flesh, these values ranged between 0.06 and 0.36 mmol kg^{-1} for 'Angeleno' and 'Black Diamond' respectively (Fig. 4). The anthocyanin concentrations are within the range reported for other red/purple plum cultivars.[7,16,17,30] The main anthocyanin in plum cultivars is cyanindin-3-glucoside, followed by cyanindin-3-rutinoside, while peonidin derivative and peonidin-3-glucoside are found in minor concentrations.[7,14] For all cultivars, in both skin and flesh, total anthocyanins were negatively correlated ($r^2 = 0.86–0.99$) with hue angle (Table 3), showing that these pigments are responsible for the colour changes associated with ripening. However, the correlations were exponential in the skin, indicating that higher anthocyanin accumulation occurred during the last 2 weeks of ripening, while the linear correlations in the flesh showed a continuous increase during on-tree ripening.

For all plum cultivars the levels of total carotenoids were determined in both skin and flesh. Similarly to anthocyanins, total carotenoids in both skin and flesh reached their maximum concentration at the last sampling date, although they started to accumulate earlier (Fig. 5). However, the absolute concentration of total carotenoids was cultivar-dependent, with the highest skin carotenoid concentration among the yellow-pigmented plums being found in 'Golden Globe' (141.93 ± 3.57 μmol kg^{-1}) and the lowest in 'Golden Japan' (32.04 ± 0.75 μmol kg^{-1}). For these cultivars a similar behaviour was found for total carotenoids in the flesh. Interestingly, 'Black Amber' and 'Larry Ann' (dark-purple plums) also showed high levels of total carotenoids in both skin and flesh (~150 and ~20 μmol kg^{-1} respectively), close to those found in 'Golden Globe'. A high linear correlation was found between total carotenoids and colour hue angle for the yellow-pigmented cultivars (Table 3) in both skin and flesh ($r^2 = 0.75–0.99$), showing that carotenoids are the main compounds responsible for the colour changes from green to orange/yellow occurring during plum ripening. Among the carotenoids, β-carotene has been found as the major carotenoid pigment in the skin and flesh of yellow or dark-purple plums, while β-cryptoxanthin occurred at much lower concentrations.[12,31] It is interesting to point out that, among the yellow plum cultivars assayed in this study, 'Golden Globe' had a high carotenoid concentration compared with other yellow cultivars such as 'Wickson',[12] 'Golden Japan', 'TC Sun' and 'Songold'. In a study of 45 (light-red and red flesh) plum genotypes, carotenoids were also present,[17] with concentrations similar to those reported for 'Black Amber' and 'Larry Ann' in the present work.

The content of total phenolics showed a similar pattern for all cultivars, with progressive increases throughout the ripening process (Fig. 6). In addition, the concentration of total phenolics was about threefold higher in the skin than in the flesh, although important differences were found among cultivars. At the last sampling date the final concentration of total phenolics in the skin ranged from 50.85 ± 0.90 mmol kg^{-1} ('Black Amber') to 7.53 ± 0.32 mmol kg^{-1} ('Golden Japan'), while in the flesh it varied it between 17.82 ± 0.36 mmol kg^{-1}

Table 2. ANOVA of dependent variables for cultivar and developmental days

Variable	Cultivar	Days
External colour hue	***	***
Flesh colour hue	***	***
Skin anthocyanins	***	***
Flesh anthocyanins	***	***
Skin carotenoids	**	**
Flesh carotenoids	**	**
Skin phenolic compounds	**	***
Flesh phenolic compounds	**	***
Skin H-TAA	**	***
Flesh H-TAA	**	***
Skin L-TAA	**	***
Flesh L-TAA	**	***

Significance levels: *** $P < 0.001$; ** $P < 0.01$.

Table 3. Correlations between bioactive compounds and colour hue angle, H-TAA or L-TAA in skin and flesh of eight plum cultivars

Cultivar	Tissue	Anthocyanins *vs* hue	Carotenoids *vs* hue	Phenolics *vs* H-TAA	Anthocyanins *vs* H-TAA	Carotenoids *vs* L-TAA
Golden Japan	Skin	–	***	***	–	***
	Flesh	–	***	***	–	***
Black Amber	Skin[a]	***	NS	***	***	***
	Flesh	***	***	***	***	***
Black Diamond	Skin[a]	***	NS	***	***	***
	Flesh	***	NS	***	***	**
Golden Globe	Skin	–	***	***	–	***
	Flesh	–	***	***	–	***
TC Sun	Skin	–	***	***	–	***
	Flesh	–	***	**	–	***
Larry Ann	Skin[a]	***	–	***	***	***
	Flesh	***	***	***	***	***
Songold	Skin	–	***	***	–	***
	Flesh	–	**	***	–	**
Angeleno	Skin[a]	***	NS	***	***	***
	Flesh	***	***	***	***	***
All cultivars	Skin[a]	***	*	***	*	**
	Flesh	**	NS	***	NS	**

For all parameters the correlations were linear, except for
[a] anthocyanins *vs* hue in the skin which was exponential. Significance levels of correlation coefficients (R^2):
*** $P < 0.001$;
** $P < 0.01$;
* $P < 0.05$. NS, no correlation: – , absence of anthocyanin data.

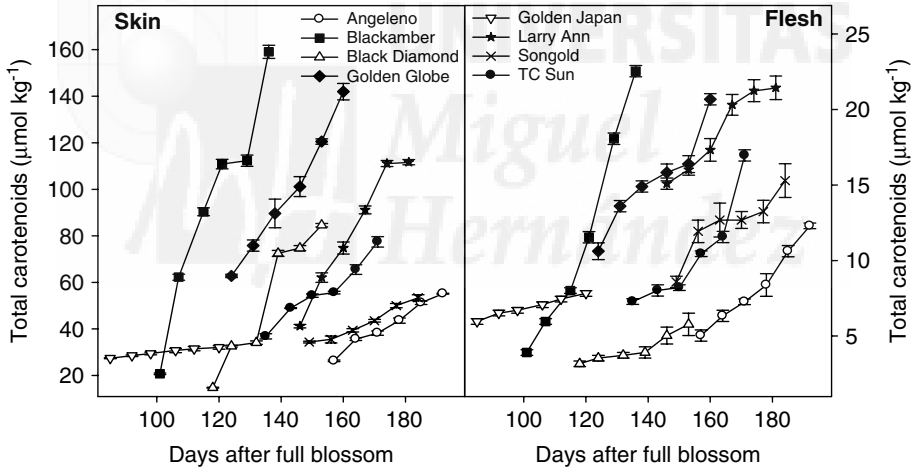

Figure 5. Evolution of total carotenoid content in skin and flesh during last 6 weeks of fruit development and ripening on tree of eight plum cultivars. Data are mean ± standard error.

('Black Amber') and 3.94 ± 0.09 mmol kg^{-1} ('Larry Ann'). The contents of total phenolics in the eight plum cultivars are within the range found in other cultivars, and the dark-purple cultivars generally had higher phenolic contents than the yellow cultivars.[12,15,32] The main phenolic compound in plum cultivars is 3-*O*-caffeoylquinic acid, followed by cyanidin-3-rutinoside, while quercetin-3-rutinoside occurs in lower concentrations.[14]

TAA was assayed in hydrophilic (H-TAA) and lipophilic (L-TAA) fractions. As shown above for the bioactive compounds, both H-TAA and L-TAA increased throughout the ripening process on tree in both skin and flesh tissues for all plum cultivars (Figs 7 and 8). TAA (H-TAA + L-TAA) of the skin was generally higher in the dark-purple plums than in the yellow plums. H-TAA was about twofold higher than L-TAA in the dark-purple cultivars, while the opposite was found in the yellow cultivars, with the exception of 'Songold' which had a threefold higher H-TAA than L-TAA. H-TAA was always lower (~1.5–2-fold) than L-TAA in the flesh, except for 'Black Diamond'. In addition, H-TAA was highly correlated ($r^2 = 0.76–0.99$) with total phenolics in the skin and flesh of all cultivars (Table 3), indicating that phenolics are important bioactive compounds contributing to hydrophilic antioxidant activity, in accordance with previous reports on other plum, peach and nectarine cultivars.[12,14–16] In addition, in the dark-purple cultivars, total anthocyanins were

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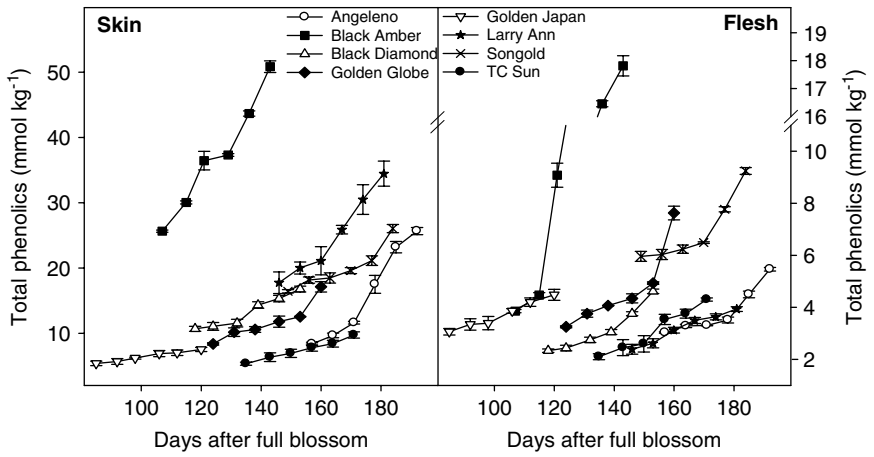

Figure 6. Evolution of total phenolic content in skin and flesh during last 6 weeks of fruit development and ripening on tree of eight plum cultivars. Data are mean ± standard error.

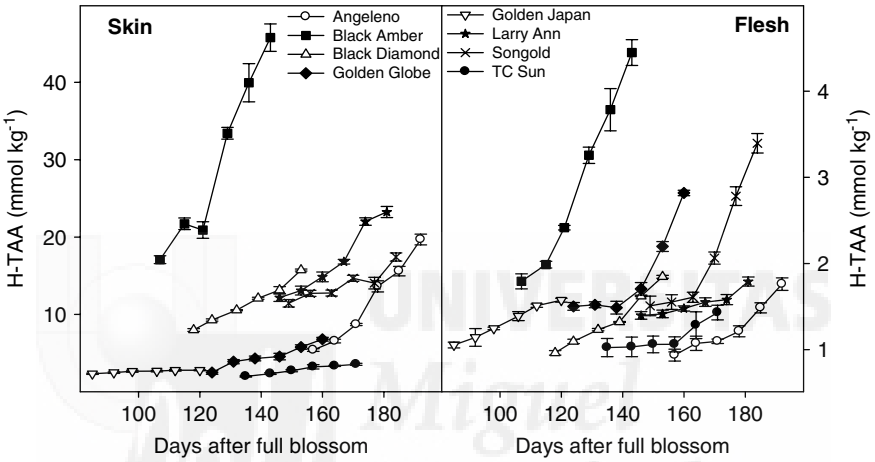

Figure 7. Evolution of total antioxidant activity in hydrophilic fraction (H-TAA) of skin and flesh during last 6 weeks of fruit development and ripening on tree of eight plum cultivars. Data are mean ± standard error.

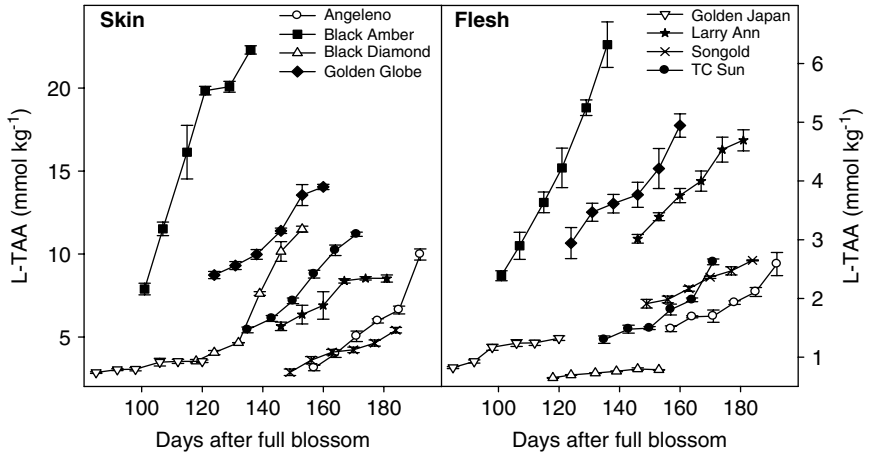

Figure 8. Evolution of total antioxidant activity in lipophilic fraction (L-TAA) of skin and flesh during last 6 weeks of fruit development and ripening on tree of eight plum cultivars. Data are mean ± standard error.

also highly correlated with H-TAA, showing that anthocyanins might contribute to antioxidant activity more than other phenolic compounds. Finally, L-TAA was highly correlated with total carotenoids ($r^2 = 0.73–0.99$) in both skin and flesh tissues. As far as we know, this is the first study in which H-TAA and L-TAA have been independently assayed in plums, and thereafter L-TAA has been correlated with total carotenoids, although evidence exists about this issue for tomato.[33] Among the cultivars assayed in this work, 'Black Amber' had the highest antioxidant activity in both skin and flesh (for both H-TAA and

L-TAA) and also had the greatest accumulation of total bioactive compounds (phenolics, anthocyanins and carotenoids), although the differences found in these compounds among cultivars could not justify the large differences in antioxidant activity. For example, 'Black Amber' had an approximately 2.5-fold higher total antioxidant activity than 'Larry Ann', while its concentration of bioactive compounds was only 1.5-fold greater. This behaviour would indicate a synergistic effect of all the compounds contributing to TAA.

In conclusion, plums harvested at the commercial maturity stage do not reach their maximum concentration of bioactive compounds, since keeping them 1 week longer on tree led to significant increases in these compounds and the corresponding total antioxidant activity (by 10–20% on average). Thus, in order to achieve the maximum health-beneficial effects of plum consumption and their optimal organoleptic and nutritive properties, it would be advisable to harvest the fruits at the fully ripe stage.

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Sensory, Nutritive and Functional Properties of Sweet Cherry as Affected by Cultivar and Ripening Stage

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In this article 11 commercial sweet cherry cultivars were selected to evaluate sensory, nutritive and functional properties over the maturation process on tree. Fruit quality was significantly different among cultivars and maturity stages at harvest, with the highest quality scores being found in the harvest which was 4 days beyond current commercial harvest maturity for all the cultivars tested. Taking into account all of the measured parameters (weight, firmness, color, acidity and total soluble solids), 'NY-6479', 'Prime Giant' and 'Sunburst' could be classified as having the highest quality in terms of sensory attributes. However, 'Cristalina' and 'Sonata' had the highest functional quality, as determined by the measurement of bioactive compound content and antioxidant capacity. We conclude that a delay of a few days in harvesting of sweet cherries would lead to achieve maximal nutritional (highest sugar and organic acid contents), sensory (greatest firmness and color development) and functional (greatest phenolics content, anthocyanins and antioxidant capacity) quality to provide both eating enjoyment and health benefits to the consumer.

Key Words: sweet cherry, ripening, antioxidants, phenolics, quality

INTRODUCTION

Sweet cherry is one of the most appreciated fruits by consumers due to its precocity and excellent quality. Spain is one of the main cherry producers in Europe, with a production of 115,000 ton in 2003, which represents 20% of the total in the European Union (FAO, 2005). The concept of 'quality' depends on the product itself and the consumer's preferences, and for sweet cherry it is widely accepted that the main characteristics related to fruit quality are fruit weight, color, firmness, sweetness, sourness, flavor and aroma (Romano et al., 2006). In sweet cherry, the ripening process is characterized by color changes, from green to red, which can be followed by the evolution of L^*, a^* and b^* parameters and the color indices Chroma and Hue. However, the industry has a standard color chart used for this purpose, the most common being that from the Centre Technique Interprofessionel de Fruits et Légumes (CTIFL, Paris). Red color development in sweet cherry is used as indicator of quality and ripening, and is due to accumulation and profile of anthocyanins (Gao and Mazza, 1995; Mozetič et al., 2004; Serrano et al., 2005a). Sweetness in cherry fruit is mainly due to glucose and fructose, while sourness is primarily due to the presence of malic acid (Serrano et al., 2005a; Usenik et al., 2008). Fruit firmness is also appreciated by consumers, together with 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 (Crisosto et al., 2003).

Nowadays, especially in developed countries, fruits and vegetables are appreciated not only by their sensory and nutritional properties, but also by their additional health benefits. In fact, critical and epidemiological studies have established an inverse correlation between the intake of fruit and vegetables and the occurrence of several degenerative diseases, such as cancer, cardiovascular illness and even Alzheimer's disease, due to their content in some bioactive compounds (Kris-Etherton et al., 2002; Scalbert et al., 2005; Schreiner and Huyskens-Keil, 2006). Among these compounds there are vitamins (A, C and E), carotenoids and phenolics,

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including anthocyanins, due to their antioxidant properties (Kaur and Kapoor, 2001; Tomás-Barberán and Espín, 2001).

There are some papers about sensory, nutritive and functional properties of sweet cherry at harvest time, showing important differences among cultivars (Girard and Koop, 1998; Gonçalves et al., 2004; Kim et al., 2005; Usenik et al., 2008). In addition, some parameters related to fruit quality, such as fruit weight, color and anthocyanins, firmness, and sugar and acid content have been also evaluated on different cultivars during fruit development on tree (Crisosto et al., 2002; Mozetič et al., 2004; Usenik et al., 2005; Muskovics et al., 2006). Harvesting is usually performed based on the attainment of acceptable fruit size, color and concentration of soluble solids. However apart from our previous paper, with sweet cherry cultivar '4–70' (Serrano et al., 2005a), there is no available information about the changes in the content of health-promoting compounds during sweet cherry development and ripening on tree. In this sense, the aim of this work was to analyze sensory, nutritive and functional properties during the ripening on tree of 11 sweet cherry cultivars with interest in Spain, the majority of them being studied for the first time. This information could be useful to pick each cultivar with the maximum overall quality, in order to satisfy the demand of consumers for taste, nutrition and health beneficial effects. In addition, results would also serve as a basis for selection of sweet cherry cultivars with both high quality fruits and health beneficial effects, since in the last decades cultivars have been screened mainly on the basis of field growth factors and on a few fruit quality attributes such as size, color, texture and flavor.

MATERIALS AND METHODS

Materials

Plant Material and Experimental Design

The experiment was carried out along the developmental cycle during the 2007 spring period, in a commercial plot located at 'Finca Los Frutales' (Villena, Alicante, Spain). Eleven different sweet cherry cultivars (Table 1) from 10 year old trees on 'Santa Lucía' rootstock were selected. After fruit set, three trees were selected for each cultivar and then 10 fruits were labeled around the equatorial perimeter of each tree. These marked fruits served to evaluate the growth by measuring three linear dimensions of the fruit: polar, suture and cheek diameters. At 3–4 day intervals along the development process, 30 similar fruits to those labeled on tree were taken, and then immediately transferred to laboratory for further analytical determinations. The commercial harvest date (CH) for each cultivar was determined according to the Technician's company and based on size and color using the CTIFL chart. Thus, the scores at CH were 3 for 'Brooks' and 'Somerset', 5 for 'Cristalina' and 'Sonata' and 4 for the remaining cultivars. In addition, some fruits were kept 4 days further on tree to take the last sample (CH + 4 days). Fruit weight, firmness, and color were measured individually in each fruit, and data are the mean ± SE ($n = 30$). When the fruit had an appropriate size (the last six sampling dates) five subsamples of six fruits were made at random, and then the edible portion was cut in small pieces to obtain five homogenous subsamples for each cultivar and sampling date. Five grams were used for total soluble solids and titratable acidity determination and the remaining tissue was immediately frozen in liquid N_2 and milled for total phenolics, anthocyanins and antioxidant activity determination at the last four sampling dates.

Table 1. Dates of full blossom and harvesting and total days from full blossom to reach harvest ripening stage for sweet cherry cultivars in 2007 year.

Cultivar	Date of full blossom	Date of commercial harvesting	Total days
Brooks	23 March	4 June	74
Cristalina	12 April	14 June	63
Newstar	24 March	4 June	73
No. 4 or Santina	8 April	11 June	60
Somerset or no. 52	6 April	11 June	66
No. 57 or 13N 7-19	9 April	14 June	66
NY-6479 or Picota	3 April	14 June	69
Prime Giant	20 March	11 June	83
Sonata	12 April	14 June	63
Sunburst	15 April	14 June	57
Sweetheart	30 March	21 June	79

Methods

Fruit Weight, Firmness and Color

The weight for each fruit was determined using a digital balance (ST-360 Gram Precision) with two significant figures and results were the mean ± SE. Fruit firmness was determined using a TX-XT2i Texture Analyzer (Stable Microsystems, Godalming, UK) interfaced to a personal computer, with a flat steel plate mounted on the machine. For each fruit, the cheek diameter was measured and then a force that achieved a 3% deformation of the fruit diameter was applied. Results were expressed as the ratio between this force and the covered distance (N/mm) and were the mean ± SE. This determination of firmness as the slope of the force-deformation curve has been chosen as the most characteristic parameter for textural changes in cherry fruits (Serrano et al., 2005a; Muskovics et al., 2006). Three color determinations were made on each

fruit at 120° interval along the equatorial perimeter using the Hunter Lab System (L^*, a^*, b^*) in a Minolta colorimeter CR200 model (Minolta Camera Co., Osaka, Japan). In addition, a/b, Chroma index (Chroma $= (a^2 + b^2)^{1/2}$) and Hue angle (Hue $=$ arctan (b/a)) were calculated. Results were the mean ± SE.

Total Soluble Solids and Total Acidity

Total Soluble Solids (TSS) were determined in duplicate from the juice obtained from each subsample with a digital refractometer Atago PR-101 (Atago Co. Ltd., Japan) at 20 °C and results expressed as °Brix. Total acidity (TA) was determined from the above juice by potentiometric titration with 0.1 N NaOH up to pH 8.1, using 1 mL of diluted juice in 25 mL distilled H_2O and results were the mean ± SE expressed as g of malic acid equivalent per 100g fresh weight.

Total Anthocyanins, Total Phenolics and Antioxidant Activity

Total anthocyanins were determined as previously reported (Serrano et al., 2005a). Two grams of fruit tissue were homogenized in 4 mL methanol and left 1 h at −18 °C. Extracts were centrifuged at 10,000 × *g* for 15 min at 4 °C and the supernatant was loaded onto a C18 Sep-Pak® cartridge, previously conditioned with 5 mL methanol, 5 mL pure water and then with 5 mL 0.01 N HCl. Cartridge was washed with 5 mL pure water and then eluted with acidified MeOH (0.01% HCl). Absorbance of the collected fraction was measured at 530 nm and total anthocyanins were calculated using cyanidin-3-glucoside (molar absorption coefficient of 23,900 L/cm · mol and molecular weight of 449.2 g/mol). Results were expressed as mg cyanindin 3-glucoside equivalent per 100g fresh weight, and were the mean ± SE of determinations made in duplicate in each one of the five subsamples.

Total phenolics were extracted according to Tomás-Barberán et al. (2001) using water: methanol (2:8) containing 2 mM NaF (1:5 w/v) and quantified using the Folin-Ciocalteu reagent (Singleton et al., 1999). Briefly, a suitable volume (25–100 µL) of extracts was mixed with 2.5 mL of water-diluted Folin-Ciocalteau. The mixture was incubated for 2 min at room temperature and 2 mL of sodium carbonate (75 g/L) were added and shaken. Finally, mixture was incubated at 50 °C for 15 min and absorbance was measured at 760 nm. A calibration curve was performed with gallic acid and results were expressed as mg gallic acid equivalent per 100g fresh weight. Results were the mean ± SE of determinations made in duplicate in each one of the five subsamples.

For antioxidant activity quantification 1 g of cherry flesh was homogenized with 5 mL of 50 mM Na-phosphate buffer pH 7.5 and 3 mL of ethyl acetate, centrifuged at 10,000 × *g* for 15 min at 4 °C and then the aqueous and organic phases were separated and used to quantify hydrophilic and lipophilic total antioxidant activity (H-TAA and L-TAA), respectively, according to Arnao et al., (2001). The method is based on the capacity of different fruit components to scavenge the $ABTS^{\bullet+}$ radicals (2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), which have been previously generated by the horse radish peroxidase enzyme (HRP) and its oxidant substrate (hydrogen peroxide). The reaction mixture contained 1.5 mM ABTS, 15 µM hydrogen peroxide and 0.25 µM HRP in a total volume of 2 mL of 50 mM glycine-HCl buffer (pH 4.5), for H-TAA or in ethyl acetate for L-TAA. 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 cherry fruit extract was added and the observed decrease in absorbance was determined. A calibration curve was performed with Trolox ((R)-(+)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), as standard antioxidant for both H-TAA and L-TAA and results are expressed as mg of Trolox equivalent per 100g fresh weight. Results were the mean ± SE of determinations made in duplicate in each one of the five subsamples.

Statistical Analysis

Data for the analytical determinations were subjected to a two-way analysis of variance (ANOVA). Sources of variation were cultivar and developmental sampling dates. LSDs ($p < 0.05$) were calculated for mean separations and are shown in the Figures. Polynomial linear or quadratic regressions were performed between color parameters and anthocyanin concentration, as well as among anthocyanins or phenolics and H-TAA. The regressions were carried out taking into account data for all cultivars and sampling dates. All analyses were performed with SPSS software package v. 12.0 for Windows (2001).

RESULTS AND DISCUSSION

Changes in Sensory and Nutritional Parameters

It is known that environmental factors and orchard management (choice of rootstock, pruning, fertilization and irrigation) affect cherry fruit quality, in terms of different concentration of nutritive and bioactive compounds (Predieri et al., 2004; Gonçalves et al., 2006). However, in this work all cherry cultivars were in the same farm, under similar environmental conditions and cultural practices and even on similar rootstocks and tree age. Then, differences in quality parameters

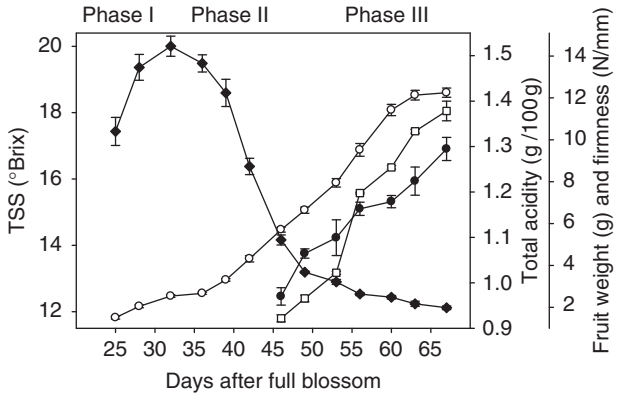

Figure 1. Evolution of fruit weight, firmness, total acidity and TSS content during development and ripening on tree of 'Sonata' cultivar. Data are the mean ± SE ($n = 30$ for firmness and fruit weight; $n = 5$ for acidity and TSS). LSD $_{(P<0.05)} = 0.14$ for fruit weight, 0.21 for total acidity, 0.27 for TSS and 0.28 for firmness. (○) Fruit weight, (□) TSS, (●) Total acidity, (◆) Firmness.

among cultivars that will be commented below should be attributed to genetic characteristics of each cultivar.

Cherry fruit weight increased along development on tree, as it is shown in Figure 1 for 'Sonata' as an example, in which the second and third phases of double sigmoid curve for stone fruit growth can be observed. The final fruit weight and the time from full blossom to harvesting were significantly different depending on cultivar (Tables 1 and 2). Thus, 'Sunburst' had faster development, with only 57 days from full blossom to harvesting, while 'Prime Giant' had the slowest with 83 days. Final fruit weight was also different among cultivars, ranging from 9 g in 'Sweetheart' to 14 g in 'Sunburst' and 'No. 57', although for all cultivars similar values were obtained in CH and CH + 4 days. This means that at CH all cherry fruits had reached their final size (Table 2), which were higher as than the majority of other cultivars studied by others (Usenik et al., 2008). According to morphological properties, Beyer et al. (2002) distinguished five typical shape characteristics of cherries: kidney, flat-round, round, oblong and chordate. In this study, most cultivars had chordate shape ('Santina', 'Somerset', 'No. 57', 'NY-6479', 'Prime Giant' and 'Sunburst'), 3 had round shape ('Newstar', 'Sonata' and 'Sweetheart'), while 'Brooks' and 'Cristalina' had kidney and flat-round shape, respectively.

Texture is one of the most important attributes in sweet cherry and it is often used as quality assessment, although there are considerable genotypic differences, as can be observed in Table 2. In previous reports, it has been found that late cultivars were generally firmer than early ones (Chistesen, 1995; Esti et al., 2002). This was true for 'Sweetheart', which was both the firmest (3.15 ± 0.12 N/mm at CH) and the latest cultivar, while 'Brooks' and 'Newstar' were early-season cultivars and showed an intermediate firmness, and the softest was 'No 57', with 1.85 ± 0.10 N/mm at CH and considered mid-season cultivar (Tables 1 and 2). For all cultivars, fruit firmness reached the highest value at the second phase of fruit growth, which has been associated to pit hardening in sweet cherry (Muskovics et al., 2006). After that, fruit firmness decreased sharply as fruit weight increased, as it is shown in Figure 1 for 'Sonata', which simply reflects cell enlargement during fruit growth. However, softening in the last days of ripening has been attributed to increases in β-galactosidase activity (Gerardi et al., 2001), unlike in most of fruits, in which softening is dependent on pectin depolymerization due to polygalacturonase activity (Batisse et al., 1996).

For all sweet cherry cultivars, TSS and TA started to increase when fruit had around 40–50% of its final size and went on until the last sampling date, as it is shown for 'Sonata' in Figure 1. However, significant differences were found among cultivars and between CH and CH + 4 days for each cultivar (Table 2). At CH the highest TSS content was found in 'Sunburst' (19.90 ± 0.12 °Brix) followed by 'NY-6479' and 'Prime Giant' (≈19.5 °Brix) and the lowest in 'Santina' (15.95 ± 0.15 °Brix). Nevertheless, at CH + 4 days the higher levels were found in 'Sweetheart' and 'NY-6479' (≈21.5 °Brix) while 'Santina' still had the lowest TSS (16.62 ± 0.15 °Brix). The TSS levels found for these cultivars were in agreement with those reported for other sweet cherries harvested at commercial ripening stage, for which values between 11 and 25 °Brix have been reported (Girard and Kopp, 1998; Esti et al., 2002; Serrano et al., 2005a and b). The main sugars found in cherry cultivars have been glucose and fructose, followed by sorbitol and sucrose (Girard and Koop, 1998; Serrano et al., 2005a; Usenik et al., 2008). TA reached the highest levels, close to 1.30 g per 100g at CH + 4 days in 'Newstar', 'NY-6479' and 'Sonata, while 'Brooks' and 'Santina' showed the lowest acidity, ≈0.80 g per 100g (Table 2). In sweet cherry as well as in other *Prunus* species, such as plum, peach, apricot and nectarines, malic acid has been found to be the major organic acid contributing to total acidity, which differed greatly among cultivars (Crisosto, 1994; Girard and Koop, 1998; Zuzunaga et al., 2001). However, in stone fruits apart from cherries, acidity decreased over the development and ripening, while an accumulation was observed for all cherry cultivars, in agreement with the reported increase in total acidity as harvesting date was delayed in 'Lapins' and '4–70' cherries (Drake and Elfving, 2002; Serrano et al., 2005a).

The color indices (Hue angle, Chroma and a/b) of the skin showed similar evolutions for all sweet cherry cultivars which are displayed in the 'Sonata' example in

Table 2. Fruit properties at CH and CH + 4 days.

	Fruit weight		TSS (°Brix)		Total acidity		Firmness	
Cultivar	CH	CH + 4 days	CH	CH + 4 days	CH	CH + 4 days	CH	CH + 4 days
Brooks	11.93 ± 0.42 a A	11.95 ± 0.33 a A	19.05 ± 0.21 a A	20.82 ± 0.19 b A	0.75 ± 0.02 a A	0.84 ± 0.02 b A	2.47 ± 0.08 a A	2.38 ± 0.10 a A
Cristalina	10.55 ± 0.29 a B	10.60 ± 0.44 a B	17.54 ± 0.31 a B	19.37 ± 0.51 b B	0.85 ± 0.03 a B	0.89 ± 0.01 a B	2.26 ± 0.09 a B	2.17 ± 0.04 a B
Newstar	11.75 ± 0.33 a A	11.81 ± 0.44 a A	18.14 ± 0.24 a C	20.72 ± 0.06 b A	1.21 ± 0.02 a C	1.29 ± 0.02 b C	2.24 ± 0.09 a B	2.10 ± 0.04 a B
Santina	10.14 ± 0.33 a B	10.22 ± 0.21 a B	15.95 ± 0.15 a D	16.62 ± 0.15 b C	0.79 ± 0.04 a A	0.81 ± 0.03 a A	2.58 ± 0.09 a A	2.27 ± 0.11 a A
Somerset	10.90 ± 0.20 a B	10.92 ± 0.24 a B	17.84 ± 0.12 a B	19.4 ± 0.11 b B	0.92 ± 0.02 a BD	0.96 ± 0.05 a BD	2.24 ± 0.11 a B	2.11 ± 0.09 a B
No 57	13.91 ± 0.44 a C	13.82 ± 0.32 a C	18.55 ± 0.24 a C	19.70 ± 0.35 b B	0.99 ± 0.02 a D	1.04 ± 0.03 a D	1.85 ± 0.10 a C	1.62 ± 0.09 b C
NY-6479	9.40 ± 0.20 a D	9.42 ± 0.15 a D	19.60 ± 0.06 a E	21.42 ± 0.10 b D	1.25 ± 0.03 a C	1.32 ± 0.02 a C	2.83 ± 0.13 a D	2.60 ± 0.2 a D
Prime Giant	13.36 ± 0.53 a C	13.45 ± 0.55 a C	19.55 ± 0.14 a E	19.90 ± 0.12 b B	1.12 ± 0.01 a E	1.14 ± 0.01 a E	2.42 ± 0.13 a A	2.31 ± 0.09 a A
Sonata	12.13 ± 0.23 a E	12.25 ± 0.22 a E	17.44 ± 0.06 a B	18.05 ± 0.29 b E	1.20 ± 0.03 a C	1.29 ± 0.02 b C	2.17 ± 0.12 a B	1.98 ± 0.09 a B
Sunburst	13.96 ± 0.26 a C	13.99 ± 0.21 C a	19.90 ± 0.12 a E	20.62 ± 0.05 b A	0.92 ± 0.01 a B	0.93 ± 0.02 a B	2.23 ± 0.10 a B	2.08 ± 0.08 b B
Sweetheart	9.02 ± 0.23 a D	9.15 ± 0.21 a D	17.90 ± 0.20 a B	21.77 ± 0.45 b D	1.18 ± 0.03 a CE	1.19 ± 0.05 a CE	3.15 ± 0.12 a E	2.82 ± 0.09 b E

Mean values followed by different small letters within a row are significantly different at $p < 0.05$ level.
Mean values followed by different capital letters within columns are significantly different at $p < 0.05$ level.

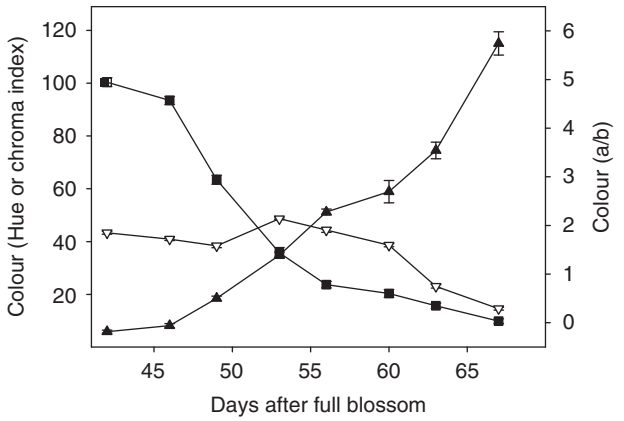

Figure 2. Evolution of color indices (Hue angle, Chroma and a/b) in 'Sonata' sweet cherry along the ripening process on tree. Data are the mean ± SE ($n = 30$). $LSD_{(P<0.05)} = 2.45$ for Hue angle, 1.67 for Chroma and 0.34 for a/b. (■) Hue angle, (▽) Chroma, (▲) Color a/b.

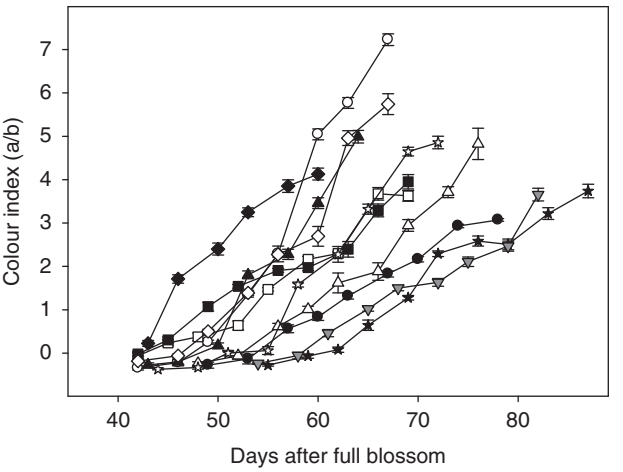

Figure 3. Evolution of color index (a/b) in the different sweet cherry cultivars along the ripening process on tree. Data are the mean ± SE ($n = 30$). $LSD_{(P<0.05)} = 0.31$. (●) Brooks, (○) Cristalina, (△) Newstar, (▲) Santina, (□) Somerset, (■) No 57, (☆) NY-6479, (★) Prime Giant, (◇) Sonata, (◆) Sunburst, (▼) Sweetheart.

Figure 2. Hue angle decreased sharply between 45 and 55 days from full blossom while the decrease was at much slow rate afterwards. Contrarily, Chroma index increased, reaching a plateau and decreased afterwards during the last days of ripening, which means an increase in the tonality of the fruit color. However, *a*/*b* could be a better index to describe the ripening process in sweet cherry, since it showed a continuous increase until the last sampling date. Significant differences were found among the studied cultivars in the final values of *a*/*b* index, which ranged from 3.07 ± 0.03 in 'Brooks' to 7.23 ± 0.14 in 'Cristalina', which had the highest bright red (score 3 of CITFL chart) and dark red (score 5 of CITFL chart) colors, respectively (Figure 3). The results about parameters related to fruit ripening, such as soluble solids and total acidity accumulation, decrease in firmness and skin color changes, showed that some ripening processes in sweet cherry started to change at an early stage during development of phase III.

Evolution on Functional Properties

Total anthocyanins were determined at the four last sampling dates in all cultivars, since it has been shown that the main anthocyanins accumulation occurred in the last two weeks of cherry development (Mozetič et al., 2004). Anthocyanin concentration increased sharply in all cultivars, reaching final concentration between 39.55 ± 2.58 and 224.65 ± 5.57 mg cyanidin-3-glucoside per 100g for 'Brooks' and 'Cristalina', respectively (Figure 4). Taking into account the data obtained at CH, the lowest anthocyanin concentration was found for 'Brooks', 'Somerset', 'Prime Giant' and 'Sweetheart' which are considered as light-colored cultivars, with a 3 score of the CTIFL color chart.

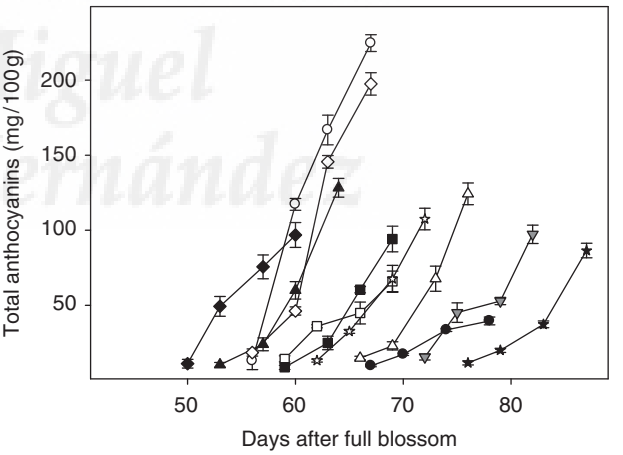

Figure 4. Evolution of total anthocyanin concentration (mg cyanidin 3-glucoside equivalent per 100g) in the different sweet cherry cultivars along the ripening process on tree. Data are the mean ± SE ($n = 5$). $LSD_{(P<0.05)} = 8.95$. (●) Brooks, (○) Cristalina, (△) Newstar, (▲) Santina, (□) Somerset, (■)No. 57, (☆) NY-6479, (★) Prime Giant, (◇) Sonata, (◆) Sunburst, (▼) Sweetheart.

'Cristalina' and 'Sonata' had the highest anthocyanin content and were dark-colored (5 score of the CTIFL color chart), while the remaining had intermediate anthocyanin concentration and a value of 4 (medium-colored cultivars). The predominant anthocyanins in cherry are cyanidin-3-rutinoside and

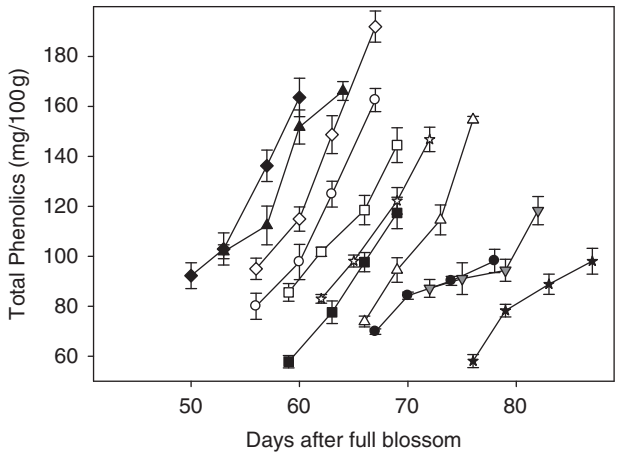

Figure 5. Evolution of total phenolic concentration (mg gallic acid equivalent per 100g) in the different sweet cherry cultivars along the ripening process on tree. Data are the mean ± SE ($n = 5$). $LSD_{(P<0.05)} = 7.42$. (●) Brooks, (○) Cristalina, (△) Newstar, (▲) Santina, (□) Somerset, (■) No. 57, (☆) NY-6479, (★) Prime Giant, (◇) Sonata, (◆) Sunburst, (▼) Sweetheart.

cyanidin-3-glucoside, while peonidin- and pelargonidin-(3-glucoside and 3-rutinoside) have been found at very low concentrations (Gonçalves et al., 2004; Mozetič et al., 2004; 2006; Chaovanalikit and Wrolstad, 2004; Usenik et al., 2008).

When linear regression was performed between anthocyanin concentration and color index a/b taking into account data from all cultivars and sampling dates, a positive linear correlation was found ($r^2 = 0.899$) and this could be considered as an easy and reliable index for anthocyanin concentration in cherries, in general. In addition, the measurement of this color index could be a good tool to predict the levels of anthocyanins in sweet cherry cultivars.

Total phenolics increased in a similar way with anthocyanins. A sharp increase during the last sampling dates was observed, with significant differences among cultivars (Figure 5). The highest phenolic concentration at the last sampling date was found in 'Sonata' (191.90 ± 6.23 mg gallic acid equivalent per 100g) and the lowest in 'Brooks' (98.14 ± 4.64 mg/100 g). These levels of total phenolics were within the same concentration range to those found in other cherry cultivars at commercial harvesting, in which concentration from 90 to 200 mg/100 g have been reported (Mozetič et al., 2002; Kim et al., 2005). The major polyphenols in sweet cherry are anthocyanins followed by the hydroxycinnamic acid's derivatives neochlorogenic acid and 3′-*p*-coumaroylquinic acid (Mozetič et al., 2002, Chaovanalikit and Wrolstad, 2004). Since phenolic compounds contribute to fruit quality in terms of modifying color, taste, aroma and flavor (Tomás-Baberán and Espín, 2001), those cultivars with higher phenolics content will have higher quality. In addition, taking into account data from all cultivars and the last four harvest dates, a highly positive correlation was found between total anthocyanins and total phenolics concentration using a polynomial quadratic equation ($r^2 = 0.813$). Thus, it could be concluded that in these sweet cherry cultivars, anthocyanins are the major phenolics, according to previous reports in other cultivars (Gao and Mazza, 1995; Chaovanalikit and Wrolstad, 2004).

TAA was quantified in hydrophilic (H-TAA) and lipophilic (L-TAA) extracts separately. It could be observed that both H-TAA and L-TAA increased along the ripening process for all sweet cherries. For all of them, H-TAA was higher than L-TAA (ca. 80% of TAA in 'Cristalina' and ≈50% in 'Prime Giant'), showing that the major contributors to antioxidant activity are hydrophilic compounds (Figure 6). Nevertheless, important differences were found among cultivars. Thus, at the last sampling date, the highest levels of H-TAA were found in 'Sonata' and 'Cristalina' (≈130 mg/100 g) and the lowest in 'Brooks' (69.67 ± 2.50 mg/100 g), while for L-TAA 'Sonata' showed the highest level (74.66 ± 2.68 mg/100 g) and the lowest (≈35 mg/100 g) were found in 'Brooks', 'Santina', 'Cristalina' and 'NY-6479'. However, no correlations were found between H-TAA and L-TAA in these cherry cultivars. This is the first time that antioxidant activity in both hydrophilic and lipophilic extracts has been measured during sweet cherry fruit ripening on tree and no literature is available for comparative purposes. The only reports in which L-TAA and H-TAA have been quantified separately are those of Wu et al. (2004), in a wide range of fruits and vegetables at CH (including 4 cherry cultivars although no names or maturity stages were reported), and Arnao et al. (2001), in vegetable soups, showing that H-TAA contributed about 70–90% of the total antioxidant activity.

A high positive correlation was obtained between H-TAA and both, phenolic and anthocyanin concentrations ($y = 1.33x - 9.83$; $r^2 = 0.841$ and $y = 0.41x + 45.7$; $r^2 = 0.753$, respectively) taking into account data for all cultivars and the four last sampling dates. Thus, in sweet cherry the main contributors to H-TAA are phenolic compounds and especially anthocyanins. The correlation between antioxidant activity and phenolic compounds has been also found in several studies comparing a wide range of fruits and vegetables (Wang et al., 1996; Kaur and Kapoor, 2001; Wu et al., 2004). Specifically, in sweet cherry cultivars, it has also been found that there is a good correlation between total phenolics and TAA (Serrano et al., 2005a; Usenik et al., 2008), although they were determined only in hydrophilic extracts. This indicates that when sweet cherry is developing the intensity of red color, the anthocyanins and other phenolic compounds could also account for

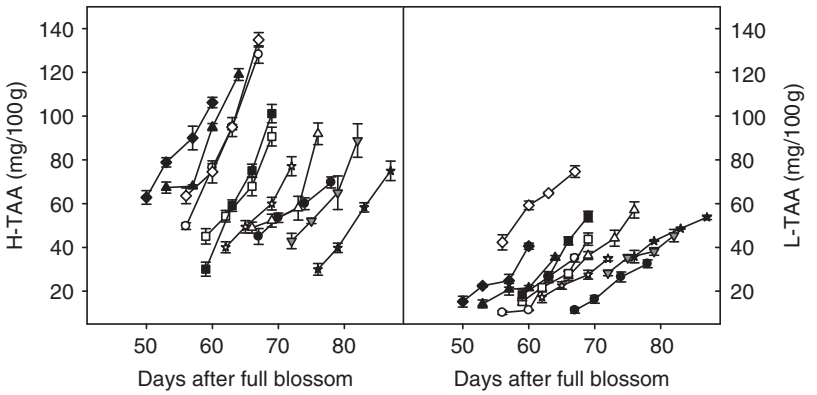

Figure 6. Hydrophilic (H-TAA) and lipophilic (L-TAA) total antioxidant activity (mg Trolox equivalent per 100g) evolution in the different sweet cherry cultivars along the ripening process on tree. Data are the mean ± SE ($n = 5$). $LSD_{(P<0.05)} = 6.94$ for H-TAA and 4.15 for L-TAA. (●) Brooks, (○) Cristalina, (△) Newstar, (▲) Santina, (□) Somerset, (■)No. 57, (☆) NY-6479, (★) Prime Giant, (◇) Sonata, (◆) Sunburst, (▼) Sweetheart.

their antioxidant activity and health beneficial effects (Scalbert et al., 2005).

It has been shown that sour and sweet cherry anthocyanins have the potential to directly interfere with intestinal tumor development (Kang et al., 2003), a strong antidegenerative activity in neuronal cells (Kim et al., 2005) and a beneficial role in the treatment of inflammatory pain (Tall et al., 2004). Thus, cherry can serve as a good source of biofunctional phytochemicals in our diet, providing health beneficial effects in humans. Moreover, ascorbic acid is a hydrophilic compound with antioxidant activity which could also account for H-TAA, as has been shown in sweet cherry '4–70' (Serrano et al., 2005a) and in other fruits, such as oranges (Pretel et al., 2004).

CONCLUSIONS

Results show that there are significant differences among sweet cherry cultivars in quality parameters related to sensory, nutritive and functional properties, and between ripening stages. Taking into account data from fruit weight, color, firmness, acidity and TSS (sensory and nutritive parameters), the cultivars more appreciated could be 'NY-6479', 'Prime Giant' and 'Sunburst'. However, 'Cristalina' and 'Sonata' exhibited the highest values of total anthocyanins and total phenolic compounds that seemed to be the main responsible for antioxidant activity properties. Finally, it is interesting to point out that for all cultivars a delay in harvesting (CH + 4 days) led to significant increases in functional compounds and antioxidant activity. In future, it would be necessary to determine the best conditions in handling, storage and commercialization to ensure that the overall cherry quality does not decrease until they reach the consumer.

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Changes in hydrophilic and lipophilic antioxidant activity and related bioactive compounds during postharvest storage of yellow and purple plum cultivars

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ABSTRACT

Eight plum cultivars (four dark-purple and four yellow) were harvested at the commercial ripening stage, and changes of fruit quality properties were evaluated during cold storage and subsequent shelf-life, with special emphasis on bioactive compounds (phenolics, anthocyanins and carotenoids) and antioxidant activity (TAA). From the eight plum cultivars, four showed the typical climacteric ripening pattern ('Blackamber', 'Larry Ann', 'Golden Globe' and 'Songold') while four behaved as suppressed-climacteric types ('Golden Japan' 'Angeleno', Black Diamond' and 'TC Sun'), the latter being described for the first time. At harvest, large variations in phytochemicals and antioxidant activity were found among cultivars in peel and pulp tissues, although phytochemical concentration and antioxidant activity were higher in the peel than in the flesh (2–40-fold depending on the bioactive compound). During storage, increases in total phenolics for all cultivars (peel and pulp), in total anthocyanin content in the peel of the dark-purple plums, and total carotenoids in the peel and pulp of the yellow cultivars were observed. This behaviour of the bioactive compounds was reflected in TAA changes, since hydrophilic-TAA (H-TAA) was correlated with both phenolics and anthocyanins, while lipophilic-TAA (L-TAA) was correlated with carotenoids. L-TAA comprised about 30–50% of the TAA in plum tissues. Carotenoids and phenolics (and among them the anthocyanins) could be the main lipophilic and hydrophilic compounds contributing to L-TAA and H-TAA, respectively. No significant loss of bioactive compounds and TAA occurred during prolonged plum storage. Moreover, for a better evaluation of the antioxidant potential of plums, the contribution to carotenoids should not be overlooked.

1. Introduction

Plums are an important group among stone fruit grown commercially in Spain. Harvest date of this crop is an essential determinant of consumer acceptability (Crisosto et al., 2004), and a number of parameters are generally used to establish the optimum ripening stage including skin colour, flesh texture, soluble solids concentration (SSC) and acidity together with developed aroma and flavour. However, large variations in these parameters can be found depending on cultivar, production area, climatic conditions and harvest season (Kader and Mitchell, 1989). In addition, two distinct patterns of ripening behaviour have been reported for plums, with cultivars showing a suppressed-climacteric phenotype such as 'Shiro', 'Rubyred' (Abdi et al., 1997) and 'Golden Japan' (Zuzunaga et al., 2001), in contrast to the typical climacteric shown for most plums.

Given the perishable nature of plum fruit, the use of cold storage, avoiding temperatures that induce chilling injury in the sensitive cultivars, is a postharvest tool to delay changes related to ripening, such as ethylene production, respiration rate, softening, pigment changes, increase in SSC and decrease in acidity (Murray et al., 2005; Guerra and Casquero, 2008). Moreover, several treatments prior to cold storage (calcium, heat, polyamines or 1-methylcyclopropene) have been reported to maintain plum quality for longer periods than low temperature alone (Valero et al., 2002a,b; Martínez-Romero et al., 2003).

Increased intake of fruit and vegetables has been associated with reduced incidence of degenerative diseases due to their antioxidant potential (Kris-Etherton et al., 2002; Prior, 2003; Schreiner and Huyskens-Keil, 2006). In this sense, plums are considered a fruit class with high amounts of bioactive compounds or phytochemicals such as vitamins (A, C and E), anthocyanins and other phenolic compounds, and carotenoids (Stacewicz-Sapuntzakis et al., 2001), which contribute to the antioxidant capacity. On the other hand, large variations in the concentration of bioactive compounds at commercial harvesting depending on cultivar have been

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reported (Los et al., 2000; Tomás-Barberán et al., 2001; Gil et al., 2002; Kim et al., 2003; Cevallos-Casals et al., 2006). No literature on the changes occurring in the above bioactive compounds during storage of plums is available. The aim of this paper was to determine the changes in bioactive compounds during storage of a wide range of plum cultivars, both dark-purple and yellow pigmented. In addition, total antioxidant activity (TAA) has been determined in two separate fractions (hydrophilic and lipophilic) from peel and flesh tissues and the contribution of the several bioactive compounds in each extract will be discussed.

2. Material and methods

2.1. Plant material and experimental design

Eight Japanese type plum cultivars (*Prunus salicina* Lindl.) were selected, four of them had dark-purple peel: 'Angeleno', 'Black Amber', 'Black Diamond' and 'Larry Ann', and four had yellow peel: 'Golden Japan', 'Golden Globe', 'Songold' and 'TC Sun'. Among these cultivars, seven had yellow-orange flesh while 'Black Diamond' had red flesh. The plum cultivars were harvested from a farm (Los Frutales, Villena, Alicante, Spain) at commercial ripening stage based on size, colour and firmness.

For each cultivar, about 400 plums were manually picked and transferred immediately to the laboratory. Then, 240 homogeneous fruit (size, colour and with absence of any defect) were selected for each cultivar and randomly sorted into 12 lots of 20 fruit. All fruit were stored in a controlled cold chamber at 2 °C and RH of 85% in darkness. After 0, 7, 14, 21, 28 and 35 d cold storage, two lots were taken randomly. One was immediately analysed and the other was stored for a further 4 d at 20 °C to simulate commercial procedure (shelf-life, SL), in which the same analyses were carried out as follows.

For each sampling date and cultivar, fruit firmness and colour were measured individually. Following these determinations, five subsamples of four fruit each were made from each lot, in which ethylene production was quantified. The fruit from each subsample were then manually peeled to separate the peel from the flesh. The flesh tissue was cut in small pieces and a portion used to determine soluble solids concentration and titratable acidity. The separate peel and flesh were then immediately frozen and ground in liquid N_2. The samples were stored in at −40 °C until analysis of total phenolics, total anthocyanins, total carotenoids and total antioxidant activity was carried out.

2.2. Ethylene production

Ethylene production was measured by placing each subsample of four fruit in a 2-L glass jar hermetically sealed with a rubber stopper for 30 min. One millilitre of the atmosphere was withdrawn with a gas syringe, and the ethylene was quantified using a Shimadzu™ GC-2010 gas chromatograph (Kyoto, Japan), equipped with a flame ionisation detector (FID) and a 3 m stainless steel column with an inner diameter of 3.5 mm containing activated alumina of 80/100 mesh. Carrier gas was helium, column temperature was 90 °C, and injector and detector temperatures were 150 °C. Results were the mean ± SE of determinations for each subsample and expressed as ng kg^{-1} s^{-1}.

2.3. Fruit firmness and colour

Fruit firmness was measured on the fruit shoulder using a flat steel plate coupled with a texturometer (TX-XT2i Texture Analyzer, Stable Microsystems, UK) interfaced to a personal computer. A bevelled holder prevented bruising of the opposite side. For each fruit, the diameter was measured and then a force that achieved a 3% deformation of the fruit diameter was applied. Results were expressed as the force-deformation (N mm^{-1}) and were the mean ± SE.

Colour was determined in both peel and flesh of each fruit using the CIE Lab System in a Minolta colorimeter CR200 model using D65 illuminant (Minolta Camera Co., Japan). Results were the mean ± SE of three determinations for each fruit and expressed as Hue angle.

2.4. Soluble solids concentration, titratable acidity and ripening index

Total soluble solids concentration was determined in duplicate 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 %. Titratable acidity (g of malic acid equivalent per 100 g^{-1} fresh weight) was determined also in duplicate by automatic titration (785 DMP Titrino, Metrohm) with 0.1N NaOH up to pH 8.1, using 1 mL of diluted juice in 25 mL distilled H_2O. The ratio between soluble solids concentration and titratable acidity was considered as the ripening index (RI).

2.5. Bioactive compounds

Total anthocyanins were determined according to García-Viguera et al. (1999) adapted as previously reported (Serrano et al., 2005). Total anthocyanin was 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 expressed as mg kg^{-1} fresh weight, and were the mean ± SE of determinations made in duplicate in each one of the five subsamples.

Total carotenoids were extracted in duplicate according to Mínguez-Mosquera and Hornero-Méndez (1993). Briefly, 1 g of skin or 2 g of flesh tissues were extracted with acetone and shaken with diethyl ether and 10% NaCl to separate the two phases. The lipophilic phase was washed with Na_2SO_4 (2%), saponified with 10% KOH in methanol, and the pigments were subsequently extracted with diethyl ether, evaporated and then made up to 25 mL with acetone. Total carotenoids were estimated by reading the absorbance at 450 nm in a UNICAM Helios-α spectrophotometer (Cambridge, UK), and expressed as mg of β-carotene equivalent kg^{-1} fresh weight, taking into account the $\varepsilon^{1\%}_{cm} = 2560$ and the results were the mean ± SE.

Total phenolics were extracted according to Tomás-Barberán et al. (2001) using water:methanol (2:8) containing 2 mM NaF and quantified using the Folin–Ciocalteu reagent (Singleton et al., 1999) and results (mean ± SE) were expressed as mg gallic acid equivalent kg^{-1} fresh weight of determinations made in duplicate in each subsample.

Total antioxidant activity was quantified also in duplicate for each subsample according to Arnao et al. (2001), which enables the determination of TAA due to both hydrophilic (H-TAA) and lipophilic (L-TAA) compounds in the same extraction. Briefly, 1 g of peel or 2 g of flesh tissues were homogenized in 5 mL of 50 mM phosphate buffer pH 7.8 and 3 mL of ethyl acetate, and then centrifuged at 15,000 rpm for 15 min at 4 °C. The upper fraction was used for L-TAA while the lower fraction for H-TAA quantification. For 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), in which $ABTS^{\bullet+}$ radicals are generated and monitored at 730 nm. The decrease in absorbance after adding the skin or flesh extracts was proportional to TAA of the sample. A calibration curve (0–20 nmol) was

Table 1
Values of soluble solids concentration (%SSC), acidity (g eq. malic acid 100 g^{-1}), ripening index (RI, SSC/acidity ratio), firmness (N mm^{-1}) and colour of the peel and the flesh (Hue angle) at harvest and after 35 d of cold storage at 2 °C + 4 d at 20 °C (SL) in eight plum cultivars.

		Angeleno	Black Amber	Black Diamond	Larry Ann	Golden Japan	Golden Globe	Songold	TC Sun
SSC	At H	11.43 ± 0.06 aA	11.00 ± 0.07 aA	12.38 ± 0.07 aB	9.80 ± 0.15 aC	10.68 ± 0.05 aAC	13.43 ± 0.06 aD	13.95 ± 0.24 aD	14.85 ± 0.12 aE
	35 + SL	13.70 ± 0.05 bA	13.03 ± 0.09 bA	13.25 ± 0.03 bA	12.83 ± 0.08 bA	10.53 ± 0.02 aB	13.75 ± 0.17 aA	15.90 ± 0.04 bC	17.10 ± 0.14 bD
Acidity	At H	1.08 ± 0.02 aA	1.24 ± 0.01 aB	0.84 ± 0.01 aC	0.92 ± 0.05 aAC	1.60 ± 0.01 aD	0.58 ± 0.01 aE	0.72 ± 0.04 aC	0.72 ± 0.01 aC
	35 + SL	0.60 ± 0.04 bA	0.67 ± 0.02 bA	0.46 ± 0.05 bB	0.57 ± 0.02 bA	0.79 ± 0.06 bC	0.27 ± 0.01 bD	0.42 ± 0.02 bB	0.45 ± 0.01 bB
RI	At H	10.56 ± 0.27 aA	8.86 ± 0.09 aB	14.67 ± 0.19 aC	10.65 ± 0.84 aA	6.68 ± 0.05 aD	23.16 ± 0.05 aE	19.42 ± 0.68 aF	20.55 ± 0.43 aF
	35 + SL	22.83 ± 1.12 bA	19.95 ± 0.76 bA	30.14 ± 1.45 bB	22.58 ± 0.99 bA	13.59 ± 0.09 bC	50.27 ± 1.03 bD	38.28 ± 1.92 bE	37.85 ± 0.72 bE
Firmness	At H	8.67 ± 0.25 aA	6.66 ± 0.45 aB	9.93 ± 0.28 aC	5.42 ± 0.56 aB	7.24 ± 0.54 aD	8.71 ± 0.45 aA	5.74 ± 0.26 aB	8.89 ± 0.34 aA
	35 + SL	3.24 ± 0.18 bA	2.18 ± 0.13 bB	2.79 ± 0.21 bA	1.75 ± 0.14 bB	2.08 ± 0.15 bB	1.62 ± 0.11 bB	1.86 ± 0.21 bB	3.07 ± 0.27 bA
Peel Hue	At H	15.45 ± 0.71 aA	9.91 ± 0.72 aB	22.69 ± 1.81 aC	19.20 ± 1.83 aD	107.29 ± 0.53 aE	88.10 ± 1.01 aF	95.39 ± 0.49 aG	93.34 ± 0.62 aG
	35 + SL	5.46 ± 0.95 bA	2.14 ± 0.49 bB	11.15 ± 1.07 bC	7.35 ± 0.44 bD	100.88 ± 0.49 bE	68.87 ± 0.92 bF	84.30 ± 0.74 bG	85.13 ± 1.09 bG
Flesh Hue	At H	91.12 ± 0.46 aA	88.37 ± 0.25 aA	43.96 ± 1.06 aB	58.95 ± 1.35 aC	104.42 ± 0.47 aD	88.63 ± 0.52 aA	94.67 ± 0.52 aE	86.30 ± 0.46 aA
	35 + SL	69.01 ± 1.86 bA	62.56 ± 1.70 bB	12.50 ± 1.47 bC	20.03 ± 1.47 bD	98.74 ± 0.68 bE	74.34 ± 0.47 bF	78.23 ± 0.72 bF	76.56 ± 0.68 bG

For each parameter, different small letters showed significant differences ($p < 0.05$) between data at harvest (H) and after 35 d of cold storage + 4 d at 20 °C (35 + SL) for each cultivar, while different caps letters within each row showed significant differences ($p < 0.05$) among cultivars.

performed with Trolox ((R)-(+)-6-hydroxy-2,5,7,8-tetramethylcroman-2-carboxylic acid, Sigma, Madrid, Spain) in both aqueous or methanolic media for H-TAA and L-TAA, respectively. The results are expressed as the mean ± SE in mg of Trolox equivalent kg^{-1} fresh weight.

2.6. Statistical analysis

Experimental data were subjected to ANOVA analysis. 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). Linear regressions were performed between total antioxidant activity (either hydrophilic or lipophilic) and the bioactive compounds taking into account all sampling data (either peel or flesh).

3. Results

3.1. Physico-chemical parameters and ethylene production

Variables related to fruit quality such as SSC, acidity, firmness and colour (peel or flesh) were analysed at harvest and during cold storage and the subsequent SL. Since these parameters changed in a continuous way during storage, Table 1 shows the results obtained at harvest and at the end of the experiment (35 d at 2 °C + SL). Cultivars differed in quality variables at commercial harvest, with a range of 10–15% SSC and 0.6–1.6 g 100 g^{-1} acidity, and higher Hue values in yellow compared to dark-purple plums. During storage, acidity levels showed significant reductions for all cultivars, with losses averaging 40–45%, while SSC significantly increased with the exception of 'Golden Japan' and 'Golden Globe' plums. For all cultivars, softening occurred during storage with firmness losses of between 60% and 80% depending on cultivar, while Hue angle significantly diminished in both peel and pulp tissues, the decrease being higher in the dark-purple than in the yellow cultivars.

Fruit from four cultivars ('Angeleno', 'Black Diamond', 'Golden Japan' and 'TC Sun') had very low ethylene production (below 200 ng kg^{-1} s^{-1}) in both cold storage and SL. Of the remaining four cultivars, all had fruit with more ethylene production especially after SL, and the red-purple types were higher (>2800 ng kg^{-1} s^{-1}) than the gold types (Fig. 1).

3.2. Bioactive compounds

The anthocyanin content was only detected in fruit of the red-purple cultivars in both tissues (peel and flesh). For all cultivars, the levels of anthocyanins were always higher in the peel (20–40-fold) than in the pulp with significant differences among cultivars. At harvest, the highest peel anthocyanin concentration was found in 'Black Amber' (4370 ± 180 mg kg^{-1}) while the lowest occurred in 'Black Diamond' (1310 ± 100 mg kg^{-1}). With respect to flesh, the anthocyanin levels ranged between 34 ± 4 and 177 ± 7 mg kg^{-1} for 'Angeleno' and 'Black Diamond, respectively (Fig. 2). However, a similar change during postharvest storage was found for all cultivars. Thus, significant increases were found in the peel anthocyanins for all cultivars, while they remained unchanged in the flesh of 'Angeleno' and 'Black Amber' or with slight reductions in 'Black Diamond' and 'Larry Ann'.

The content of total phenolics was generally 2–5-fold higher in the peel than in the flesh for all cultivars. In addition, the red-purple cultivars had significantly higher phenolic concentrations than the yellow plums in the peel, while in the flesh the content of total phenolics was not dependent on the fruit colour (Fig. 3). Among red-purple plums, 'Black Amber' had the highest phenolic content (5210 ± 180 mg kg^{-1}) and 'Black Diamond' the lowest (2700 ± 130 mg kg^{-1}), while 'Songold' was the yellow cultivar with the highest concentration (1990 ± 140 mg kg^{-1}) and 'Golden Japan' with the lowest (1010 ± 80 mg kg^{-1}) total phenolics. During storage, either in cold or after SL, the same pattern of total phenolics was found for all cultivars, that is, significant increases from the initial values to those obtained after 35 d at 2 °C + SL (end of the experiment). This increase was also detected in the flesh phenolics, although the differences among cultivars were not so noticeable when compared with peel.

The determination of total carotenoids (expressed as β-carotene) showed that these pigments were present in the peel and flesh of either yellow or red-purple plums (Fig. 4). It is interesting to highlight that two red-purple cultivars, 'Larry Ann' and 'Black Amber', had the highest carotenoid content in both tissues: peel (99 ± 7 and 62 ± 5 mg kg^{-1}, respectively) and pulp (11 ± 0.2 and 10 ± 0.2 mg kg^{-1}, respectively). Among yellow plums, the highest content of carotenoids was observed in 'Golden Globe' in both peel and flesh (47 ± 0.7 and 8 ± 0.5 mg kg^{-1}, respectively), while the lowest was in 'Golden Japan' (13 ± 0.6 and 2 ± 0.1 mg kg^{-1}, for peel and flesh, respectively). During storage, the carotenoid behaviour seemed to be dependent on the cultivar peel colour, since significant increases were found in yellow plums while remaining

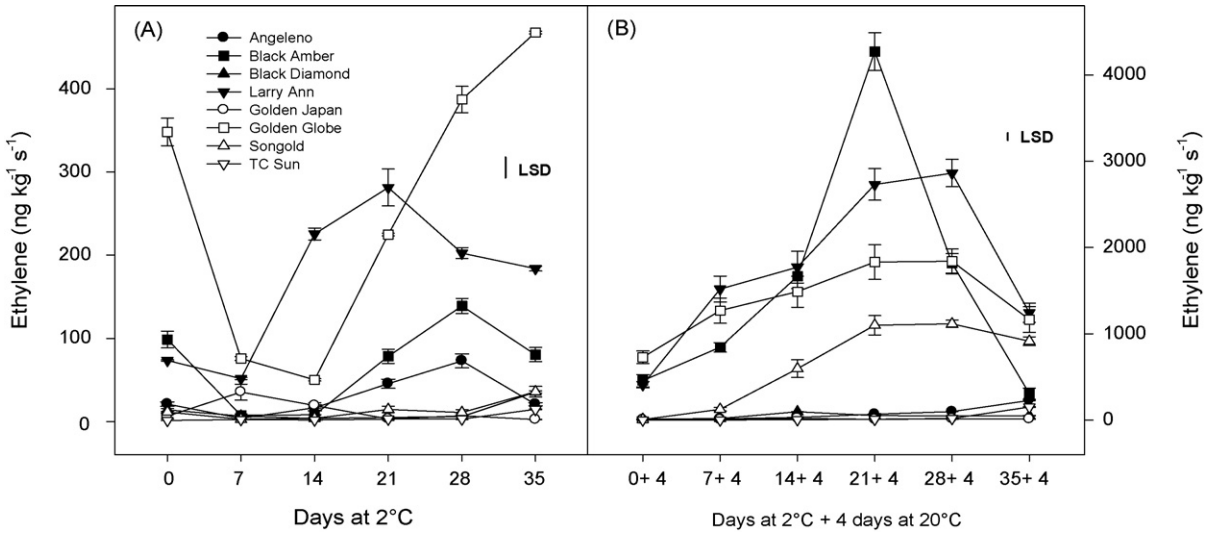

Fig. 1. Ethylene production rate during cold storage (A) and subsequent shelf-life (B) for the eight plum cultivars. Data are the mean ± SE and the LSD ($p < 0.05$) values are shown.

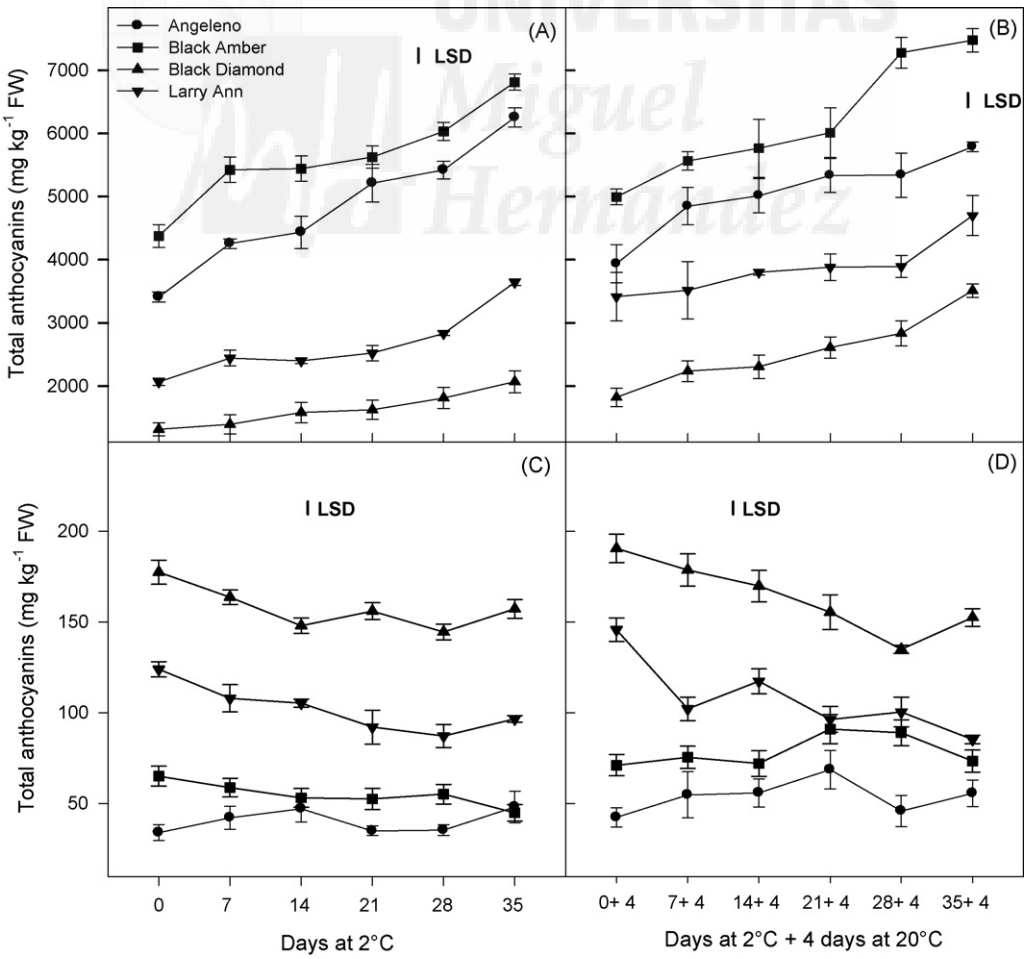

Fig. 2. Changes of total anthocyanin concentration in the peel during cold storage (A) and subsequent shelf-life (B) or in the pulp during cold storage (C) and subsequent shelf-life (D) of the dark-purple plums. Data are the mean ± SE and the LSD ($p < 0.05$) values are shown.

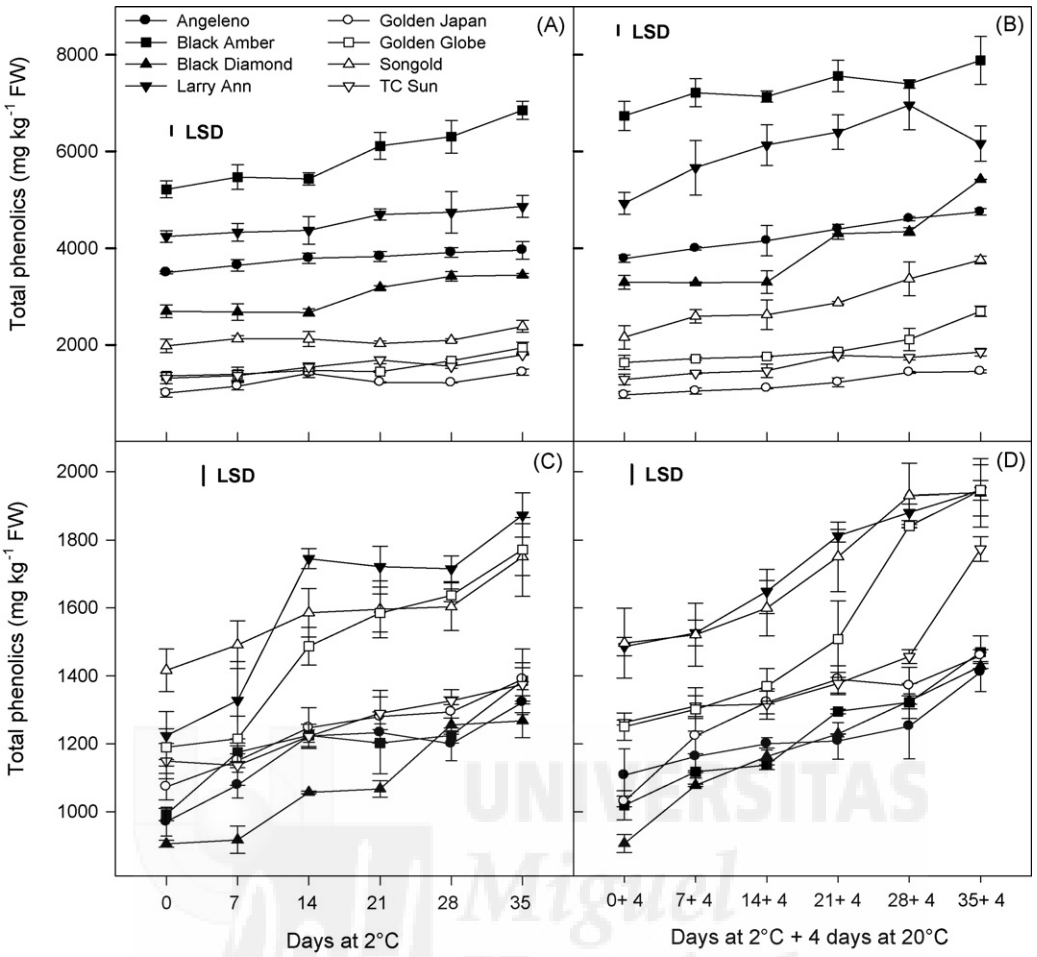

Fig. 3. Changes in total phenolic concentration in the peel during cold storage (A) and subsequent shelf-life (B) or in the pulp during cold storage (C) and subsequent shelf-life (D) of the eight plum cultivars. Data are the mean ± SE and the LSD ($p < 0.05$) values are shown.

unchanged in all dark-purple ones, for both peel and flesh tissues.

3.3. Total antioxidant activity

The total antioxidant activity was measured separately as hydrophilic (H-TAA) and lipophilic (L-TAA) fractions and both types were higher in peel than in flesh for all cultivars. With respect to H-TAA, the levels at harvest in the peel were significantly higher in the dark-purple than in the yellow plums, ranging from 5980 ± 560 to 594 ± 26 mg kg^{-1}, for 'Black Amber' and 'TC Sun', respectively. However, differences in H-TAA among cultivars were lower in the flesh, since their values ranged from 454 ± 12 mg kg^{-1} in 'Larry Ann' to 260 ± 30 mg kg^{-1} in 'TC Sun' (Fig. 5). During postharvest storage, H-TAA increased significantly in the peel of 'Black Amber', 'Black Diamond' and 'Larry Ann' cultivars, and in the flesh of 'Larry Ann' and 'Songold' cultivars, while no significant changes occurred in the remaining cases.

L-TAA at harvest was significantly different depending on cultivar; although for all of them the levels in the peel were 2–4 times higher than those found in the flesh (Fig. 6). Thus, the highest values in peel and flesh tissues were found in 'Larry Ann' fruit (4880 ± 160 and 1470 ± 90 mg kg^{-1}, respectively), while the lowest L-TAA in peel was recorded in 'Golden Japan' (720 ± 70 mg kg^{-1}) and in the flesh of 'Black Diamond' (190 ± 10 mg kg^{-1}) fruit. During postharvest storage, the yellow plums 'Golden Globe' and 'Songold' exhibited significant increases in the L-TAA of the peel, while no significant changes were found for the remaining yellow cultivars. However, in the dark-purple cultivars, significant decreases occurred. With respect to the flesh, no significant changes were obtained for the majority of the cultivars, and only 'Angeleno' showed a significant increase in L-TAA from the levels at harvest (430 ± 30 mg kg^{-1}) to those obtained after 35 d at 2 °C + SL (950 ± 40 mg kg^{-1}).

To know which of the bioactive compounds were contributing to TAA, linear regressions were performed taking into account all data (peel and pulp) obtained from all cultivars during cold storage and after SL. As expected, the results revealed that H-TAA was highly correlated with the content of total phenolics ($y = 1.52x - 163$; $R^2 = 0.949$) and at a lower dimension with anthocyanins ($y = 1.28x + 56$; $R^2 = 0.842$), while L-TAA was highly correlated with total carotenoids ($y = 46.72x + 33.55$; $R^2 = 0.882$).

4. Discussion

The eight plum cultivars were harvested at commercial ripening stage, although the levels in SSC, acidity, firmness and colour showed significant differences among cultivars. The average values are in agreement with those reported in a wide range of plum cultivars (Murray et al., 2005; Menniti et al., 2006; Crisosto et al., 2007; Paz et al., 2008). Although it has been reported that early cultivars have lower SSC than late ones in Californian plums (Crisosto et al., 2007), this did not seem to occur in Spanish cultivars, since two late plums ('Larry Ann' and 'Songold') showed the minimum and the maximum SSC.

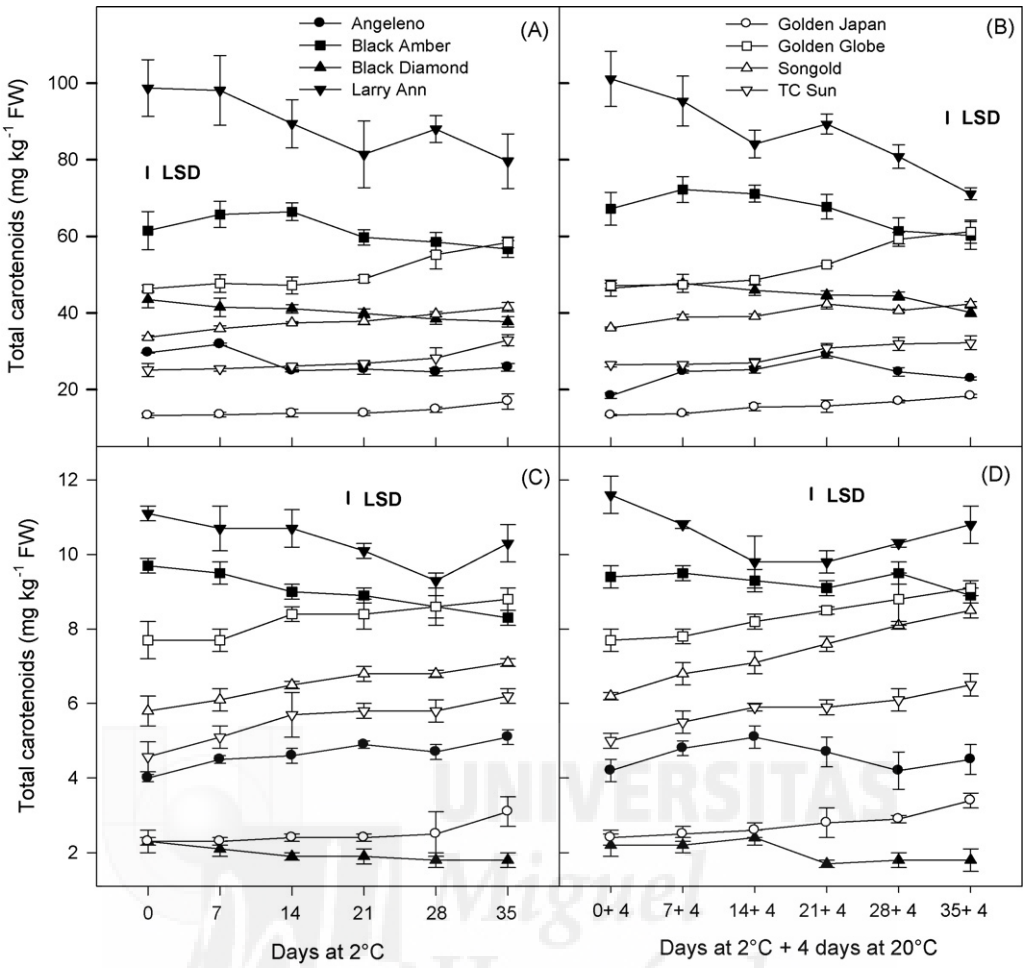

Fig. 4. Changes in total carotenoid concentration in the peel during cold storage (A) and subsequent shelf-life (B) or in the pulp during cold storage (C) and subsequent shelf-life (D) of the eight plum cultivars. Data are the mean ± SE and the LSD ($p < 0.05$) values are shown.

For all cultivars, the typical changes related to ripening (softening, colour changes, decrease in acidity and increase in both SSC and RI) occurred during cold storage and after SL, the magnitude of the change being similar for all plums and within the range of previous reports (Abdi et al., 1997; Martínez-Romero et al., 2003; Serrano et al., 2003; Valero et al., 2003; Murray et al., 2005; Menniti et al., 2006). Although these changes were similar for all cultivars, their ethylene production was clearly different, since after cold storage and subsequent SL, the ethylene production was high in the red cultivars 'Black Amber' and 'Larry Ann' and in the yellow ones 'Golden Globe' and 'Songold', while in 'Angeleno' and 'Black Diamond (red) and in 'Golden Japan' and 'TC Sun' (yellow) the ethylene production was very low. From these results, it can be concluded that the physiological ripening pattern was different depending on cultivar, since 'Angeleno', 'Black Diamond, 'Golden Japan' and 'TC Sun' behaved as suppressed-climacteric phenotypes without any increase in ethylene production during ripening. This behaviour has already been described for 'Golden Japan' (Zuzunaga et al., 2001; Abdi et al., 1997), 'Angeleno' (Khan et al., 2007; Candan et al., 2008) and for 'Black Diamond' (Serrano et al., 2003), while the suppressed-climacteric of 'TC Sun' plum is described for the first time in this paper. In contrast, 'Black Amber', 'Larry Ann', 'Golden Globe' and 'Songold' exhibited the typical climacteric ripening pattern reported for most plum cultivars (Abdi et al., 1997; Zuzunaga et al., 2001; Martínez-Romero et al., 2003; Serrano et al., 2003; Valero et al., 2003).

As commented above, there are numerous reports on the physico-chemical changes during plum storage. In addition, there is some evidence on the bioactive compounds and antioxidant properties of several plum cultivars harvested at commercial ripening stage as will be discussed later. However, as far as we are aware, this is the first paper in which the changes of these bioactive compounds and antioxidant activity during cold storage and subsequent SL are evaluated in a wide range of plums, both dark-purple and yellow ones. Moreover, all the literature on this issue deals with the antioxidant activity of hydrophilic plum extracts (H-TAA), while we have gone a further step with the analysis of antioxidant activity due to lipophilic compounds (L-TAA).

Anthocyanins were present only in the peel and pulp of the dark-purple plums although their levels at harvest were affected by cultivar, with concentrations within the range of previous studies (Tomás-Barberán et al., 2001; Cevallos-Casals et al., 2006; Manganaris et al., 2007; Vizzotto et al., 2007). The main anthocyanin reported for plum is cyanidin 3-glucoside followed by cyanidin 3-rutinoside, while peonidin 3-glucoside is found at low concentrations (Tomás-Barberán et al., 2001; Chun et al., 2003). More recently, another five minor anthocyanins have been identified: cyanidin-3-galactoside, 3-(6″-acetoyl)glucoside, pelargonidin 3-glucoside, cyanidin 3-xyloside, and cyanidin 3-(6″-acetoyl)glucoside (Wu and Prior, 2005).

The changes in anthocyanin content during cold storage + SL were similar for all dark-purple cultivars, showing a continuous

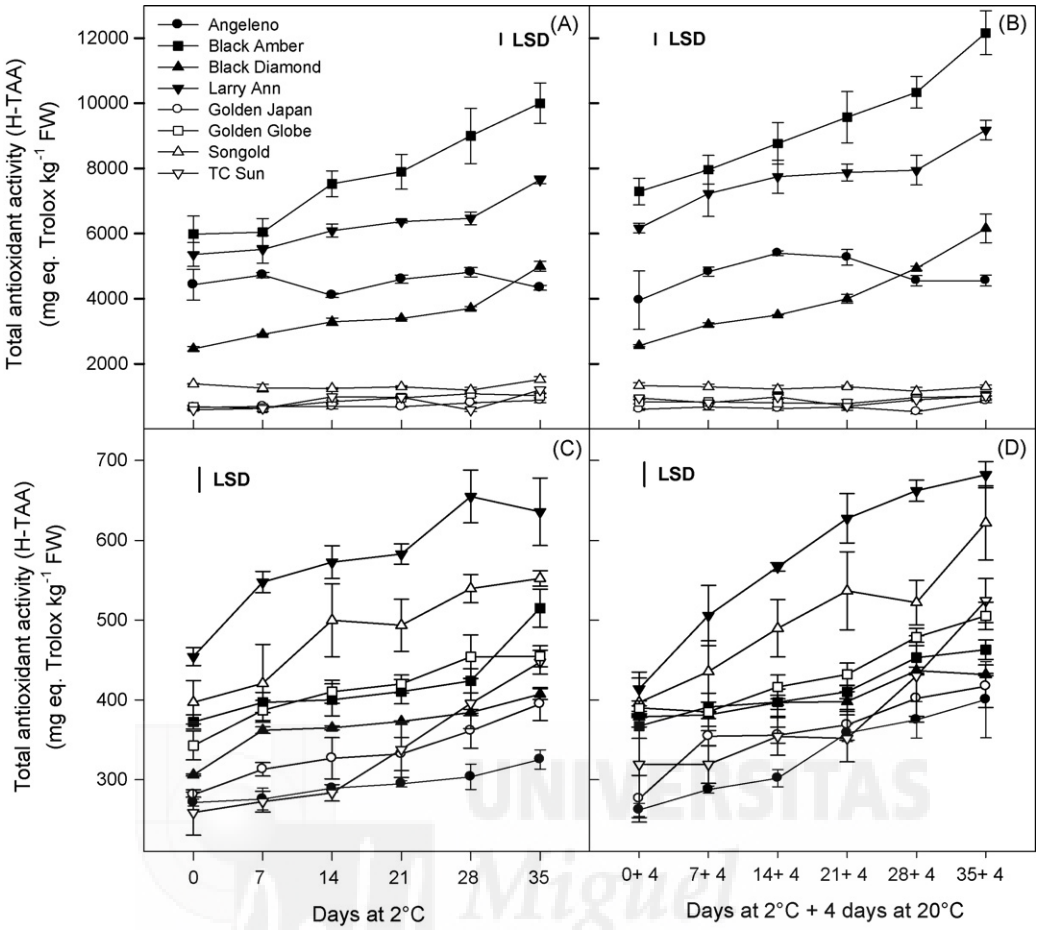

Fig. 5. Changes in total antioxidant activity from the hydrophilic extract (H-TAA) in the peel during cold storage (A) and subsequent shelf-life (B) or in the pulp during cold storage (C) and subsequent shelf-life (D) of the eight plum cultivars. Data are the mean ± SE and the LSD ($p < 0.05$) values are shown.

increase in the peel while remaining unchanged for most cultivars. The increase in peel anthocyanin was parallel to the reduction in Hue values, showing that these pigments contribute to the colour changes associated with the postharvest ripening process. Manganaris et al. (2007) reported increases in anthocyanin content in the pulp of 'Harrow Sun' plum as a consequence of chilling injury development at 5 °C, which was manifested as flesh reddening. However, in our plums no symptoms of chilling injury (internal browning or gel breakdown) were observed during storage. Thus, the enhancement of anthocyanins is attributed to normal ripening, as has been found in other commodities such as blueberry (Zheng et al., 2003), raspberry and strawberry (Kalt et al., 1999), fresh prunes (Hamauzu and Cume, 2005), and cherry (Gonçalves et al., 2004).

The total phenolics results revealed the same behaviour for all dark-purple and yellow plums, that is, a significant increase during storage plus SL, although the phenolic concentration in the peel was always higher in dark-purple than in yellow cultivars, while in the flesh this aspect could not be observed. Plums contain large amounts of phenolic compounds, and among them neochlorogenic acid (3-*O*-caffeoylquinic acid) is considered the major polyphenol followed by anthocyanins in a wide range of cultivars (Chun et al., 2003; Kim et al., 2003; Lombardi-Boccia et al., 2004). The pattern of phenolic changes during postharvest storage seems to be variable among species (and even cultivars) and affected by several postharvest treatments (Brovelli, 2006; Hamauzu, 2006). Low temperature storage has been found to increase total phenolic content in pomegranate arils (Mirdehghan et al., 2007) and apple (Leja et al., 2003), probably due to stimulation of the activity of some enzymes involved in phenolic biosynthesis by cold storage (Hamauzu, 2006). In addition, phenolic changes during storage seem to be dependent on the ripening stage at harvest, since total phenolics decreased in full red strawberry compared to white varieties (Shin et al., 2008).

Carotenoids form a large class of lipophilic molecules synthesised by plants and acting as coloured pigments in fruit. In plums, we have found carotenoids in the eight cultivars for both peel and pulp. Interestingly, the highest concentration of total carotenoids in the peel was found in 'Black Amber' and 'Larry Ann', which are dark-purple coloured. Thus, although the colour was due to anthocyanins, these cultivars are also enriched with yellow-orange pigments. However, the levels of carotenoids increased only in the yellow cultivars for both peel and flesh tissues during storage, which was correlated to the decrease in Hue colour parameter. This increase in carotenoid content has been also observed in stored kiwifruit (Tavarini et al., 2008), watermelon (Perkins-Veazie and Collins, 2006) and sapote (Alia-Tejacal et al., 2007). For comparative purposes, no references are available in plums about carotenoid changes during storage, since the levels of these pigments have been only determined at the commercial ripening stage. In these reports, important differences have been found depending on cultivar, although the levels were always higher in the peel than in the flesh, with the main carotenoid being β-carotene followed by β-cryptoxanthin (Gil et al., 2002; Lombardi-Boccia et al., 2004).

In recent years, increasing attention has been paid by consumers to the health-beneficial effects of fruit and vegetables intake due to their content in bioactive molecules with antioxidant

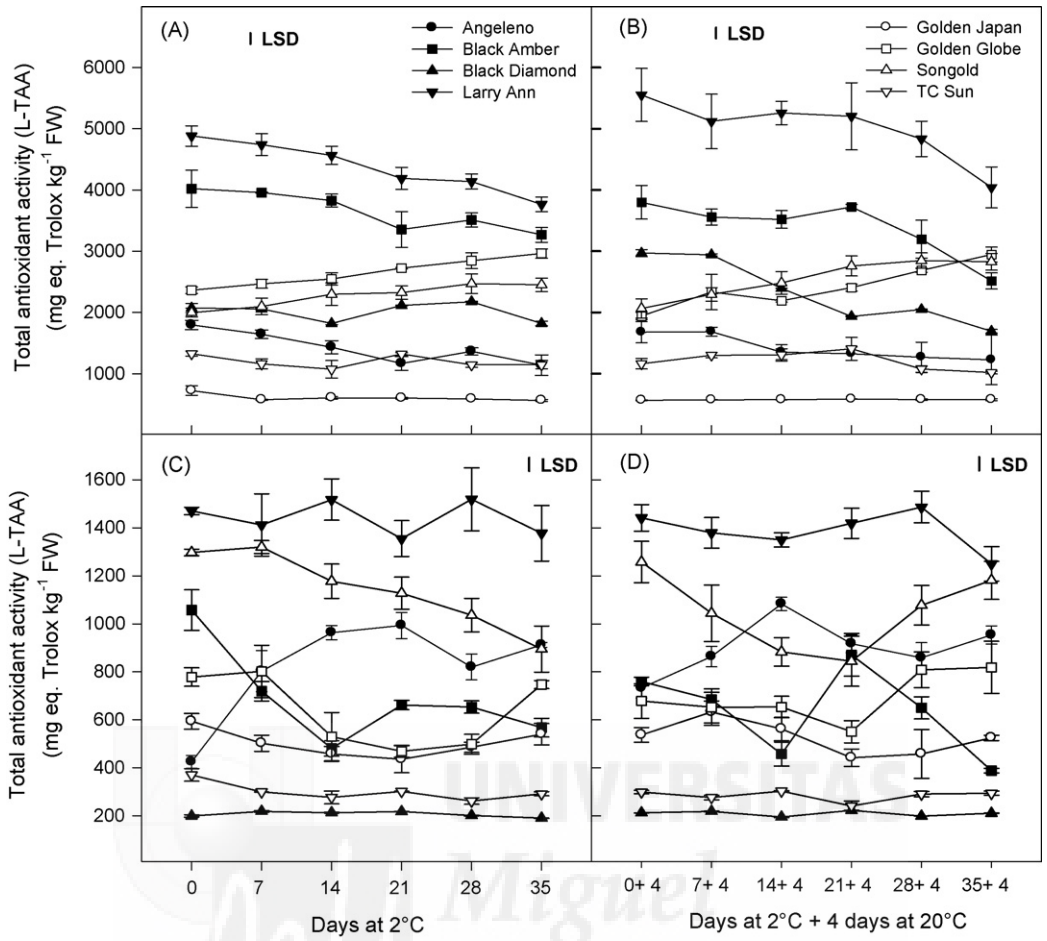

Fig. 6. Changes in total antioxidant activity from the lipophilic extract (L-TAA) in the peel during cold storage (A) and subsequent shelf-life (B) or in the pulp during cold storage (C) and subsequent shelf-life (D) of the eight plum cultivars. Data are the mean ± SE and the LSD ($p < 0.05$) values are shown.

properties, which can protect the human body against degenerative diseases (Brandt et al., 2004; Hung et al., 2004; Chen et al., 2007). Thus, currently the antioxidant content is becoming an increasingly important measure of quality in relation to fruit and vegetables. Among the substances with antioxidant activity, anthocyanins and other polyphenols (Espín et al., 2007; Stevenson and Hurst, 2007; Dorais et al., 2008), carotenoids (Perera and Yen, 2007), and several vitamins (A, C and E) are included (Hounsome et al., 2008). Most of the assays to measure the antioxidant capacity of fruit and vegetables have been carried out on hydrophilic extracts and only recently the antioxidant activity derived from water-soluble or lipo-soluble molecules separately is being evaluated in a few fruit and vegetables (Wu et al., 2004; Cano and Arnao, 2005; Scalzo et al., 2005; Cho et al., 2007). In plums, we have observed that H-TAA was higher than L-TAA in the peel, while the contrary occurred for the pulp. It is interesting that in plum, L-TAA comprised between 30% and 50% of the total antioxidant activity (sum of H-TAA and L-TAA), while in other fruit the contribution of L-TAA was much lower, as has been reported for strawberry, peach, apricot and apple (Scalzo et al., 2005). Taking into account the results of the linear regressions, the bioactive compounds that are contributing to H-TAA plums were mainly total phenolics, and within this group the anthocyanins were also important antioxidants. We found the results of the relationship between carotenoids and L-TAA very interesting, especially in the dark-purple cultivars, since the carotenoid concentration was much lower than total phenolics (ca. 10-fold) but exhibited an antioxidant activity (L-TAA) just twice as low (H-TAA). In previous studies, the relationship between total phenolics and antioxidant activity in a wide range of plum cultivars harvested at commercial ripening has been reported (Chun et al., 2003; Kim et al., 2003; Cevallos-Casals et al., 2006; Rupasinghe et al., 2006; Vizzotto et al., 2007). However, in these papers just the antioxidant activity of the hydro-soluble extracts was evaluated and the contribution of lipophilic substances, such as carotenoids and tocopherols which are important plant antioxidants (Cho et al., 2007), was not estimated.

In conclusion, with this paper we demonstrate that there are great variations in the content of the bioactive compounds and antioxidant activity depending on plum cultivar, although on a general basis, there are not important losses during prolonged cold storage and subsequent shelf-life. The peel of yellow cultivars had lower H-TAA than for red-purple fruit, which was correlated with phenolic compounds. Moreover, for a better evaluation of the antioxidant potential of plums, the contribution of carotenoids should be taken into account, especially when comparative studies among fruit and vegetables are carried out, since 'Larry Ann' and 'Black Diamond' had the highest L-TAA, attributable to the higher carotenoid concentration in both peel and pulp tissues.

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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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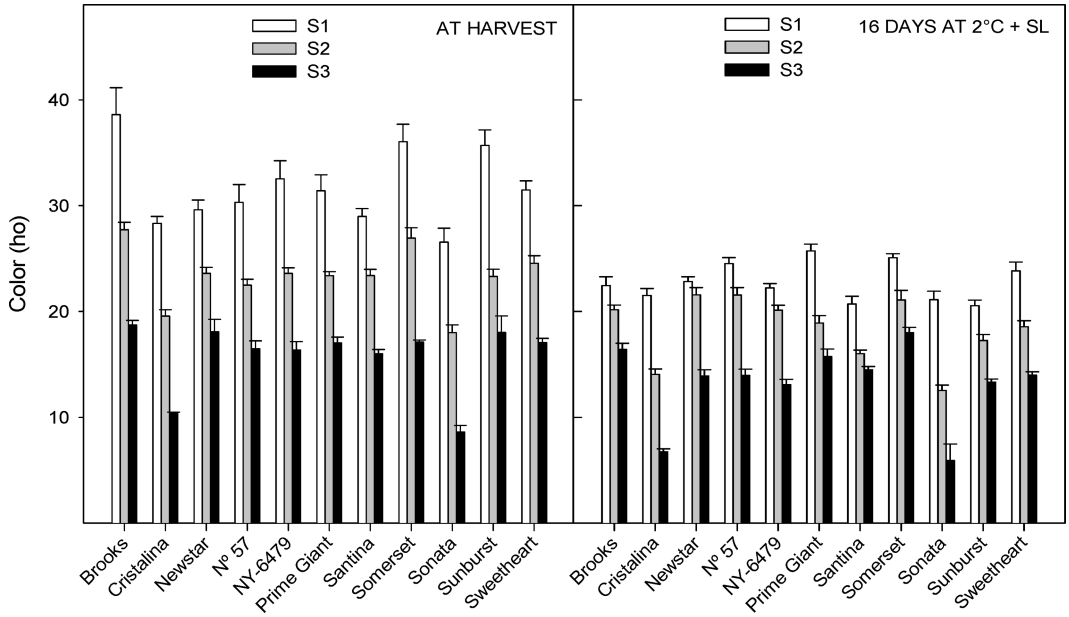

Figure 1. Color hue angle (ho) at harvest and after 16 days at 2 °C + 2 days at 20 °C (SL) of 11 sweet cherry cultivars harvested at three maturity stages (S1, S2, and S3). Data are the mean ± SE.

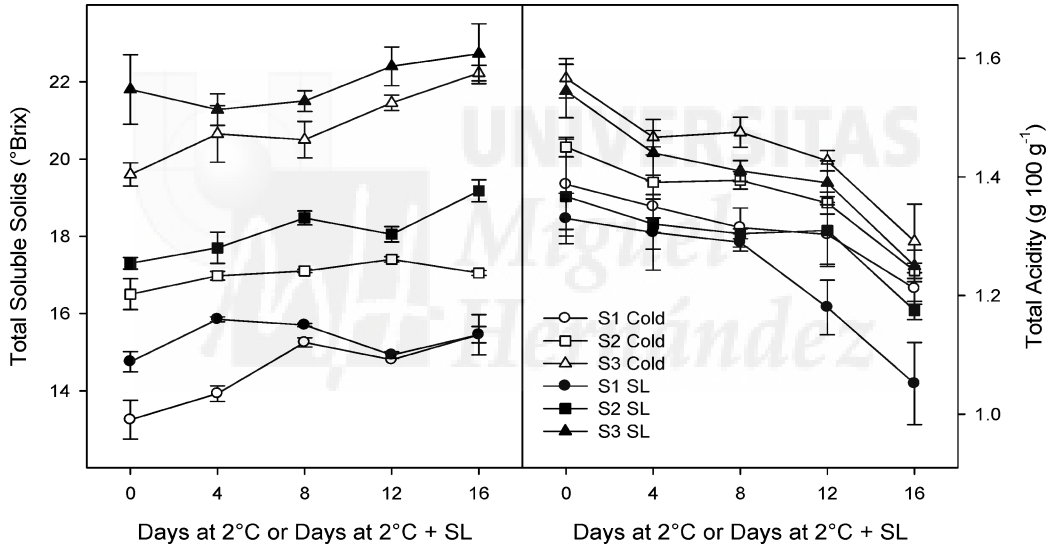

Figure 2. Evolution of total soluble solids and total acidity during storage at 2 °C or at 2 °C + 2 days at 20 °C (SL) of NY-6479 sweet cherry cultivar harvested at three maturity stages (S1, S2, and S3). Data are the mean ± SE.

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

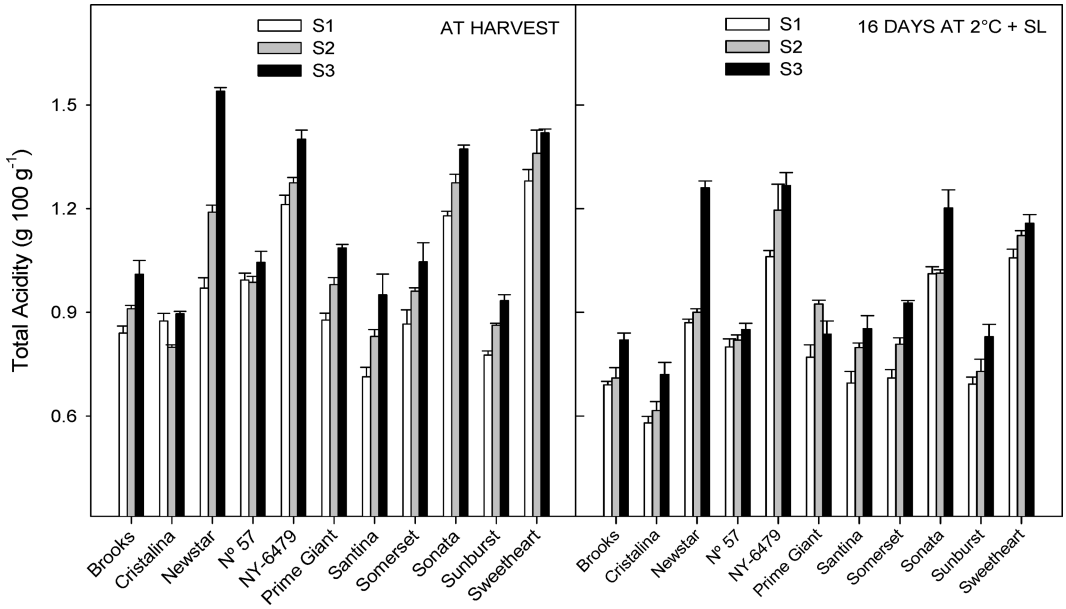

Figure 3. Total acidity at harvest and after 16 days at 2 °C (cold) + 2 days at 20 °C (SL) of 11 sweet cherry cultivars harvested at three maturity stages (S1, S2, and S3). Data are the mean ± SE.

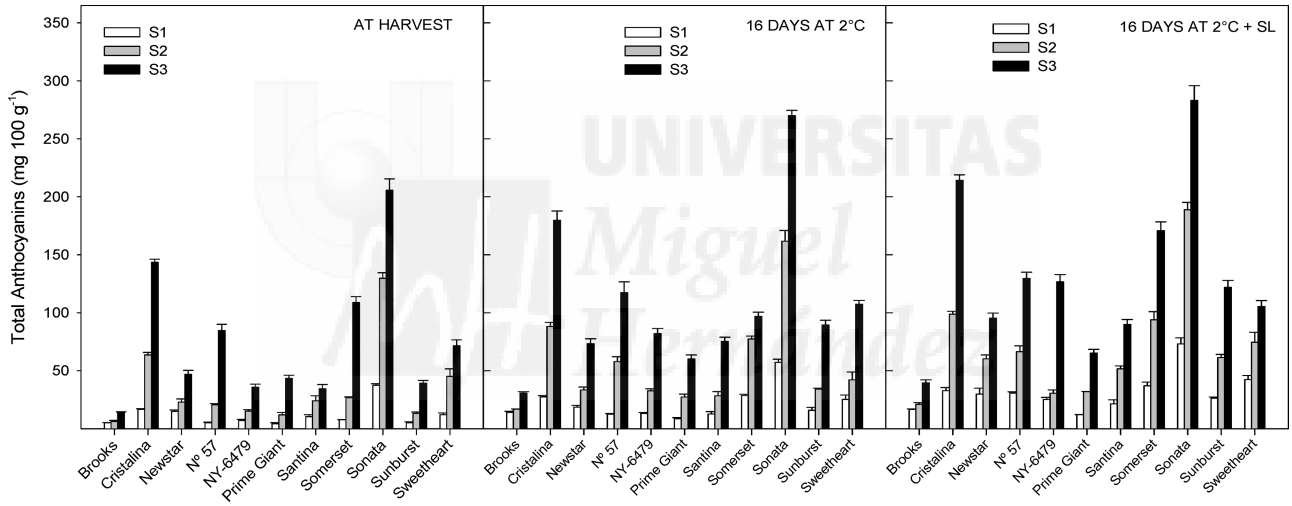

Figure 4. Total anthocyanins at harvest, after 16 days of storage at 2 °C and after 16 days at 2 °C + 2 days at 20 °C (SL) of 11 sweet cherry cultivars harvested at three maturity stages (S1, S2, and S3). Data are the mean ± SE.

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.

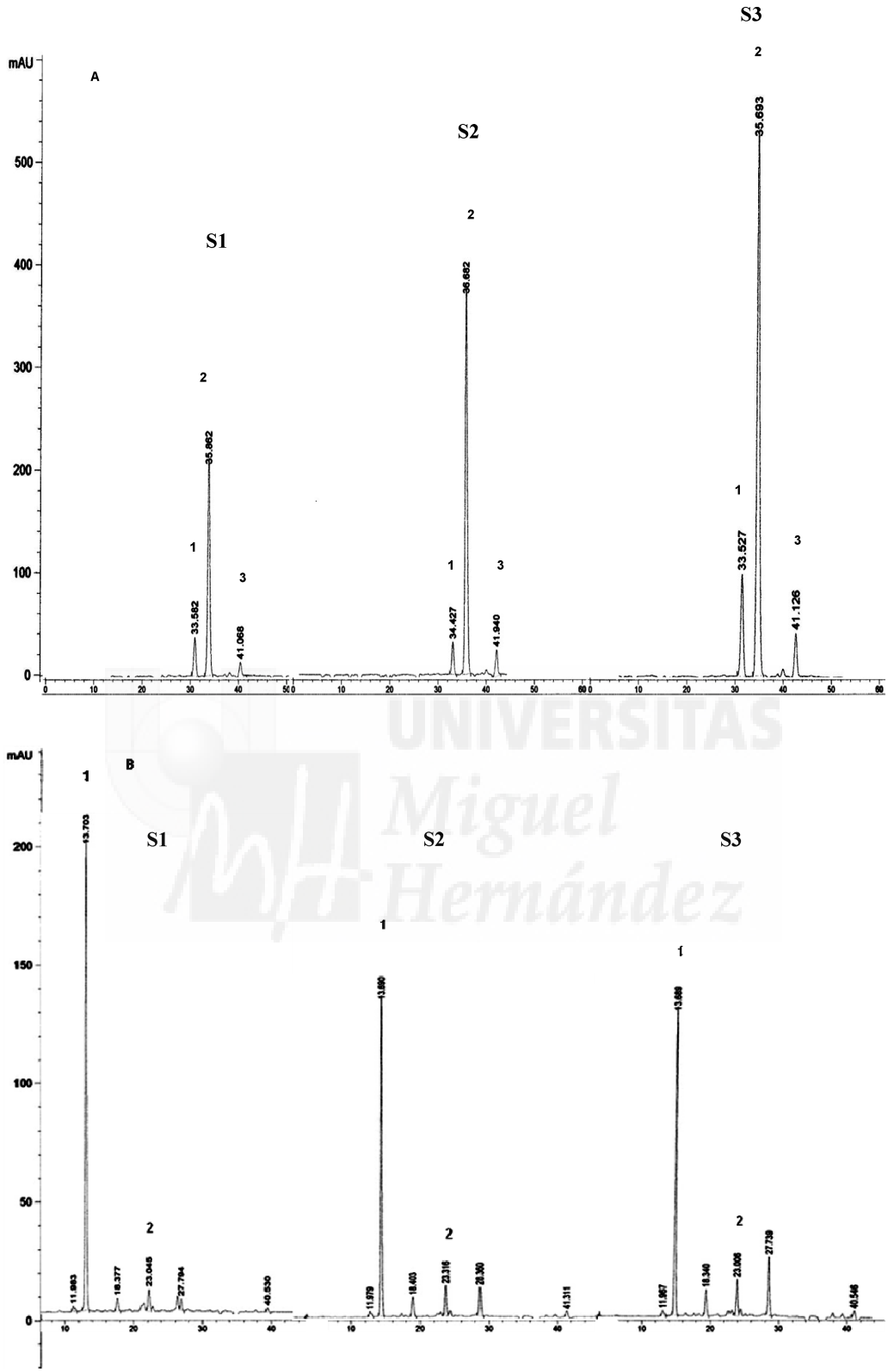

Figure 5. HPLC-DAD chromatograms for anthocyanins (**A**) and hydroxycinnamic acids (**B**) in Sonata sweet cherry harvested at 3 maturity stages (S1, S2, and S3). For anthocyanin peaks: (1) cyanidin 3-glucoside, (2) cyanidin 3-rutinoside, and (3) pelargonidin 3-rutinoside. For hidroxycinnamic acids: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

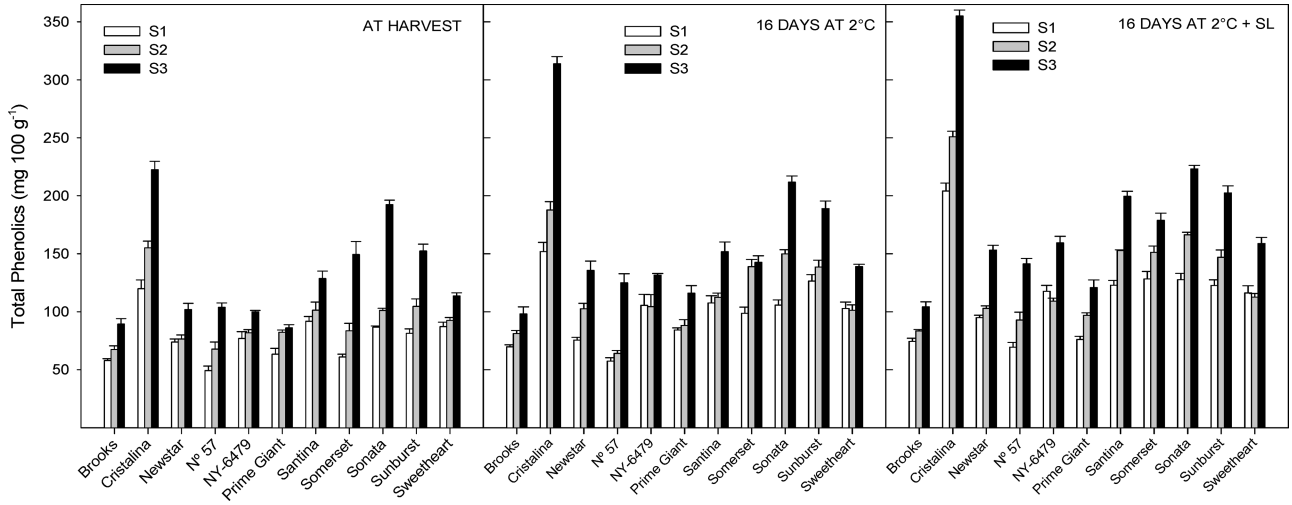

Figure 6. Total phenolics at harvest, after 16 days of storage at 2 °C and after 16 days at 2 °C + 2 days at 20 °C (SL) of 11 sweet cherry cultivars harvested at three maturity stages (S1, S2, and S3). Data are the mean ± SE.

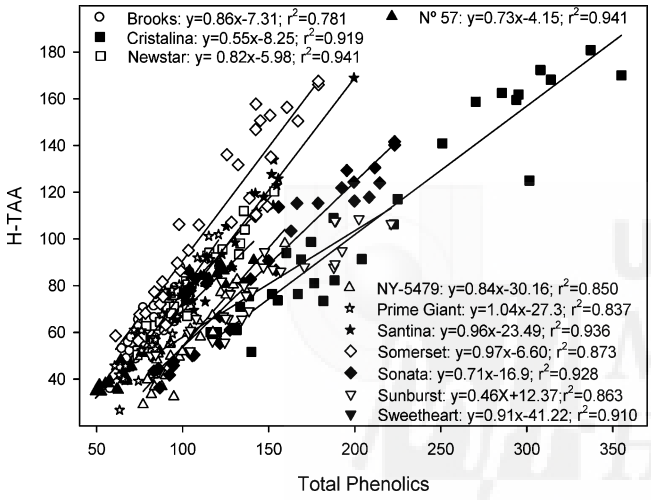

Figure 7. Correlation between hydrophilic total antioxidant activity (H-TAA) and total phenolics in 11 sweet cherry cultivars, taking into account data for all maturity stages and sampling data during storage at 2 °C and subsequent shelf life.

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

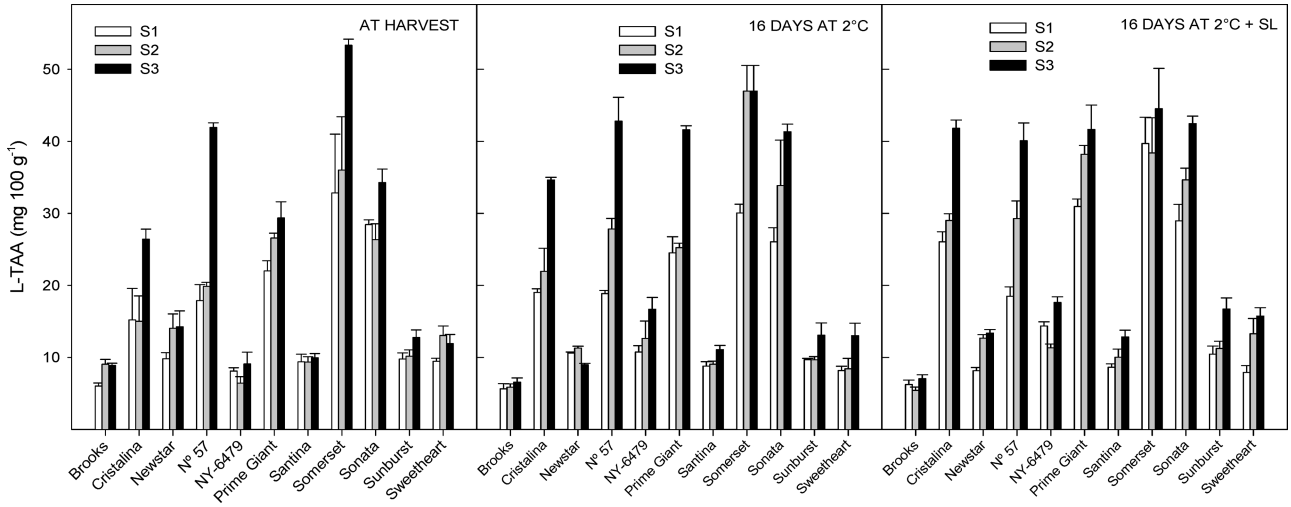

Figure 8. Lipophilic total antioxidant activity (L-TAA) at harvest, after 16 days of storage at 2 °C and after 16 days at 2 °C + 2 days at 20 °C of 11 sweet cherry cultivars harvested at three maturity stages (S1, S2, and S3). Data are the mean ± SE.

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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Modified atmosphere packaging of yellow and purple plum cultivars. 1. Effect on organoleptic quality

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ABSTRACT

The effect of modified atmosphere packaging (MAP) on maintaining plum quality was assayed on 4 plum cultivars (2 with yellow and 2 with purple skin) thermo-sealed in baskets with 2 distinct films (M and H) which differed in permeability. Fruit stored with macroperforated film served as a control and lost their quality attributes very rapidly, manifested by accelerated colour changes, softening, decrease in acidity and increase in total soluble solids. The use of MAP retarded these changes, the efficacy being higher in the fruit packed with film M compared with film H as a result of the delay in postharvest ripening, which could be attributed to the effect of MAP on reducing ethylene production rates. With the use of these packages, the storage time with fruit having high quality attributes could be increased 3–4 weeks more as compared with control plums.

1. Introduction

Plums are climacteric fruit with a limited postharvest storage life due to acceleration of quality loss, affecting some properties such as colour, texture, total soluble solids (TSS) and total acidity (TA). The use of cold storage is an appropriate mean to delay ripening by reducing ethylene production, respiration rate, pigment changes, softening, the increase in TSS and the reduction in TA (Crisosto et al., 2007; Guerra and Casquero, 2008; Díaz-Mula et al., 2009). Several attempts have been made to maintain plum quality during storage by combining cold storage and 1-methylcyclopropene (Valero et al., 2003), polyamine (Valero et al., 2002), heat and calcium (Serrano et al., 2004) treatments.

In some commodities, the use of modified atmosphere packaging (MAP) has shown positive effects on maintaining produce quality, such as in table grape (Martínez-Romero et al., 2003), broccoli (Serrano et al., 2006) and sweet cherry (Serrano et al., 2005), among others. MAP consists of sealing a certain amount of fruit or vegetable by using plastic films with selective permeability to CO_2, O_2 and water vapour diffusion. The commodity respiration increases CO_2 and decreases O_2 concentrations inside the packages, and transpiration rate increases water pressure. These modifications lead to reduction of weight loss, respiration rate and ethylene production, as well as to retard changes in properties related to the ripening process, and in turn, postharvest quality can be maintained for longer periods (Alam and Goyal, 2006; Artés et al., 2006; Valero and Serrano, 2010). However, little information exists on the use of MAP to preserve plum fruit quality. Recently, Guan and Dou (2010) reported some effects in reducing chilling injury symptoms in the sensitive plum cultivar 'Friar' using polyvinyl chloride (PVC) film, and Cantín et al. (2008) showed that the use of commercial box liners (LifeSpan) in the same cultivar retarded fruit quality deterioration. On the other hand, MAP exacerbated the effect of 1-MCP in reducing ethylene production and softening during cold storage of 'Tegan Blue' plums (Khan and Sing, 2008).

The aim of this paper was to perform a broad study to evaluate the effect of MAP on preserving fruit quality with 4 plum cultivars, 2 with purple ('Blackamber' and 'Larry Ann') and 2 with yellow skin ('Golden Globe' and 'Songold'). To fulfil this aim, fruit were placed in polypropylene baskets (rigid packages) and then thermo-sealed with two films with different gas permeabilities, or a macroperforated film (control). Gas composition inside the packages, fruit ethylene production and parameters related to quality (firmness, colour, TSS and TA) were analyzed weekly during 35 days of cold storage at 2 °C.

2. Material and methods

2.1. Plant material and experimental design

Two purple 'Blackamber' (BA) and 'Larry Ann' (LA) and two yellow skin 'Golden Globe' (GG) and 'Songold' (SG) plum (*Prunus salicina* Lindl.) cultivars were used. Fruit were harvested at the commercial ripening stage according to fruit properties at har-

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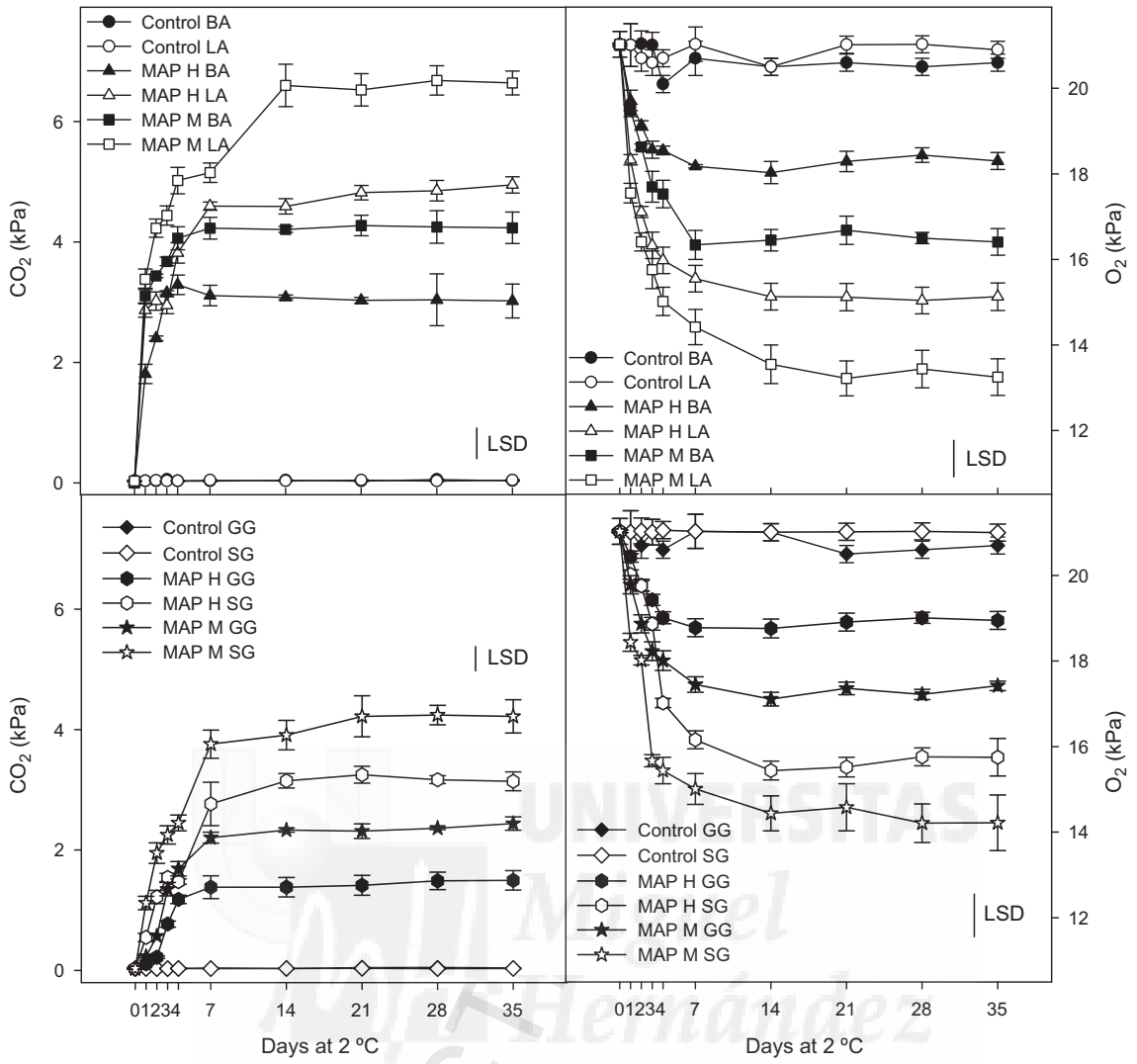

Fig. 1. Changes in CO_2 and O_2 concentrations inside MAP packages (with film H and M) containing the different plum cultivars: 'Blackamber' (BA), 'Larry Ann' (LA), 'Golden Globe' (GG) and 'Sungold' (SG). Data are the mean ± SE ($n = 5$). Least significant differences (LSDs) are shown.

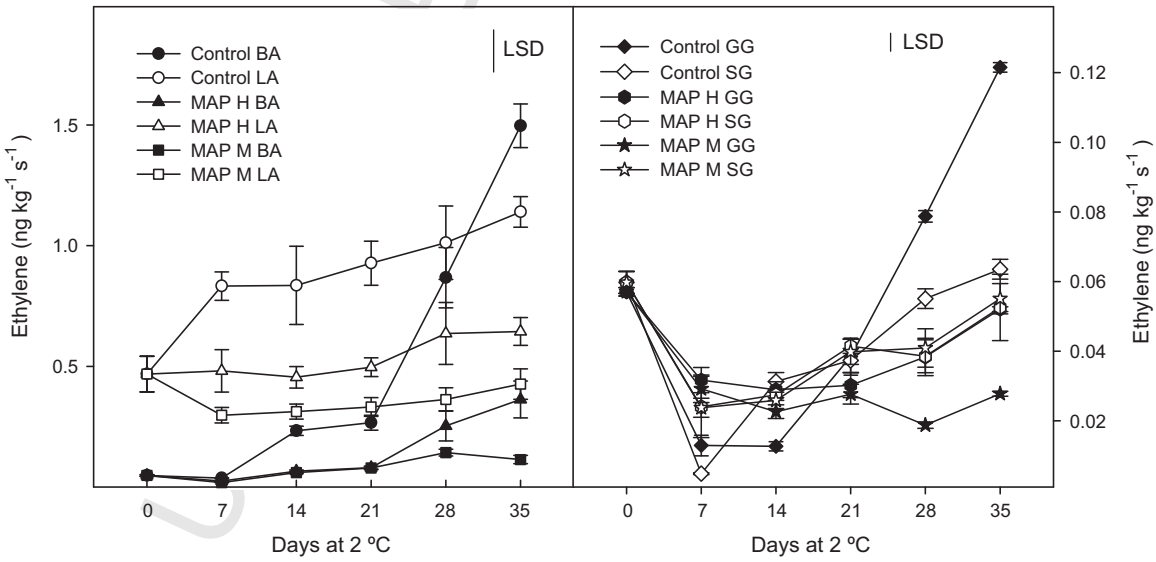

Fig. 2. Ethylene production rates of plums, 'Blackamber' (BA), 'Larry Ann' (LA), 'Golden Globe' (GG) and 'Sungold' (SG), during storage at 2 °C after removing the fruit from MAP packages (with film H and M). Data are the mean ± SE ($n = 5$). Least significant differences (LSDs) are shown.

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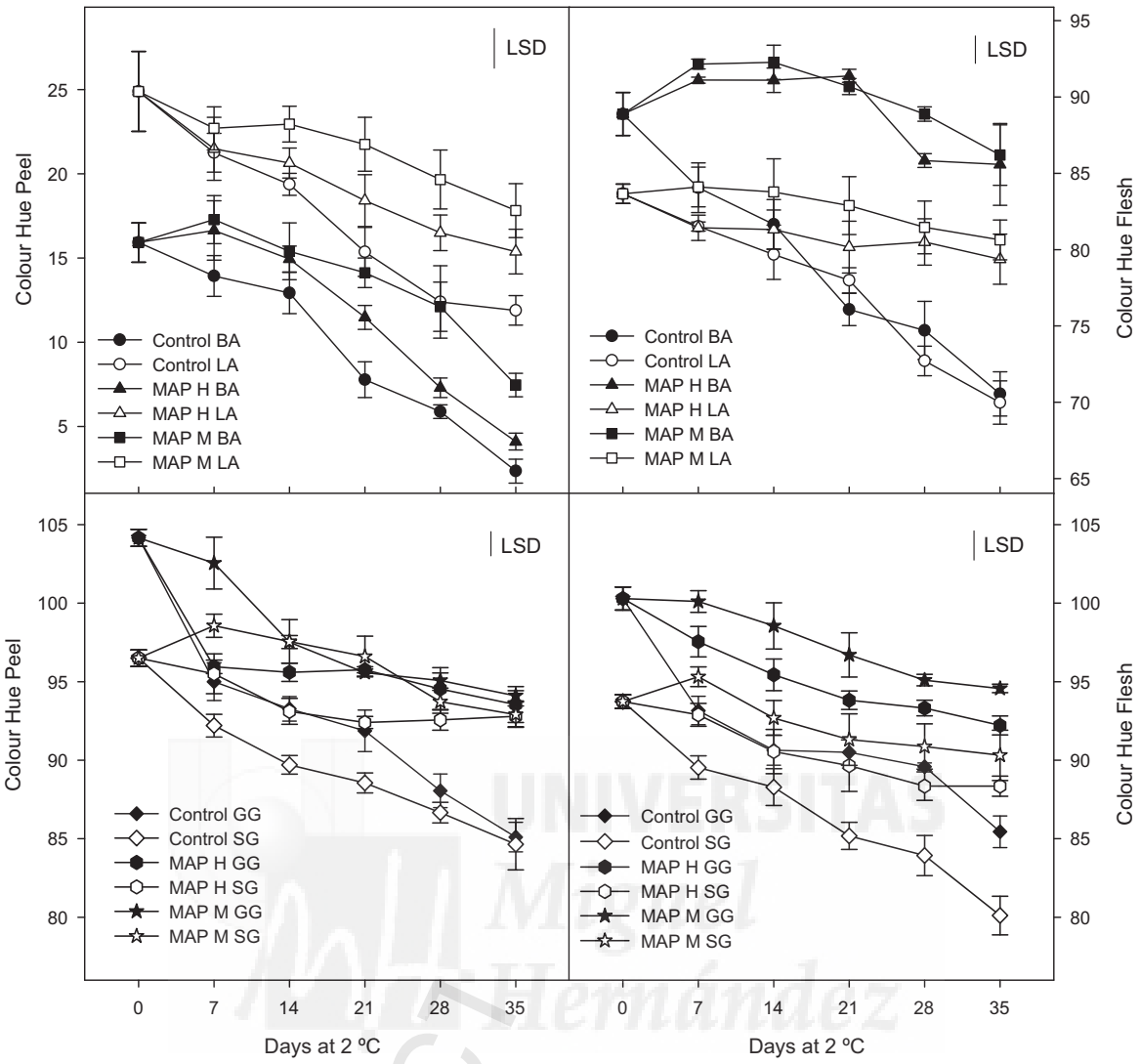

Fig. 3. Colour (Hue angle) changes in peel and flesh during storage of different plum cultivars, 'Blackamber' (BA), 'Larry Ann' (LA), 'Golden Globe' (GG) and 'Sungold' (SG), under MAP conditions (with film H and M). Data are the mean ± SE (n = 48). Least significant differences (LSDs) are shown.

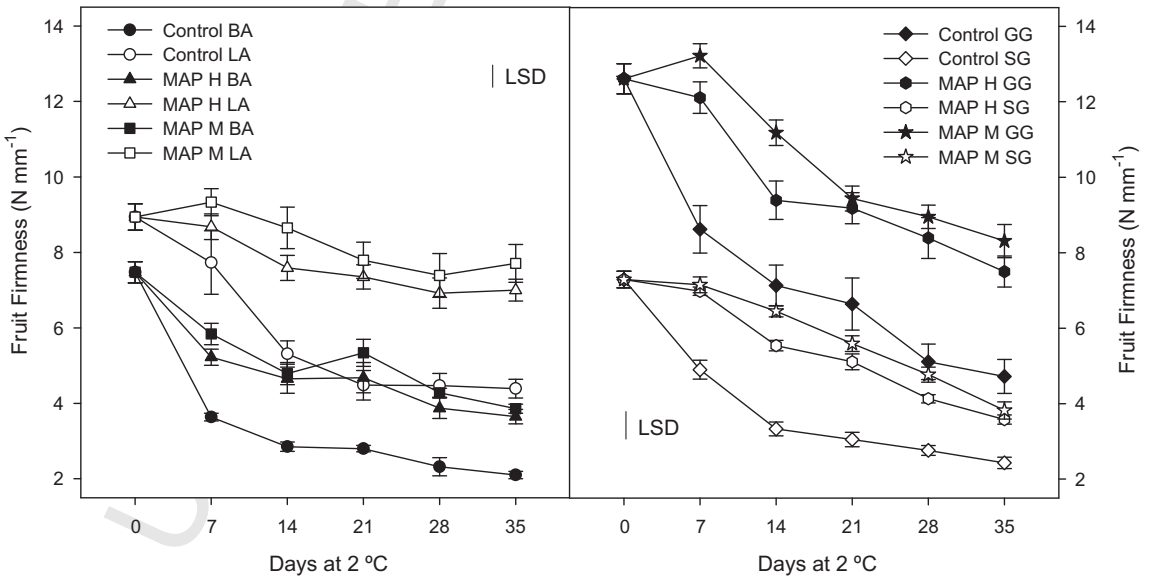

Fig. 4. Fruit firmness changes during storage of different plum cultivars, 'Blackamber' (BA), 'Larry Ann' (LA), 'Golden Globe' (GG) and 'Sungold' (SG), under MAP conditions (with film H and M). Data are the mean ± SE (n = 24). Least significant differences (LSDs) are shown.

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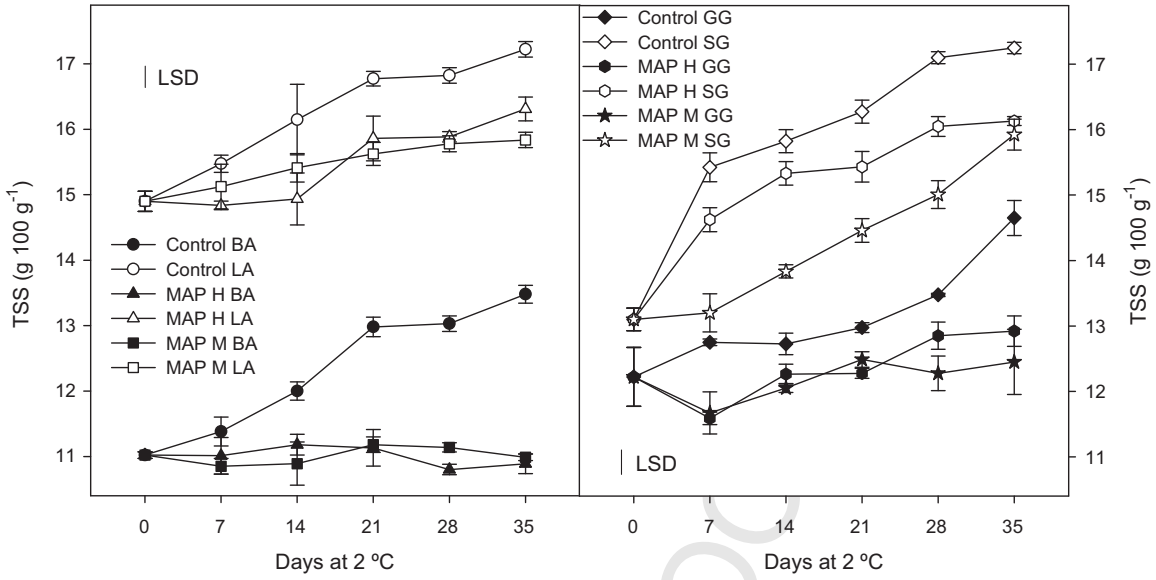

Fig. 5. Total soluble solids (TSS) changes during storage of different plum cultivars, 'Blackamber' (BA), 'Larry Ann' (LA), 'Golden Globe' (GG) and 'Sungold' (SG), under MAP conditions (with film H and M). Data are the mean ± SE ($n = 6$). Least significant differences (LSDs) are shown.

vest previously reported (Díaz-Mula et al., 2008). For each plum cultivar, about 650 fruit were manually picked and immediately transferred to the laboratory under cold conditions. Then, 504 fruit were selected based on homogeneous colour and size, and absence of visual defects, and distributed at random into 63 lots of 8 fruit (average weight 750–800 g mass for all cultivars). Three lots from each cultivar were used to determine physicochemical properties at harvest (day 0). The remaining lots were individually deposited in polypropylene baskets and divided into 3 batches for the following modified atmosphere packages (MAP): macro-perforated film as control, film H and film M, which were effective for MAP storage of broccoli and table grapes (Martínez-Romero et al., 2003; Serrano et al., 2006), and appropriate in preliminary studies on plums (data not shown). Baskets were thermo-sealed on top with the different films (total area of 336 cm^2, 14 cm × 24 cm).

The films properties were: macro-perforated polypropylene film (with 32 holes of 1.5 mm diameter per dm^2, and total perforated area of 0.56%); Film H was composed of polyester (12 μm)-polypropylene (50 μm), and Film M was composed of polyester (12 μm)-polypropylene (60 μm). Macro-perforated polypropylene was purchased from Plásticos del Segura S.L. (Spain), while Films H and M were purchased from Amcor Flexibles (Amcor, Barcelona, Spain) with permeability to O_2 < 100 mL O_2 m^{-2} day^{-1} atm^{-1}. All baskets were stored at 2 °C and 90% RH for 35 days. For each cultivar and film type, 5 lots were used to follow the gas composition inside the packages, which had silicone septa on the film surfaces, and 3 lots were chosen weekly at random for analytical measurements.

2.2. Gas composition

CO_2 and O_2 concentrations were quantified in duplicate in each basket by withdrawal of 1 mL of headspace atmosphere using an airtight syringe, and injected into a gas chromatograph GC 14B

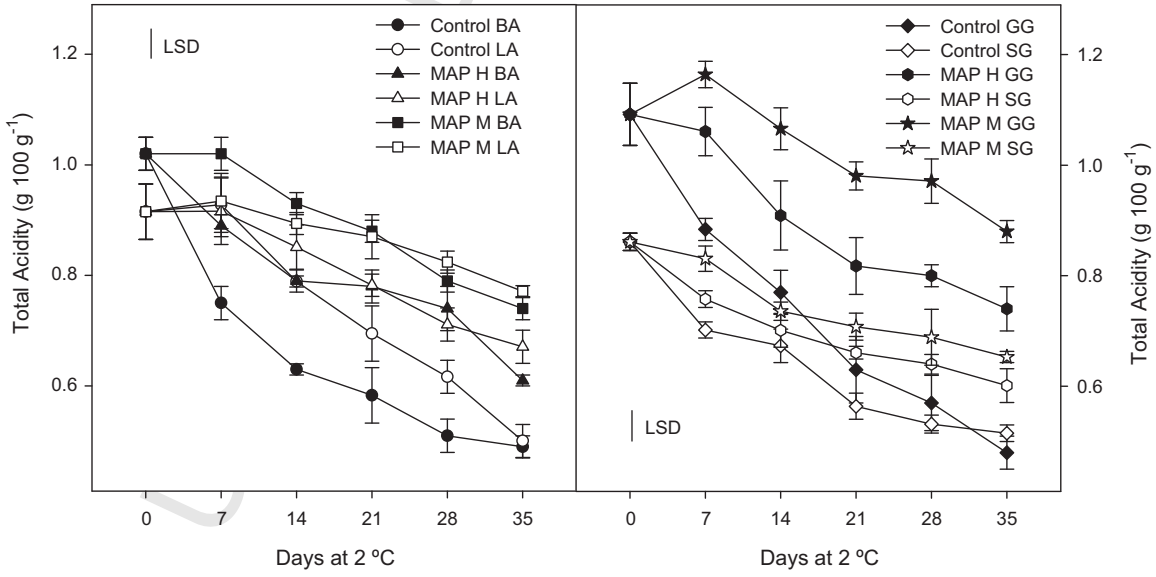

Fig. 6. Total acidity (TA) changes during storage of different plum cultivars, 'Blackamber' (BA), 'Larry Ann' (LA), 'Golden Globe' (GG) and 'Sungold' (SG), under MAP conditions (with film H and M). Data are the mean ± SE ($n = 6$). Least significant differences (LSDs) are shown.

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(Shimadzu, Tokyo, Japan) equipped with a thermal conductivity detector (TCD). CO_2 and O_2 were separated on a molecular sieve 5A column, 80–100 mesh (Carbosieve SII, Supelco Inc., Bellefonte, USA), of 2 m length and 3 mm i.d. Oven and injector temperature were 50 and 110 °C, respectively. Helium was used as carrier gas at a flow rate of 50 mL min^{-1}. Results (mean ± SE) were expressed as kPa O_2 and kPa CO_2 inside the baskets.

2.3. Respiration rate and ethylene production

For each sampling date, packages were opened and after 2 h ethylene production was measured at cold temperature (2 °C) by placing each replicate of 8 fruit in a 2-L glass jar hermetically sealed with a rubber stopper for 30 min. For ethylene quantification (in duplicate) 1 mL of the atmosphere was withdrawn with a gas syringe and then injected into a Shimadzu™ GC-2010 gas chromatograph (Kyoto, Japan), equipped with a flame ionisation detector (FID) and a 3 m stainless steel column with an inner diameter of 3.5 mm containing activated alumina of 80/100 mesh. The carrier gas was helium, at a flow rate of 30 mL min^{-1}, column temperature was 90 °C, and injector and detector temperatures were 150 °C. Results were the mean ± SE of determinations for each replicate and expressed as ng kg^{-1} s^{-1}. Similarly, respiration rate at harvest was quantified in duplicate by injecting 1 mL of the atmosphere into the gas chromatograph GC 14B described above. Results were expressed as μg kg^{-1} s^{-1} and were the mean ± SE. For day 0, respiration and ethylene rates were measured at 20 °C.

2.4. Fruit quality parameters

Fruit firmness was measured on the shoulder of each fruit using a flat steel plate coupled with a texturometer (TX-XT2i Texture Analyzer, Stable Microsystems, UK) interfaced to a personal computer. A bevelled holder prevented bruising of the opposite side. For each fruit, the diameter was measured and then a force that achieved a 3% deformation of the fruit diameter was applied. Results were expressed as the force-deformation (N mm^{-1}) and were the mean ± SE (n = 24). Colour was determined in both peel and flesh of each fruit using the CIE Lab System in a Minolta colorimeter CR200 model using D65 illuminant (Minolta Camera Co., Japan). Results were the mean ± SE (n = 48) of 2 determinations for each fruit and expressed as Hue angle.

After fruit firmness and colour determination, the fruit were peeled and a flesh tissue portion was taken along the equatorial fruit zone and then combined to obtain a homogenous juice sample for each replicate, in which total soluble solids (TSS) and total acidity (TA) were determined in duplicate. TSS concentration was determined with a digital refractometer Atago PR-101 (Atago Co. Ltd., Tokyo, Japan) at 20 °C, and expressed as g 100 g^{-1}. TA (g of malic acid equivalent per 100 g^{-1} fresh weight) 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 distilled H_2O. The ratio between soluble solids concentration and titratable acidity was considered as a ripening index (RI).

2.5. Statistical analysis

Experimental data were subjected to ANOVA analysis. Sources of variation were MAP packages and storage. The overall least significant differences (Fisher's LSD procedure, $p < 0.05$) were calculated and used to detect significant differences among packages and storage time. Values of LSD are showed in figures. All analyses were performed with SPSS software package v. 11.0 for Windows (SPSS, 2001).

3. Results and discussion

3.1. Gas composition and ethylene production

During storage, an increase in CO_2 and decrease in O_2 concentrations occurred inside the MAP packages, reaching a steady-state atmosphere after 7 days at 2 °C. Final gas concentrations were affected by the type of film used and the plum cultivar (Fig. 1). For all cultivars, the atmosphere modification was higher inside the packages with film M than with film H. For instance, in LA plums CO_2 concentrations at equilibrium were ≈6.5 and 5 kPa, while O_2 concentrations were ≈13 and 15 kPa, for film M and H, respectively. For each particular film (M or H), differences existed among cultivars in the equilibrium atmosphere. Thus, when film H was used, the highest CO_2 levels were found for LA (≈5 kPa), followed by SG (≈3.2 kPa), BA (≈3 kPa) and GG (≈1.5 kPa). For O_2 levels, in film H, the same order was obtained, with LA (≈15 kPa) and SG (≈16 kPa) being the cultivars with lower O_2 than BA and GG (≈18.5–19.0 kPa). These differences could be attributable to differences in the respiration rate at harvest, since LA showed the highest rate (12.19 ± 0.32 μg kg^{-1} s^{-1}) and GG the lowest (5.10 ± 0.21 μg kg^{-1} s^{-1}), while BA and SG had intermediate respiration rates (≈7 μg kg^{-1} s^{-1}). On the other hand, the steady-state atmospheres achieved could be considered as optimum for plum fruit, according to preliminary experiments and in agreement with previous work (Cantín et al., 2008).

With respect to ethylene production rates (measured under cold condition after opening the packages) significant differences were also found among cultivars and films, especially after the second sampling date (Fig. 2). Thus, in control fruit ethylene production was higher than in those stored under MAP conditions, the highest ethylene production being found at the end of the experiment in BA fruit (1.50 ± 0.09 ng kg^{-1} s^{-1}), followed by LA (1.14 ± 0.06 ng kg^{-1} s^{-1}) and the lowest in SG (0.06 ± 0.01 ng kg^{-1} s^{-1}). In addition, the results showed a significant inhibition in ethylene production rate in all plum cultivars which was higher in those stored under film M than under film H. For instance, in BA fruit, the ethylene production after 35 days of cold storage was 1.50 ± 0.09 ng kg^{-1} s^{-1} in control fruit and 0.36 ± 0.07 and 0.11 ± 0.02 ng kg^{-1} s^{-1} in those stored under MAP conditions with films H and M, respectively. These plum cultivars have the typical climacteric ripening pattern reported for most plum cultivars, with high ethylene production rates associated with plum ripening (Valero et al., 2002; Serrano et al., 2003; Díaz-Mula et al., 2008). For all cultivars, the effect of MAP on Q2 inhibiting ethylene production was higher in film M than in film H, which is attributed to both lower O_2 and higher CO_2 concentrations inside film M than in film H. Low O_2 is known to inhibit 1-aminocyclopropane-1-carboxylic acid oxidase (ACO), one of the key enzymes regulating ethylene biosynthesis, while CO_2 is an antagonist of ethylene action and impedes its autocatalytic synthesis when present at concentrations over 1 kPa, these effects being additive to those of low O_2 atmospheres (Artés et al., 2006). In other stone fruit, such as apricot, MAP also resulted in similar inhibition of ethylene biosynthesis (Pretel et al., 1993).

3.2. Fruit quality parameters

Colour (expressed as Hue angle) showed changes during storage which were manifested by reduction in colour Hue angle in both peel and flesh for all cultivars (Fig. 3), although initial Hue values were lower in the peel of purple (≈16 and ≈25 for BA and LA, respectively) than in yellow cultivars (≈96 and ≈104 for SG and GG, respectively). However, colour differences among cultivars at harvest were lower in the flesh than in the skin, since all were cultivars with yellow flesh, with Hue values of ≈100, 95, 90 and 85 for GG, SG,

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BA and LA, respectively. Colour changes were significantly delayed in those plums stored under MAP conditions, with generally lower changes in both peel and flesh in plums packed with film M than in those with film H. The delay in colour change associated with the postharvest ripening process has also been shown in other fruit, such as mango (Pesis et al., 2000), table grape (Martínez-Romero et al., 2003) and loquat (Amorós et al., 2008) under MAP conditions. These effects could be attributed to the delay in anthocyanin and carotenoid biosynthesis induced by MAP, according to a previous report (Artés et al., 2006), which was higher in those plums stored under the more impermeable film.

With respect to fruit firmness, differences existed at harvest among cultivars with GG being the firmest cultivar ($12.61 \pm 0.40\ N\ mm^{-1}$) followed by LA ($8.94 \pm 0.75\ N\ mm^{-1}$), while BA and SG showed the lowest firmness values ($\approx 7.4\ N\ mm^{-1}$). For all cultivars, fruit firmness decreased significantly during storage with firmness losses in control fruit being ≈72% for BA, ≈65% for GG and SG, and 51% for LA at the end of the experiment (Fig. 4). The softening process was significantly delayed by the use of MAP, with slightly higher fruit firmness retention in those plums packed in film M than in film H. The effect of MAP on delaying softening could be an ethylene-mediated effect, since it was higher in plums under film M, in which ethylene production was inhibited to a greater extent than in those plums under film H. However, a direct effect of high CO_2 and low O_2 on inhibiting cell-wall degrading enzymes could not be discounted, since delay in softening under MAP conditions has been observed in both climacteric fruit, such as kiwifruit, apricot, peach and nectarine (Pretel et al., 1993; Agar et al., 1999; Akbudak and Eris, 2004), and non-climacteric ones, such as strawberry and table grape (García et al., 1998; Martínez-Romero et al., 2003).

Total soluble solids (TSS) increased during storage of control fruit in all plum cultivars, the increases being significantly lower in those plums stored under MAP conditions. No significant differences existed between M and H films, with the exception of SG plums, in which TSS accumulation was lower in plums packaged with film M than with film H (Fig. 5). In addition, TSS remained unchanged during storage in BA plums under MAP conditions, irrespective of the film used. In contrast, total acidity (TA) decreased during storage for all plum cultivars and films assayed, although acidity losses were retarded by the use of MAP with higher acidity retention in the plums packaged with film M than in those with film H (Fig. 6). Accordingly, in peach and nectarine, the decrease in total acidity was delayed by the use of MAP, as well as the increase in TSS (Akbudak and Eris, 2004), and in loquat the decrease in individual sugars and malic acid was delayed in MAP packages as compared with fruit stored in open air (Amorós et al., 2008). These results showed a clear effect of MAP on decreasing fruit metabolism, including respiration rate, leading to maintenance of respiration substrates and in turn to a delay of the postharvest ripening process. In fact, the ratio between TSS and TA (TSS/TA) considered as a fruit ripening index, increased sharply during storage in control plums, with initial values of ≈10 (for BA and GG) and ≈16 (for LA and SG) to final values of ≈34 for LA and SG, and ≈29 for BA and GG. However, with the use of both films the values of TSS/TA were lower than in control plums, and even differences were obtained between the 2 film types, with the lowest TSS/TA ratio for those plums packaged with film M, which were ≈15 for GG and BA, ≈18 for LA and ≈24 for SG.

In conclusion, the MAP technique was useful to delay the ripening process of plum cultivars through a delay in the changes in colour, and the losses of firmness and acidity, and in turn an extension of shelf-life could be achieved (3–4 weeks more depending on the evaluated parameter) as compared with controls. In most cases the effect was higher in those plums packaged with film M than film H, probably due to the higher inhibition of ethylene production by the use of film M. However, the effect of these packages on the content of bioactive compounds and antioxidant activity of plum stored under MAP conditions deserves further research, which has not been carried out to date.

Acknowledgements

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Modified atmosphere packaging of yellow and purple plum cultivars. 2. Effect on bioactive compounds and antioxidant activity

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ABSTRACT

Changes in bioactive compounds (total phenolics and total carotenoids, and individual anthocyanins) as well as total antioxidant activity (TAA) in separate fractions; hydrophilic (H-TAA) and lipophilic (L-TAA), in the peel and the flesh of 2 purple and 2 yellow skin plum cultivars under modified atmosphere packaging (MAP) conditions, using two films with different gas permeability, were studied. Results revealed that in all cultivars, total phenolics and H-TAA increased in the peel and flesh during storage, as well as the two identified anthocyanins: cyanidin-3-glucoside and cyanidin-3-rutinoside in the purple cultivars. These changes were significantly delayed in fruit stored under MAP conditions. Total carotenoids and L-TAA increased in the yellow cultivars (in both peel and flesh) while decreases were observed in the purple cultivars, these changes also being delayed by the use of MAP. Positive correlations were found between H-TAA and total phenolics and between L-TAA and total carotenoids.

1. Introduction

Modified atmosphere packaging (MAP) has been poorly studied in plums, although recent papers have shown efficacy of MAP in alleviating chilling injury symptoms in 'Friar' plums through reducing softening and browning (Cantín et al., 2008; Guan and Dou, 2010), and in retarding parameters related with ripening, such as dehydration, respiration rate, colour changes, softening, acidity losses and the increase in total soluble solids (Díaz-Mula et al., 2011).

Plums are known to contain large amounts of phytochemicals and are considered a good source of natural antioxidants in our daily diet. It has been demonstrated that plums have higher total antioxidant capacity (TAA) than many other common fruit such as apple, tomato, and peach, and similar capacity to that of strawberry and blueberry (Wang et al., 1996; Wu et al., 2004; Valero and Serrano, 2010). Phenolic compounds, especially flavonoids, phenolic acids and anthocyanins, have been considered the most important compounds contributing to TAA (Gil et al., 2002; Kim et al., 2003; Cevallos-Casals et al., 2006). The TAA reported in these and other papers are only related to hydrophilic compounds, since extractions were carried out using hydrophilic solvents. However, when extraction is performed with both hydrophilic and lipophilic solvents, it can be shown that lipophilic compounds, such as carotenoids, are also important antioxidant compounds in plums, even in red-purple cultivars (Díaz-Mula et al., 2008, 2009).

Currently, there are only a few reports on the effect of MAP conditions on the content and changes in bioactive compounds with antioxidant activity in fruit and vegetables. Broccoli heads stored with polypropylene films showed lower losses in ascorbic acid, total phenolics and antioxidant activity than those stored in air (Serrano et al., 2006). In loquats, MAP was also effective in suppressing ascorbic acid losses that occurred in control fruit (Amorós et al., 2008), and in papaya, the antioxidant potential was maintained under MAP conditions due to retention of both ascorbic acid and lycopene (Singh and Rao, 2005).

In plums, there is only one report in which MAP delayed the increase in anthocyanin and phenolic content in the flesh of 'Friar' plums (Guan and Dou, 2010), and thus more in depth studies are necessary. In this sense, the aim of this paper was to study the effect of MAP on the changes in bioactive compounds (total phenolics and total carotenoids, and individual composition of anthocyanins) as well as the total antioxidant activity (TAA) in separate fractions, hydrophilic (H-TAA) and lipophilic (L-TAA), in the peel and the flesh of 4 plum cultivars. The cultivars 'Blackamber' (BA) and 'Larry Ann' (LA) have purple skin and 'Sungold' (SG) and 'Golden Globe' (GG) have yellow skin, although all of them have yellow coloured flesh.

2. Material and methods

2.1. Plant material and experimental design

Plum fruit (*Prunus salicina* Lindl.) from 'Blackamber' (BA), 'Larry Ann' (LA), 'Golden Globe' (GG) and 'Songold' (SG) cultivars were

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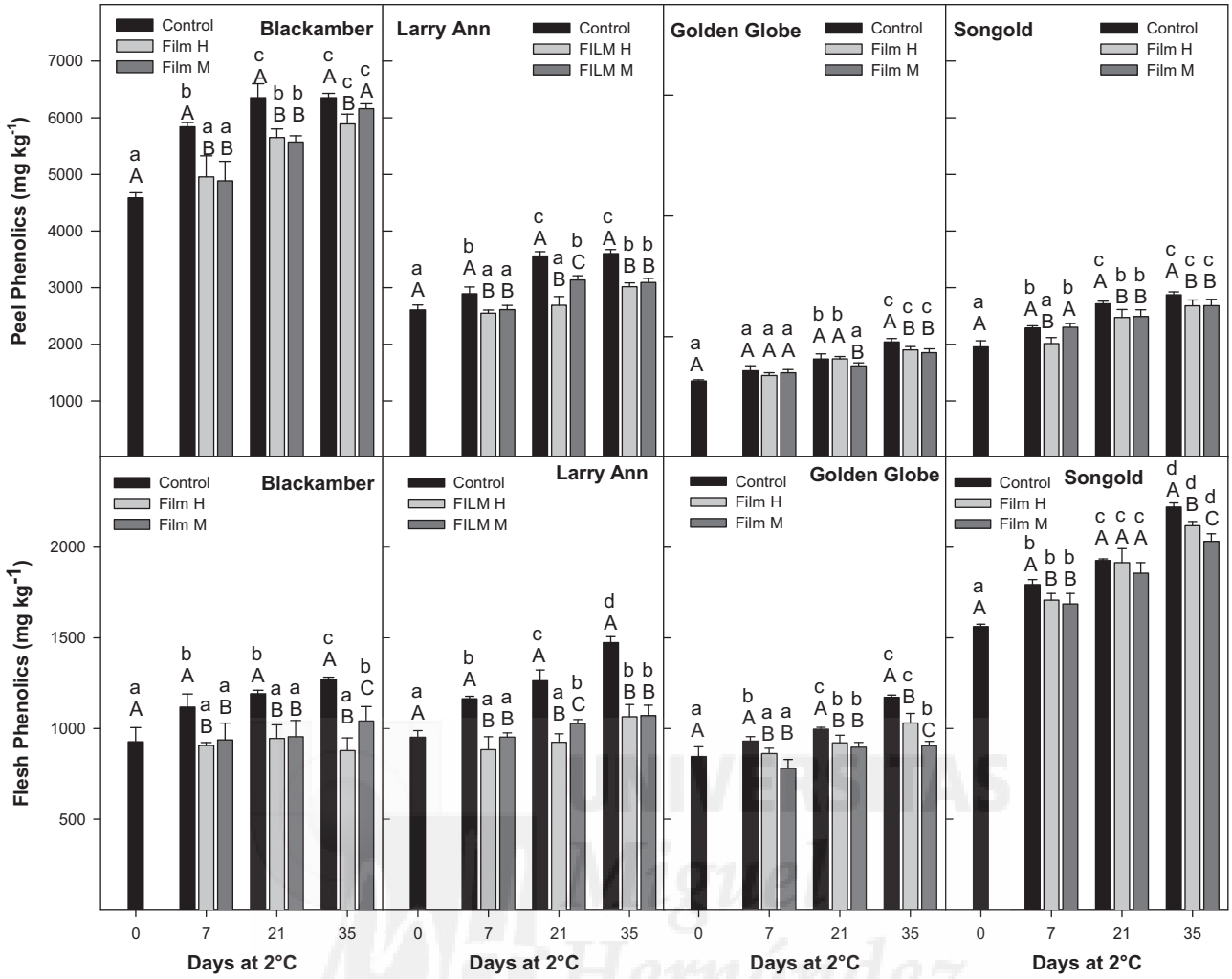

Fig. 1. Changes in total phenolics (peel and flesh) during storage at 2 °C of 4 plum cultivars under MAP conditions with 2 different films (M and H) or with a macroperforated film (control). Results are the mean ± SE ($n = 4$). Minor and capital letters show significant differences ($p < 0.05$) during storage for each treatment and among treatments for each storage time, respectively.

picked at the commercial ripening stage according to Díaz-Mula et al. (2008). Once in the laboratory, 240 fruit homogenous in colour, size and with absence of defects, were selected for each cultivar and sorted at random in 30 lots of 8 fruit. Three lots were used to analyze the fruit properties at harvest (Day 0). The remained lots were individually placed in polypropylene baskets and divided into 3 batches at random and then thermo-sealed on top (total area of 336 cm^2, 14 cm × 24 cm) with the following films: macro-perforated film as control, film H and film M, which were effective for preserving plum organoleptic quality during MAP storage (Díaz-Mula et al., 2011). The film characteristics were as follows: macro-perforated polypropylene film (purchased from Plásticos del Segura S.L., Spain), had 32 holes of 1.5 mm diameter per dm^2, and total perforated area of 0.56%; film H was composed of polyester (12 μm)-polypropylene (50 μm), and film M was composed of polyester (12 μm)-polypropylene (60 μm), and both purchased from Amcor Flexibles (Amcor, Barcelona, Spain) having a permeability to O_2 <100 mL O_2 m^{-2} day^{-1} atm^{-1}. All baskets were stored at 2 °C and 90% RH for 35 days. For each cultivar and film type, baskets were taken after 7, 21 and 35 days, and analytical determinations were made after removing the fruit from MAP and cold storage. For each sampling date and replicate, the peel and flesh for each fruit were sampled, cut into small pieces and divided into 2 subsamples. Tissues from each subsample were ground under liquid N_2 and stored at −20 °C until the bioactive compounds (phenolics, carotenoids and anthocyanins) and total antioxidant activity (TAA) in both hydrophilic and lipophilic fractions were analyzed.

2.2. Bioactive compounds and antioxidant activity determination

The method of Tomás-Barberán et al. (2001) was used for total phenolic extraction by using water:methanol (2:8) containing 2 mM NaF. The phenolic content was quantified as previously described (Díaz-Mula et al., 2008) using the Folin-Ciocalteu reagent and results (mean ± SE) were expressed as mg gallic acid equivalent kg^{-1} fresh weight.

Anthocyanins were extracted from 2 g of peel tissue by homogenization in 4 mL methanol and left 1 h at −18 °C. Extracts were centrifuged at 15,000 × *g* for 15 min at 4 °C, and the anthocyanins in the supernatant was purified onto a C18 Sep-Pak® cartridge (Waters, Madrid, Spain), as described in Serrano et al. (2005). Individual anthocyanins were eluted in a high performance liquid chromatography (HPLC, Hewlett-Packard HPLC series 1100, Agilent, Madrid, Spain) coupled to a diode array detector (DAD).

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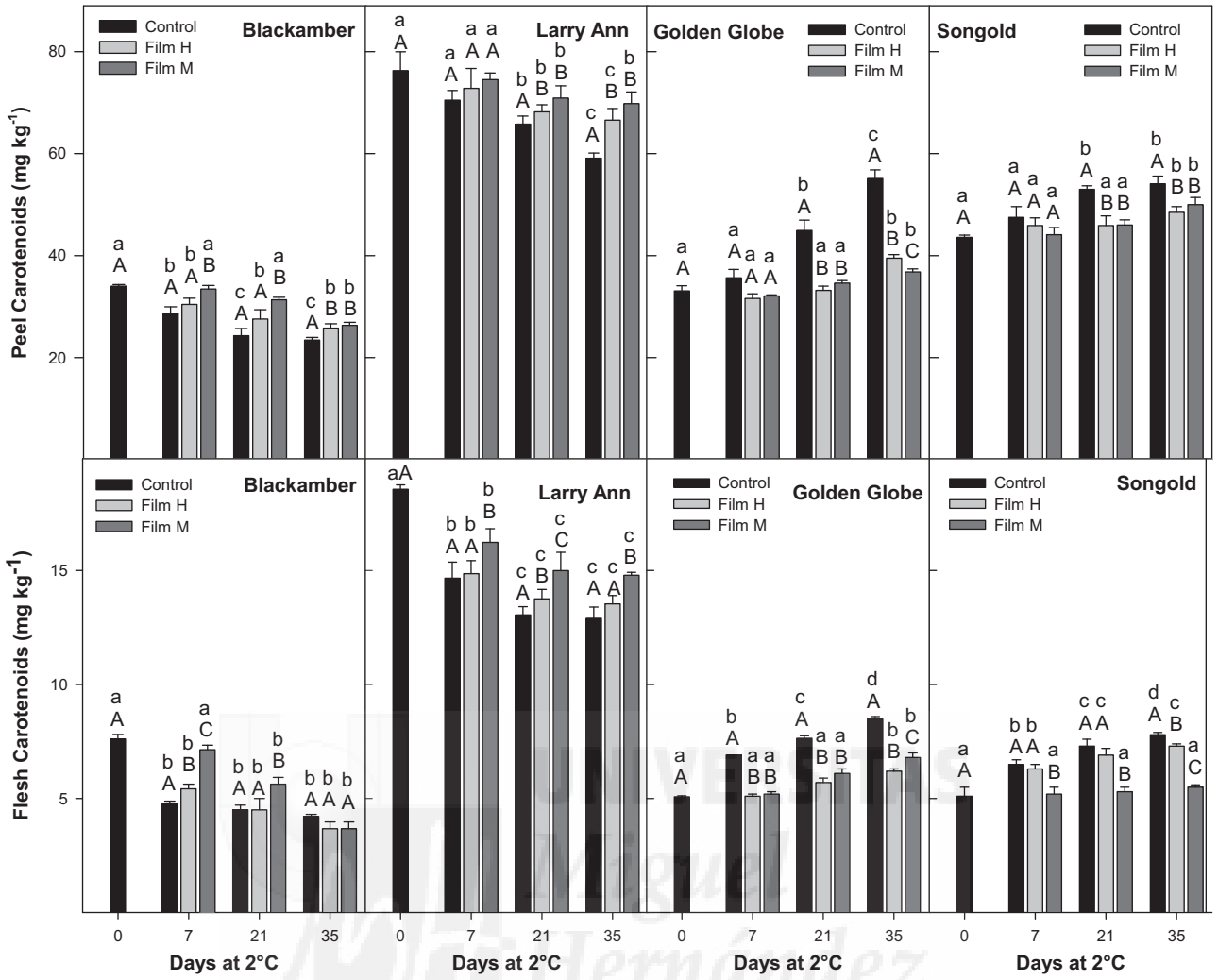

Fig. 2. Changes in total carotenoids (peel and flesh) during storage at 2 °C of 4 plum cultivars under MAP conditions with 2 different films (M and H) or with a macroperforated film (control). Results are the mean ± SE ($n = 4$). Minor and capital letters show significant differences ($p < 0.05$) during storage for each treatment and among treatments for each storage time, respectively.

1 mL from the extracts was filtered through 0.45 μm Millipore filter and then injected into a C18 Supelco column (Supelcogel C-610H, 30 cm × 7.8 mm, Supelco Park, Bellefonte, USA) and detected by absorbance at 510 nm. The peaks were eluted by the gradient proposed by Tomás-Barberán et al. (2001). The anthocyanin standards cyanidin 3-glucoside and cyanidin 3-rutinoside were kindly provided by Dr. García-Viguera (CSIC, Murcia, Spain).

Total antioxidant activity (TAA) was quantified based on Arnao et al. (2001) and slightly modified by Serrano et al. (2009), which enables determination of TAA due to both hydrophilic (H-TAA) and lipophilic (L-TAA) compounds in the same extraction. In brief, 1 g of peel or 5 g of flesh tissues were homogenized in 5 mL of 50 mM phosphate buffer pH 7.8 and 3 mL of ethyl acetate, and then centrifuged at 15,000 × *g* for 15 min at 4 °C. The upper fraction was used for L-TAA while the lower fraction for H-TAA quantification using the enzymatic system composed of the chromophore 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), horseradish peroxidase enzyme (HRP) and its oxidant substrate (hydrogen peroxide).

Total carotenoids were estimated in the lipophilic extract (Arnao et al., 2001) by reading the absorbance at 450 nm in a UNICAM Helios-α spectrophotometer (Cambridge, UK), and expressed as mg of β-carotene equivalent kg^{-1} fresh weight, taking into account the $\varepsilon_{cm}^{1\%} = 2560$ and the results were the mean ± SE.

2.3. Statistical analysis

Experimental data were subjected to ANOVA analysis. Sources of variation were MAP packages and storage. The overall least significant differences (Fisher's LSD procedure, $p < 0.05$) were calculated and used to detect significant differences among packages and storage time. All analyses were performed with SPSS software package v. 11.0 for Windows (SPSS, 2001). Linear regressions were performed between total antioxidant activity (either hydrophilic or lipophilic) and the bioactive compounds taking into account all sampling data (either peel or flesh).

3. Results and discussion

During storage, CO_2 concentrations increased and O_2 concentrations decreased inside the MAP packages and the steady-state atmosphere was reached after 7 days at 2 °C, the composition being dependent on the film and the plum respiration rate (Díaz-Mula et al., 2011). The highest atmosphere modification was for LA plums with film M, with CO_2 and O_2 concentrations at equilibrium of ≈6.5

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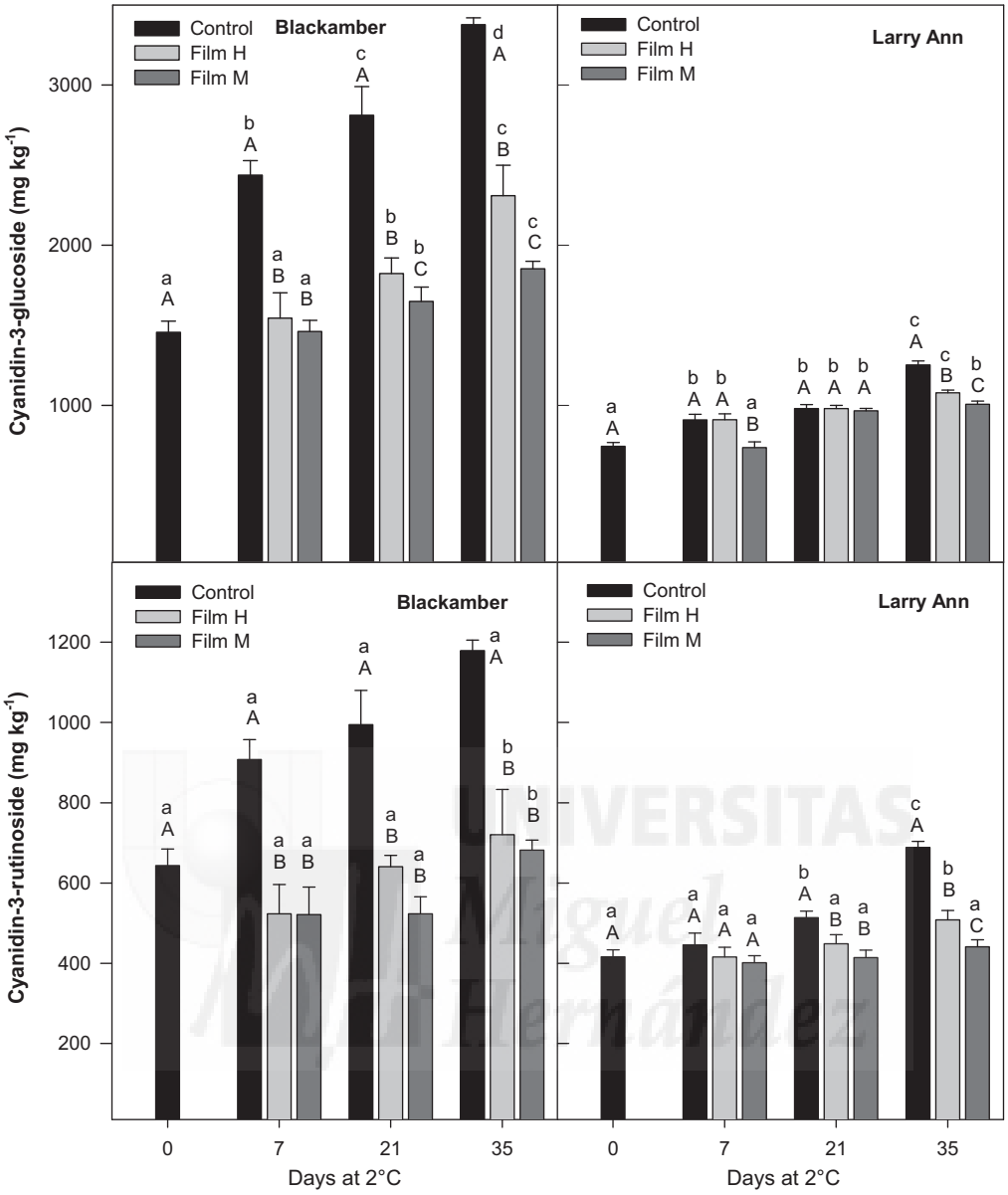

Fig. 3. Changes in anthocyanins (cyanidin-3-glucoside and cyanidin-3-rutinoside) in the peel of purple plum cultivars during storage at 2 °C under MAP conditions with 2 different films (M and H) or with a macroperforated film (control). Results are the mean ± SE ($n = 4$). Minor and capital letters show significant differences ($p < 0.05$) during storage for each treatment and among treatments for each storage time, respectively.

and ≈13 kPa, respectively, while the lowest atmosphere modification was reached in GG under film H, ≈1.5 and 19 kPa for CO_2 and O_2, respectively.

3.1. Bioactive compounds

Total phenolic concentrations at harvest were different depending on cultivar in both peel and flesh tissues. In peel tissue, total phenolic concentrations ranged from 1267 ± 19 to 4584 ± 87 mg kg^{-1}, for GG and BA plums, respectively, while for flesh tissue this range was smaller, from 846 ± 54 to 1562 ± 129 mg kg^{-1}, for GG and SG, respectively. During storage a similar trend was observed for both peel and flesh, that is an increase between 40 and 50% for all cultivars in control fruit (Fig. 1). The increases in total phenolics were delayed by the use of MAP packages, without significant differences between H and M films. Polyphenols, which are the most abundant secondary metabolites in fruit, showed a similar trend during storage in the 4 plum cultivars. These results are in agreement with previous reports on these and other plum cultivars ('Black Diamond', 'Golden Japan', 'TC Sun' and 'Angeleno'), as well as other stonefruit such as sweet cherry, peach and nectarine (Di Vaio et al., 2008; Díaz-Mula et al., 2009; Serrano et al., 2009). When low temperature storage was combined with MAP, there was a delay in phenolics accumulation, which might be due to the effect of MAP (low O_2 and high CO_2) in retarding postharvest ripening, as can be inferred by the reduced ethylene production, fruit softening, colour change and acidity loss (Díaz-Mula et al., 2011). In addition, the possible effect of low O_2 and high CO_2 on the delay in phenylalanine ammonia lyase (PAL), chalcone synthase or anthocyanidin synthase, the key enzymes in the biosynthesis pathway of phenolic compounds (Desjardins, 2008), or reduced polyphenol oxidase (PPO) or peroxidase activities (Pourcel et al., 2007), the main enzymes responsible of polyphenol degradation, should not be discounted.

As for total phenolics, differences in carotenoid concentrations existed among cultivars and types of tissue. In this regard, the peel

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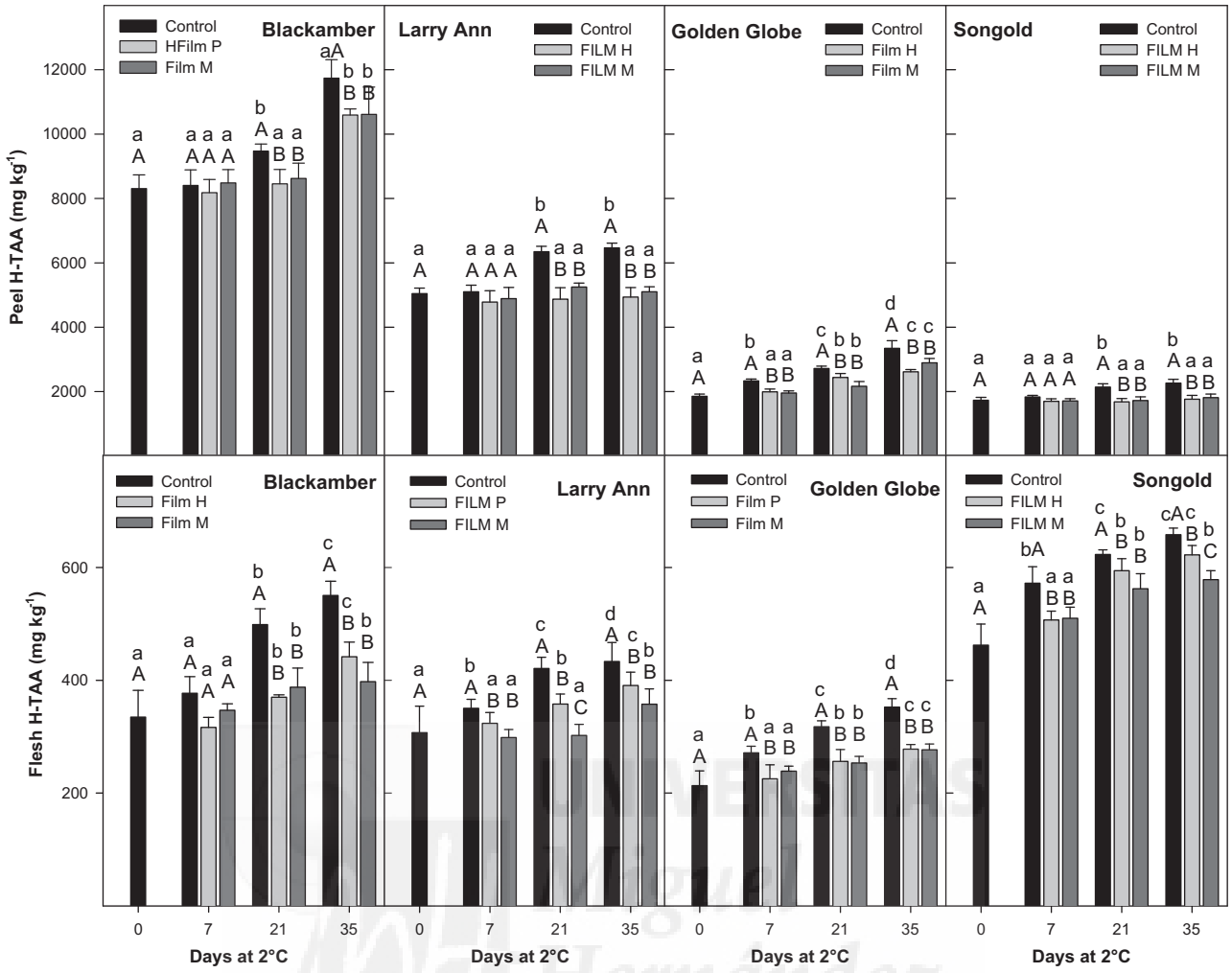

Fig. 4. Changes in total hydrophilic antioxidant activity (H-TAA) in the peel and flesh of 4 plum cultivars stored at 2 °C under MAP conditions with 2 different films (M and H) or with a macroperforated film (control). Results are the mean ± SE ($n = 4$). Minor and capital letters show significant differences ($p < 0.05$) during storage for each treatment and among treatments for each storage time, respectively.

always had 5–7-fold more carotenoids than flesh tissues, LA being the cultivar with the highest carotenoid concentrations in both peel and flesh (76.28 ± 3.17 and 18.56 ± 0.49 mg kg^{-1}, respectively), while in BA plums the lowest total carotenoid concentrations were found for both tissues (34.01 ± 0.30 and 7.62 ± 0.21 mg kg^{-1}, respectively). During postharvest storage, different behaviour was observed between yellow and purple cultivars (Fig. 2), since increases were found for both peel and flesh of plum cultivars with yellow skin, while the contrary occurred for BA and LA plums (purple skin). For all cultivars, MAP packages induced a delay in the change of total carotenoids during storage.

Anthocyanins were analyzed by HPLC–DAD and were detected in the peel and flesh of the purple plum cultivars (BA and LA), with two individual anthocyanins identified, cyanidin-3-glucoside and cyanidin-3-rutinoside, the latter found at significant lower concentrations, according to previous reports in other purple plum cultivars (Tomás-Barberán et al., 2001; Chun et al., 2003; Wu and Prior, 2005; Díaz-Mula et al., 2008). In addition, differences existed in anthocyanin concentrations between the 2 cultivars. Thus, concentrations in the peel at harvest of cyanidin-3-glucoside were 1456 ± 71 and 744 ± 24 mg kg^{-1}, for BA and LA, respectively, and 644 ± 42 and 416 ± 17 mg kg^{-1} of cyanidin-3-rutinoside (Fig. 3). In control fruit of both cultivars, significant increases in the concentration of both anthocyanins in the peel were found during storage, while these increases were retarded in those plums stored under MAP conditions, with generally no significant differences attributable to film type. However, in the flesh, concentrations at harvest were much lower (≈30 and 60 mg kg^{-1} of cyanidin-3-glucoside, for BA and LA, respectively, and ≈10 and 20 mg kg^{-1} of cyanidin-3-rutinoside) and no significant changes were observed during storage, irrespective of the treatment (data not shown).

Increases in carotenoids and anthocyanins concentrations in yellow and purple plums, respectively, are responsible for the colour changes associated with the ripening process on the tree (Díaz-Mula et al., 2008) or after cold storage (Díaz-Mula et al., 2009). However, these increases were delayed in plums stored under MAP conditions, leading to a reduction in the changes in colour as shown in our previous paper (Díaz-Mula et al., 2011). Accordingly, increases in anthocyanins in sweet cherry, strawberry, blueberry and raspberry, and in lycopene in tomato and watermelon, were lower during storage under MAP conditions than in control fruit stored in open air, due to the effect of MAP on delaying the development of the postharvest ripening process (Jones, 2007).

3.2. Total antioxidant activity

TAA was measured in both hydrophilic (H-TAA) and lipophilic (L-TAA) fractions for both peel and flesh tissues, for which H-TAA and L-TAA were always higher in the peel than in the flesh although

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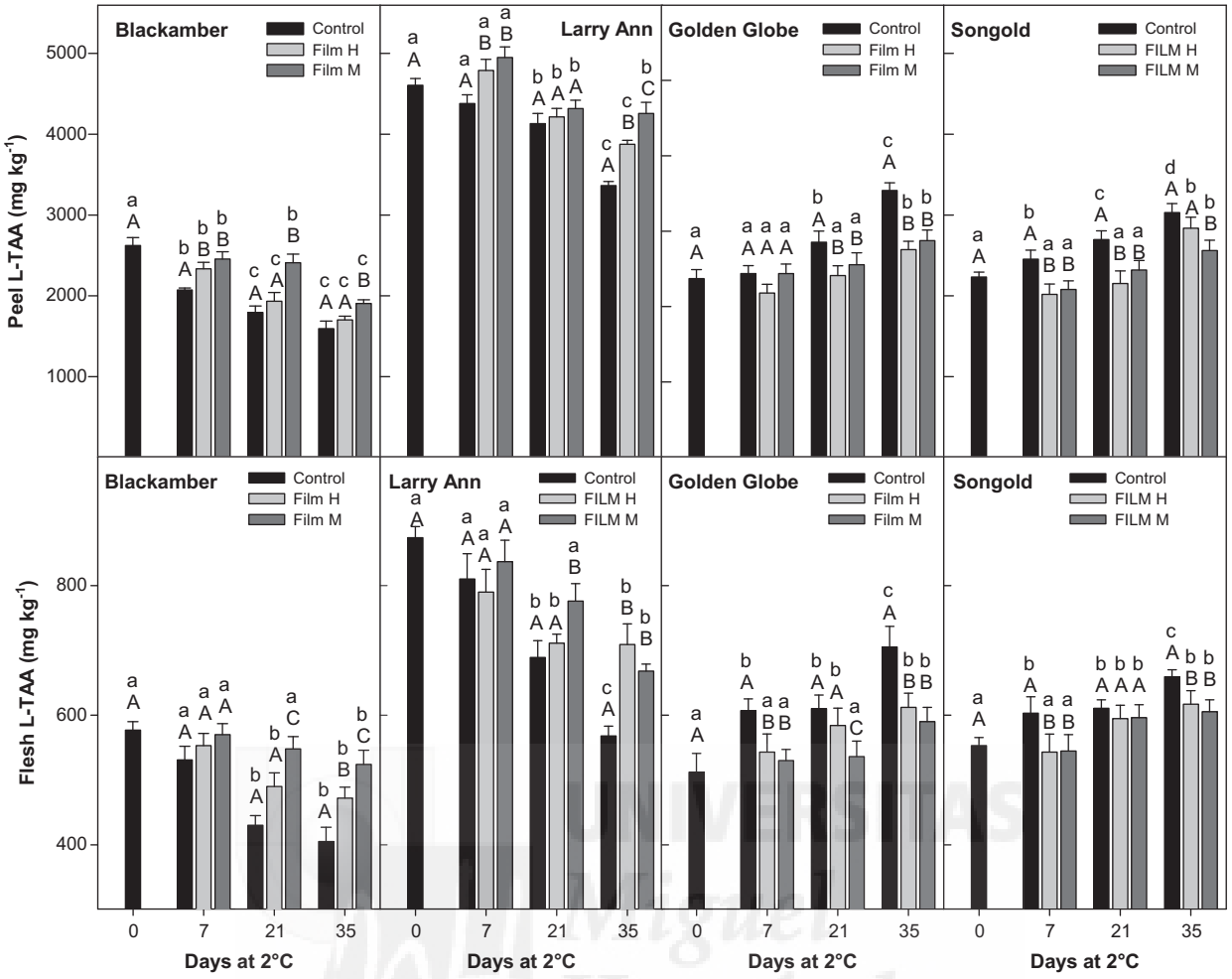

Fig. 5. Changes in total lipophilic antioxidant activity (L-TAA) in the peel and flesh of 4 plum cultivars stored at 2 °C under MAP conditions with 2 different films (M and H) or with a macroperforated film (control). Results are the mean ± SE ($n = 4$). Minor and capital letters show significant differences ($p < 0.05$) during storage for each treatment and among treatments for each storage time, respectively.

important differences existed among cultivars. Thus, H-TAA was 3–25 fold higher in the peel than in the flesh, while L-TAA was 5-fold in all cultivars (Figs. 4 and 5). In addition, H-TAA and L-TAA were also different at harvest, the BA plums having the highest H-TAA (8308 ± 428 mg kg^{-1}) and SG the lowest (1733 ± 89 mg kg^{-1}) in the peel, while in the flesh the highest H-TAA was found in SG (462 ± 37 mg kg^{-1}) and the lowest in GG (213 ± 26 mg kg^{-1}). With respect to L-TAA, lower variations were found among cultivars at harvest, with levels of ≈2500 and ≈550 mg kg^{-1} in the peel and flesh, respectively, for BA, GG and SG plum cultivars. The exception was LA plums, in which L-TAA levels were 4606 ± 83 and 874 ± 17 mg kg^{-1} in peel and flesh, respectively (Fig. 5). During storage, control fruit exhibited significant increases in H-TAA in both peel and flesh of all cultivars although these increases were retarded by the use of plastic films, but without significant differences attributable to film type. However, changes in L-TAA during storage were dependent on cultivar, since decreases were observed in the peel and flesh of purple cultivars (BA and LA) while increases occurred in the yellow ones. For all cases, a significant delay in those changes of L-TAA was observed in the plums stored under MAP conditions with both film types. It is interesting to highlight that to measure the antioxidant capacity of plums, the contribution of L-TAA should be taking into account, since the values obtained for L-TAA were as high as those of H-TAA, in both peel and flesh of these plum cultivars.

Taking into account data for all cultivars, sampling dates and storage conditions, a positive correlation was found between H-TAA and total phenolics content ($y = 1.82x - 1021$; $R^2 = 0.874$, for peel and $y = 0.27x + 67$; $R^2 = 0.826$, for flesh), and between L-TAA and total carotenoids ($y = 53.32x + 420$; $R^2 = 0.855$, for peel and $y = 22.83x + 422$; $R^2 = 0.790$, for flesh). Correlations between total phenolics and H-TAA levels at harvest have been found in other plum cultivars and stone fruit, such as peach, nectarine and sweet cherry (Gil et al., 2002; Cevallos-Casals et al., 2006; Rupasinghe et al., 2006; Vizzotto et al., 2007; Díaz-Mula et al., 2008), although during storage these correlations have only been found in our previous work (Díaz-Mula et al., 2009; Serrano et al., 2009). On the other hand, L-TAA has been also been correlated with total carotenoids in a wide range of plum cultivars during on-tree ripening as well as in tomatoes, and some vegetables and legumes (Wu et al., 2004; Lenucci et al., 2006; Cho et al., 2007; Díaz-Mula et al., 2008). As previously stated, almost no information exists on the effect of MAP on antioxidant capacity of fruit in general, and in plums particularly. Our results suggest that MAP does not impart any negative effects on TAA and just reflects the delay of the ripening process occurring in the plums stored under MAP conditions. Additionally, it is interesting to consider that H-TAA might be underestimated, since an increase in TAA has been recently reported when the extraction residues were subjected to two different acidic treatments to

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release hydrolysable tannins and non-extractable proanthocyanidins (Kristl et al., 2011).

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Postharvest Treatments with Salicylic Acid, Acetylsalicylic Acid or Oxalic Acid Delayed Ripening and Enhanced Bioactive Compounds and Antioxidant Capacity in Sweet Cherry

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ABSTRACT: Sweet cherry cultivars ('Cristalina' and 'Prime Giant') harvested at commercial ripening stage were treated with salicylic acid (SA), acetylsalicylic acid (ASA) or oxalic acid (OA) at 1 mM and then stored for 20 days under cold temperature. Results showed that all treatments delayed the postharvest ripening process, manifested by lower acidity, color changes and firmness losses, and maintained quality attributes for longer periods than controls. In addition, total phenolics, anthocyanins and antioxidant activity increased in untreated fruit during the first 10 days of storage and then decreased, while in fruits of all treatments, these parameters increased continuously during storage without significant differences among treatments. Thus, postharvest treatments with natural compounds, such as SA, ASA or OA, could be innovative tools to extend the storability of sweet cherry with higher content of bioactive compounds and antioxidant activity as compared with control fruits.

KEYWORDS: phenolics, anthocyanins, carotenoids, total antioxidant activity, salicylic acid, acetylsalicylic acid, oxalic acid, postharvest

INTRODUCTION

Sweet cherry quality is determined by attributes affecting fruit marketing appeal and consumer satisfaction. Among the attributes related to consumer purchase decision are visual appearance, fruit size and color, firmness, and especially sweetness and flavor. However, these parameters change with cultivar[1] and are associated with the ripening process.[2] On the other hand, sweet cherry has been reported to contain several phenolic compounds and anthocyanins which contribute to the antioxidant capacity.[3,4] The two dominant polyphenols in cherries are caffeoyltartaric acid and 3-*p*-coumaroylquinic acid, while the main anthocyanins are cyanidin-3-rutinoside and cyanidin-3-glucoside followed by pelargonidin-3-rutinoside, peonidin-3-rutinoside and peonidin-3-glucoside.[5,6]

Cherry fruits deteriorate rapidly during postharvest storage, and in some cases do not reach consumers with optimal quality after transport and marketing. The main causes of sweet cherry deterioration are weight loss, color changes, softening, surface pitting, stem browning, loss of acidity and slight increases in TSS.[6−8] The storage period also affects the content of bioactive compounds, with general increases in phenolic and anthocyanin concentrations associated with the postharvest ripening process.[5,6]

Consumers demand food preservation systems with absence of chemicals or pesticide residues, and thus there is increasing interest in the use of natural compounds. In the case of sweet cherry the combined use of modified atmosphere packaging and essential oils[7] or *Aloe vera* as edible coating[8] resulted in a reduction of spoilage microorganisms, maintenance of fruit quality and extension of shelf life. Other natural compounds, such as salicylic acid (SA), acetylsalicylic acid (ASA) and oxalic acid (OA), are present in fruits and vegetables, and have shown important roles in delaying the ripening process when applied as postharvest treatment.

SA and ASA retarded the ripening process in banana,[9] sugar apple fruit,[10] kiwifruit,[11] mango[12] and peach,[13] through an induction of antioxidant enzymes, such as peroxidase, catalase and superoxidodismutase and reduction of lipoxygenase activity. In pomegranate, ASA treatment reduced occurrence of chilling injury (CI) and maintained higher content in nutritive and bioactive compounds.[14] OA has been involved in controlling litchi browning,[15] and delaying the ripening process in some climacteric fruits such as mango,[16] peach,[17] and jujube fruit,[18] through an inhibition of the ethylene biosynthesis. More recently, Sayyari et al.[19] reported that application of oxalic acid alleviated CI symptoms of pomegranate, a nonclimacteric fruit.

As far as we know, few reports exist on the role of SA in increasing resistance against cherry decay,[20,21] but there is no literature about the effect of postharvest treatments with SA, ASA or OA on the sweet cherry ripening process and parameters related to fruit quality. Thus, the objective of this research was to analyze the effect of these treatments on the organoleptic parameters related to sweet cherry quality and the content of bioactive compounds and antioxidant activity during storage.

MATERIALS AND METHODS

Plant Material and Experimental Design. Sweet cherry cultivars ('Cristalina' and 'Prime Giant') were harvested at commercial ripening stage, S2 according to Serrano et al.,[6] from a commercial plot

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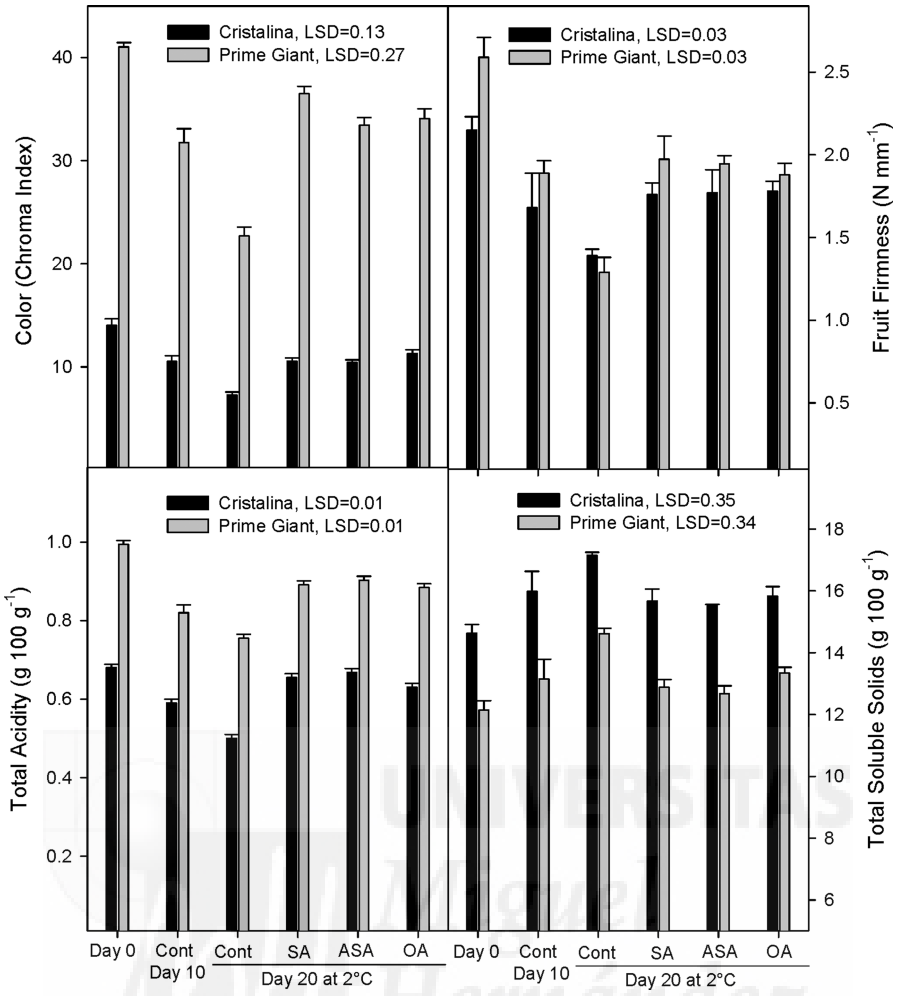

Figure 1. Color, firmness, total acidity and total soluble solids at harvest (day 0) and after 10 or 20 days of storage at 2 °C in control fruit, and after 20 days of storage in sweet cherry 'Cristalina' and 'Prime Giant' treated with salicylic acid (SA), acetylsalicylic acid (ASA) or oxalic acid (OA). Data are the mean ± SE. LSD value for each cultivar is shown inside figures.

(Finca Los Frutales, Villena Alicante, Spain), with scores of 5 and 4 for 'Cristalina' and 'Prime Giant', respectively, according to the color chart from Centre Technique Interprofessionel de Fruits et Légumes (CTIFL, Paris). For each cultivar, about 12 kg of cherries were picked and immediately transferred to the laboratory. Then, 1020 homogeneous fruits in color and size and without visual defects were selected for each cultivar and randomly grouped in 51 lots of 20 fruits. Three lots were used to analyze the properties at harvest and 48 lots for the following treatments in triplicate: control (distilled water), 1 mM salicylic acid (SA), 1 mM acetylsalicylic acid (ASA), and 1 mM oxalic acid (OA). These concentrations were chosen based on a preliminary experiment, in which higher concentrations did not show delays in the ripening process additional to the effect found with 1 mM (data not shown). Treatments were performed by dipping fruits in 10 L of solution for 10 min, and then they were left to dry at room temperature before cold storage at 2 °C and RH of 85% in darkness for up to 20 days. After 5, 10, 15, and 20 days, 3 lots from each cultivar and treatment were sampled at random from cold chambers for analytical determinations. In these fruits, color and firmness were individually measured and then the edible portion of each lot was cut in small pieces to obtain a homogeneous sample. For each sample 5 g was used to determine in duplicate total soluble solids (TSS) and total acidity (TA) and the remaining sample was frozen in liquid N_2, mixed and stored at −20 °C until total anthocyanins, total phenolics, total carotenoids, and antioxidant activity in both hydrophilic and lipophilic fractions were determined.

Ripening Parameters. Color was determined in a Minolta colorimeter (CRC200, Minolta Camera Co., Japan), using the CIELab coordinates and expressed as chroma $[(a^2 + b^2)^{1/2}]$. Fruit firmness was determined using a TX-XT2i Texture Analyzer (Stable Microsystems, Godalming, U.K.) interfaced to a personal computer, with a flat steel plate mounted on the machine. For each fruit, the cheek diameter was measured and then a force that achieved a 3% deformation of the fruit diameter was applied. Results were expressed as the mean ± SE of the force−deformation ratio (N mm^{-1}). TSS was determined from the juice obtained for each subsample with a digital refractometer (model PR-101, Atago Co. Ltd., Tokyo, Japan) at 20 °C, and results (mean ± SE) were expressed as % (°Brix). Total acidity (TA) was determined by automatic titration (785 DMP Titrino, Metrohm, Herisau, Switzerland) 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 (mean ± SE) were expressed as g of malic acid equivalent per 100 g fresh weight.

Total Antioxidant Activity Determination. Total antioxidant activity (TAA) of hydrophilic and lipophilic compounds was quantified according to Arnao et al.[22] which enables determination of TAA due to both hydrophilic and lipophilic compounds in the same extraction. Briefly, for each subsample, five grams of tissue was homogenized in 5 mL of 50 mM Na-phosphate buffer (pH = 7.8) and 3 mL of ethyl

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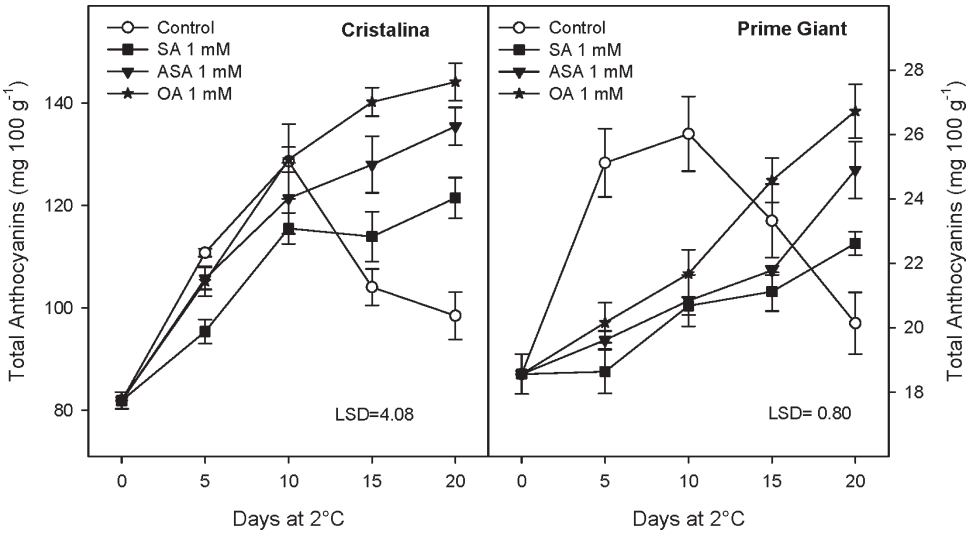

Figure 2. Total anthocyanin concentration in sweet cherry 'Cristalina' and 'Prime Giant' during storage at 2 °C in control fruits and fruits treated with salicylic acid (SA), acetylsalicylic acid (ASA) or oxalic acid (OA). Data are the mean ± SE.

acetate, and then the mixture was centrifuged at 10000*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 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 results are expressed as mg of Trolox equivalent per 100 g.

Bioactive Compound Determination. Total phenolics were extracted according to Tomás-Barberán et al.[23] 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, and results (mean ± SE) were expressed as mg of gallic acid equivalent per 100 g fresh weight. Total anthocyanins were determined according to García-Viguera et al.[24] 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 results were expressed as mg per 100 g fresh weight and were the mean ± SE. Total carotenoids were extracted according to Mínguez-Mosquera and Hornero-Méndez.[25] Briefly, 2 g of sweet cherry fruit was extracted with acetone and shaken with diethyl ether and 10% NaCl for separation of the two phases. The lipophilic phase was washed with Na_2SO_4 (2%) and saponified with 10% KOH in methanol, and the pigments were subsequently extracted with diethyl ether, evaporated and then made up to 25 mL with acetone. Total carotenoids were estimated by reading the absorbance at 450 nm according to Díaz-Mula et al. 2008[26] and expressed as mg of β-carotene equivalent per 100 g, taking into account the $\varepsilon^{1\%}_{cm} = 2560$. Analytical reagents were purchased from Sigma-Aldrich (Madrid, Spain).

Statistical Analysis. Data for the analytical determinations were subjected to analysis of variance (ANOVA). Sources of variation were storage time and treatment. Mean comparisons were performed using HSD Tukey's test to examine if differences were significant at $P < 0.05$. Linear regressions were performed between the color chroma parameter and anthocyanin concentration, as well as among the bioactive compounds and H-TAA or L-TAA taking into account data from both for cultivars and all sampling dates. All analyses were performed with SPSS software package v. 12.0 for Windows.

RESULTS AND DISCUSSION

Sweet Cherry Ripening Parameters. During storage of control fruits, color (chroma index), fruit firmness and total acidity (TA) decreased in both cultivars while total soluble solids (TSS) increased. These changes were significantly delayed in 'Cristalina' and 'Prime Giant' treated with SA, ASA and OA (Figure 1). Interestingly, the observed values after 20 days of storage in treated fruits were similar to those of control fruit just after 10 days. Thus, for both cultivars shelf life of control cherries was established at 10 days while it was extended up to 20 days in treated fruits. The loss of firmness and TA and the increase of color to dark-red have been associated with ripening of sweet cherry and loss of quality attributes.[4−6] OA and SA delayed the ripening process in climacteric fruit, such as mango,[16] peach[17] and jujube fruit,[18] as well as SA and ASA on kiwifruit[11] due to the inhibition of ethylene production. In addition, retention of fruit firmness after SA treatment has been reported in several crops due to inhibition of cell-wall degrading enzymes, such as polygalacturonase, cellulase, and pectinmethylesterase.[27] Although sweet cherry is a nonclimacteric fruit, the results show a clear effect of these treatments on delaying the ripening process and maintaining fruit quality in this fruit, as has been reported for other nonclimacteric fruit such as pomegranate treated with SA,[28] OA[19] and ASA.[14]

Bioactive Compounds and Total Antioxidant Activity. At harvest, the anthocyanin concentration differed between cultivars with values of 81.89 ± 1.62 and 18.56 ± 0.62 mg per 100 g, for 'Cristalina' and 'Prime Giant', respectively (Figure 2). For both cultivars a similar pattern was found over storage in control fruits, that is, increases until day 10 of storage followed by a decrease. The application of SA, ASA and OA led to a continuous increase in anthocyanin concentration until the end of the experiment, the values being higher for OA-treated cherries

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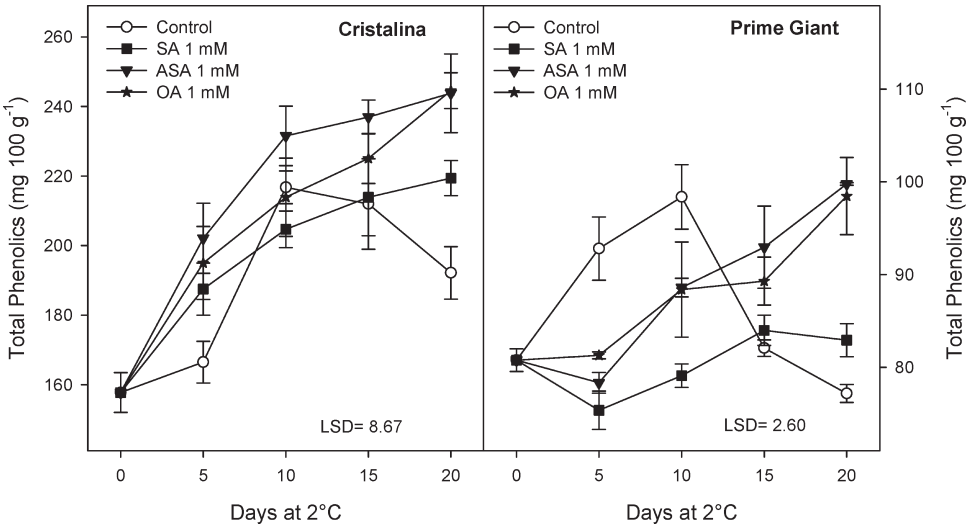

Figure 3. Total phenolics concentration in sweet cherry 'Cristalina' and 'Prime Giant' during storage at 2 °C in control and treated fruits with salicylic acid (SA), acetylsalicylic acid (ASA) or oxalic acid (OA). Data are the mean ± SE. LSD is shown inside figures.

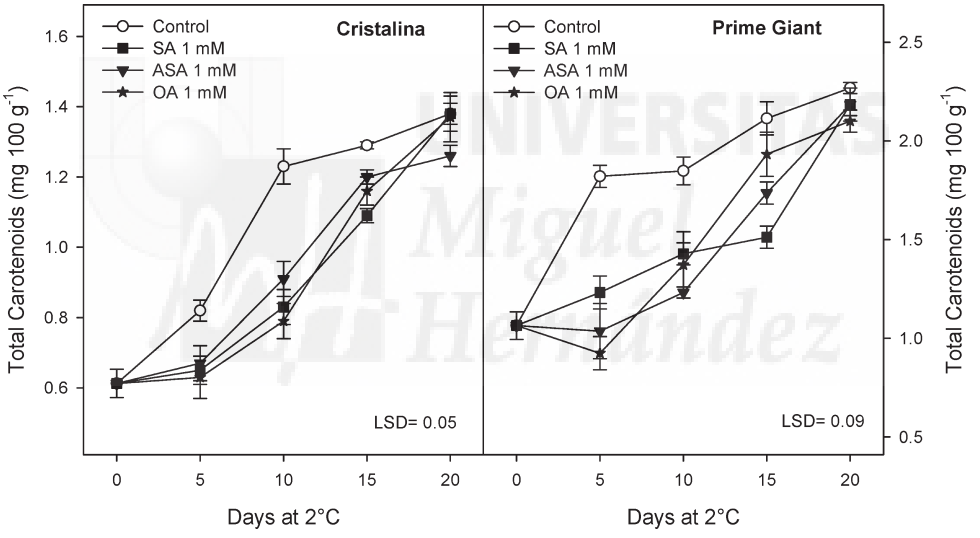

Figure 4. Total carotenoid concentration in sweet cherry 'Cristalina' and 'Prime Giant' during storage at 2 °C in control and treated fruits with salicylic acid (SA), acetylsalicylic acid (ASA) or oxalic acid (OA). Data are the mean ± SE. LSD is shown inside figures.

followed by ASA and SA treated fruit. The main anthocyanins in 'Cristalina' and 'Prime Giant' cultivars are cyanidin-3-rutinoside followed by cyanidin-3-glucoside and pelargonidin-3-rutinoside, and their changes have been related to the advancement of the ripening process, either on-tree[1,2] or during storage,[6] and negatively correlated with color parameters.[3,6] Thus, control fruits exhibited the lowest chroma index, acquiring a dark-red color after 10 days, at which the highest anthocyanin concentration was found. After this period, control cherries became even darker and however loss of anthocyanins was detected. Discrepancies exist on the changes of anthocyanins during postharvest storage. Thus, in 'Sciazza' and 'Ferrovia' cherry cultivars, over 50% of the anthocyanin concentration was lost after 15 days at 1 °C, while in 'Burlat', 'Saco', 'Summit' and 'Van' total anthocyanin concentration increased during 14 days and no changes have been found in 'Lambert Compact' cherries.[5,29] In our work, the storage period was extended up to 20 days and the fruit could be considered as over-ripe and in senescence phase, at which a diminution of total anthocyanins occurred. Accordingly, in 'Bing' cultivar the cyanidin-3-rutinoside, which is the major anthocyanin in cherries, decreased from day 15 to day 30 of storage, and the increase in color intensity was attributed to minor anthocyanins that impart dark-red color.[30] On the contrary, the clear effect of SA, ASA and OA on delaying the ripening process was reflected in the increase in anthocyanins over storage.

A similar pattern to that of total anthocyanins was found for total phenolic compounds, that, is increases in control fruits up to day 10 of storage and further decreases, while in all treated fruits total phenolics increased throughout the experiment (Figure 3). Among treatments, the highest levels of total phenolics were found in those cherries treated with OA and ASA followed by SA. In our and other sweet cherry cultivars nechlorogenic acid was the predominant hydroxycinnamic acid followed by 3-*p*-coumaroylquinic acid.[5,6,29] Phenolic compounds increased their

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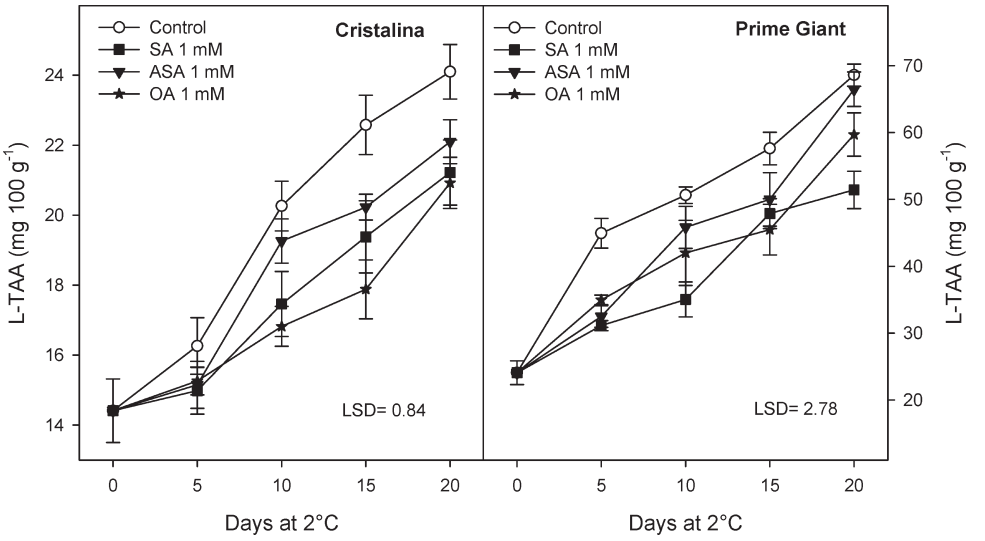

Figure 5. Lipophilic total antioxidant activity (L-TAA) in sweet cherry 'Cristalina' and 'Prime Giant' during storage at 2 °C in control and treated fruits with salicylic acid (SA), acetylsalicylic acid (ASA) or oxalic acid (OA). Data are the mean ± SE. LSD is shown inside figures.

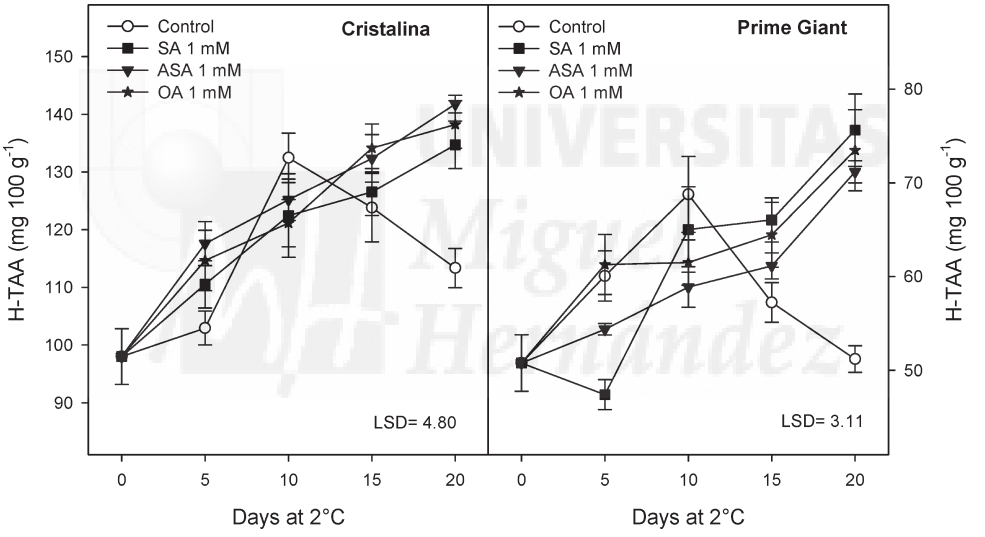

Figure 6. Hydrophilic total antioxidant activity (H-TAA) in sweet cherry 'Cristalina' and 'Prime Giant' during storage at 2 °C in control and treated fruits with salicylic acid (SA), acetylsalicylic acid (ASA) or oxalic acid (OA). Data are the mean ± SE. LSD is shown inside figures.

concentration (40−60%) during cold storage and also were related to the advancement of the ripening process. However, the change of total phenolics during postharvest storage could be affected by several factors including ripening stage at harvest, cultivar, season growth and duration of storage time, since a general increase occurred in a short period of storage for a wide range of cherry cultivars,[6] although decreases or increases were reported for 'Summit' and 'Van' cherries depending on year and season growth.[5] The data reported herein suggest that increases occurred during the first days of storage and the prolongation of storage led to significant decreases in phenolic content. Linear regression was performed between total polyphenols and anthocyanins taking into account all data (cultivar, treatment and storage), and a high correlation was found (y =1.30x + 57.23; R^2 = 0.978), which suggests that anthocyanins are the main phenolic compounds as has been shown in other cherry cultivars.[3,4,6] No literature exists on the effect of SA, ASA or OA on the content of total phenolics in fruits for comparative purposes, apart from our previous papers on pomegranate treated with OA or ASA, which alleviated CI and reduced the phenolic losses found in control fruits.[14,19]

Although carotenoids are other important bioactive constituents in fruits,[31] no evidence exist on their occurrence in sweet cherry. In both cultivars carotenoids were present but at different concentrations, with 'Prime Giant' having significantly higher total carotenoids (1.06 ± 0.07 mg per 100 g) than 'Cristalina' (0.61 ± 0.04 mg per 100 g). Along storage, an accumulation of total carotenoids was observed in both control and treated cherries for both cultivars (Figure 4). This increase was retarded in treated fruits with respect to controls, although at the end of the experiment the carotenoid concentration was similar for all cases, with final values ≈1.3 and ≈2.2 mg per 100 g, for 'Cristalina' and 'Prime Giant', respectively. In other stone fruits, such as plums, an increase of carotenoid levels was also observed

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along storage and related to the advancement of the ripening process in both yellow and red-purple cultivars.[26,32]

Total antioxidant activity (TAA) was measured in hydrophilic (H-TAA) and lipophilic (L-TAA) fractions separately, since early reports demonstrated that the contribution of L-TAA supposed about 20−30% of the TAA in a wide range of sweet cherry cultivars.[1,6] The change of L-TAA was similar to that obtained for carotenoids, that is, a continuous increase alongside the storage, although the application of SA, ASA or OA induced a significant delay in the increase of L-TAA (Figure 5). In fact, a high correlation was found between L-TAA and carotenoids (y = 29.63x − 6.71; R^2 = 0.824), which would indicate that carotenoids are the main lipophilic bioactive compounds contributing to L-TAA, although other lipophilic compounds such as tocopherols could also be present in sweet cherry and having a role as antioxidant moieties.[33]

During storage, continuous increases occurred in H-TAA for those cherries treated with SA, ASA or OA, while in control fruits H-TAA peaked at day 10 and showed decreases after that (Figure 6). The pattern of H-TAA was similar to that observed for total phenolics, and therefore high correlation was found between total phenolic and H-TAA (y = 0.51x + 16.70; R^2 = 0.975), which is in agreement with previous papers reporting that phenolic compounds are the main compounds responsible for antioxidant capacity of sweet cherry,[2,4] although ascorbic acid can also contribute to this activity.[2] Early reports have demonstrated that these treatments have also effects on antioxidant enzymes. For example, increases in catalase, peroxidase, superoxide dismutase and ascorbate peroxidase have been found in fruits such as banana, sugar apple fruit and mango after SA, ASA or OA treatment.[9,10,12] Moreover, in kiwifruit and loquat, ASA application delayed the increase in lipoxygenase associated with senescence leading to a lower production of superoxide free radicals.[11,34] During storage of these fruits, a decline in the content of free SA occurred, and thus exogenous treatments with SA or ASA led to increase its endogenous concentration and delaying of the ripening process.

Given the relationship between L-TAA-carotenoids and H-TAA-phenolics, it could be concluded that phenolic compounds, including anthocyanins, and carotenoids could be responsible for the health-beneficial effects after sweet cherry consumption in relation to its reported effect on reducing the risk of several diseases, such as cancer, diabetes, Alzheimer's and cardiovascular diseases.[35] The application of natural compounds as postharvest tools, such as SA, ASA or OA, resulted in delayed ripening rates of sweet cherry during storage, and maintained higher contents of bioactive compounds and antioxidant activity as compared with control fruit. Thus, control fruit could be stored for 10 days while this period was extended up to 20 days in treated cherries, without significant differences among treatments.

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