



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

H.M. Díaz-Mula^a, P.J. Zapata^b, F. Guillén^b, J.M. Valverde^b, D. Valero^b, M. Serrano^{a,*}

^a Department of Applied Biology, EPSO, University Miguel Hernández, Ctra. Beniel km. 3.2, 03312 Orihuela, Alicante, Spain

^b Department of Food Technology, EPSO, University Miguel Hernández, Ctra. Beniel km. 3.2, 03312 Orihuela, Alicante, Spain

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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.

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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 'Sungold' (SG) cultivars were

* Corresponding author. Fax: +34 96 6749678.

E-mail address: m.serrano@umh.es (M. Serrano).

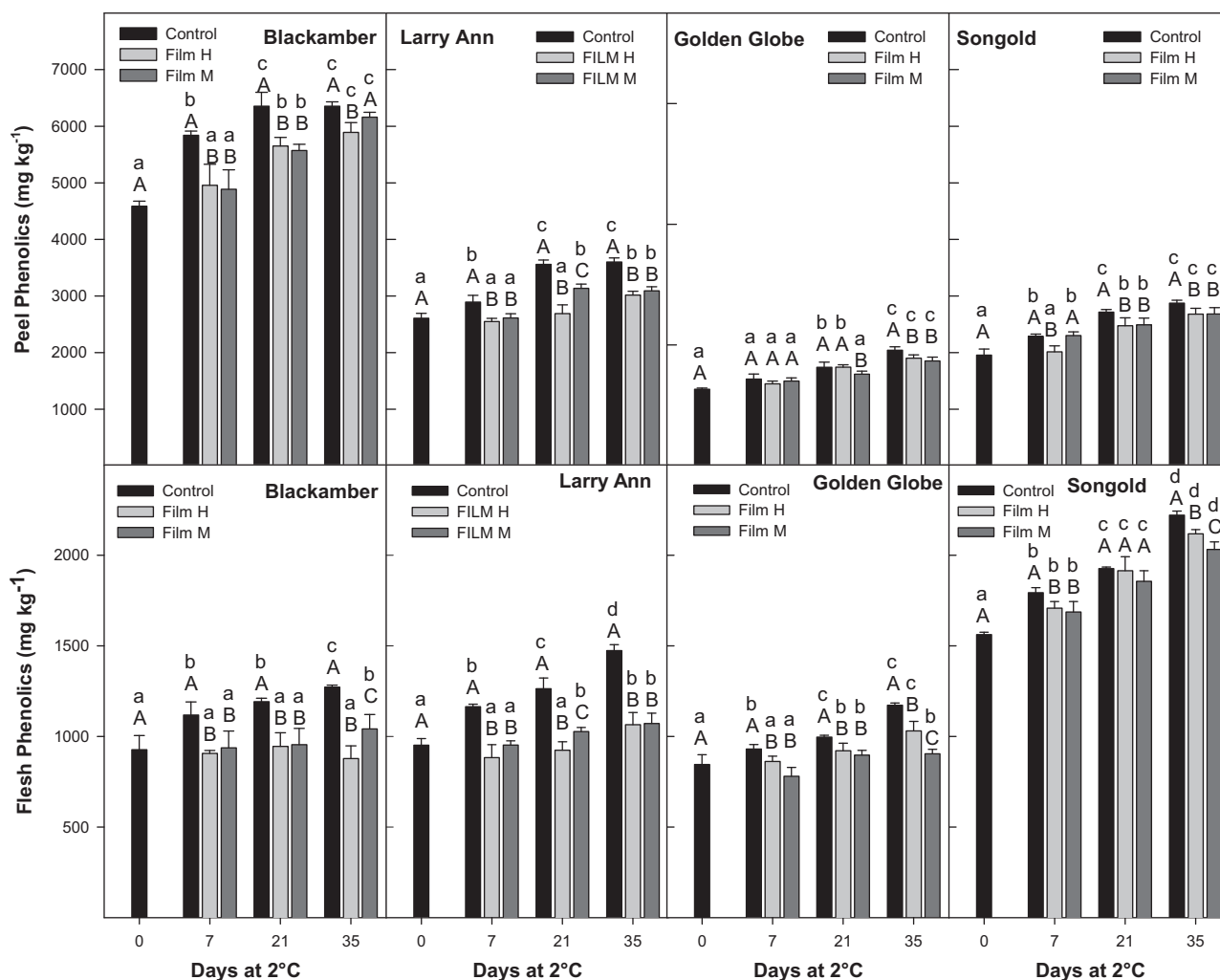


Fig. 1. Changes in total phenolics (peel and flesh) during storage at 2 °C of 4 plum cultivars under MAP conditions with 2 different films (M and H) or with a macroperforated film (control). Results are the mean \pm SE ($n=4$). Minor and capital letters show significant differences ($p < 0.05$) during 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 cm², 14 cm \times 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 dm², and total perforated area of 0.56%; film H was composed of polyester (12 μ m)-polypropylene (50 μ m), and film M was composed of polyester (12 μ m)-polypropylene (60 μ m), and both purchased from Amcor Flexibles (Amcor, Barcelona, Spain) having a permeability to O₂ <100 mL O₂ 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 N₂ 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 \pm 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 \times 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).

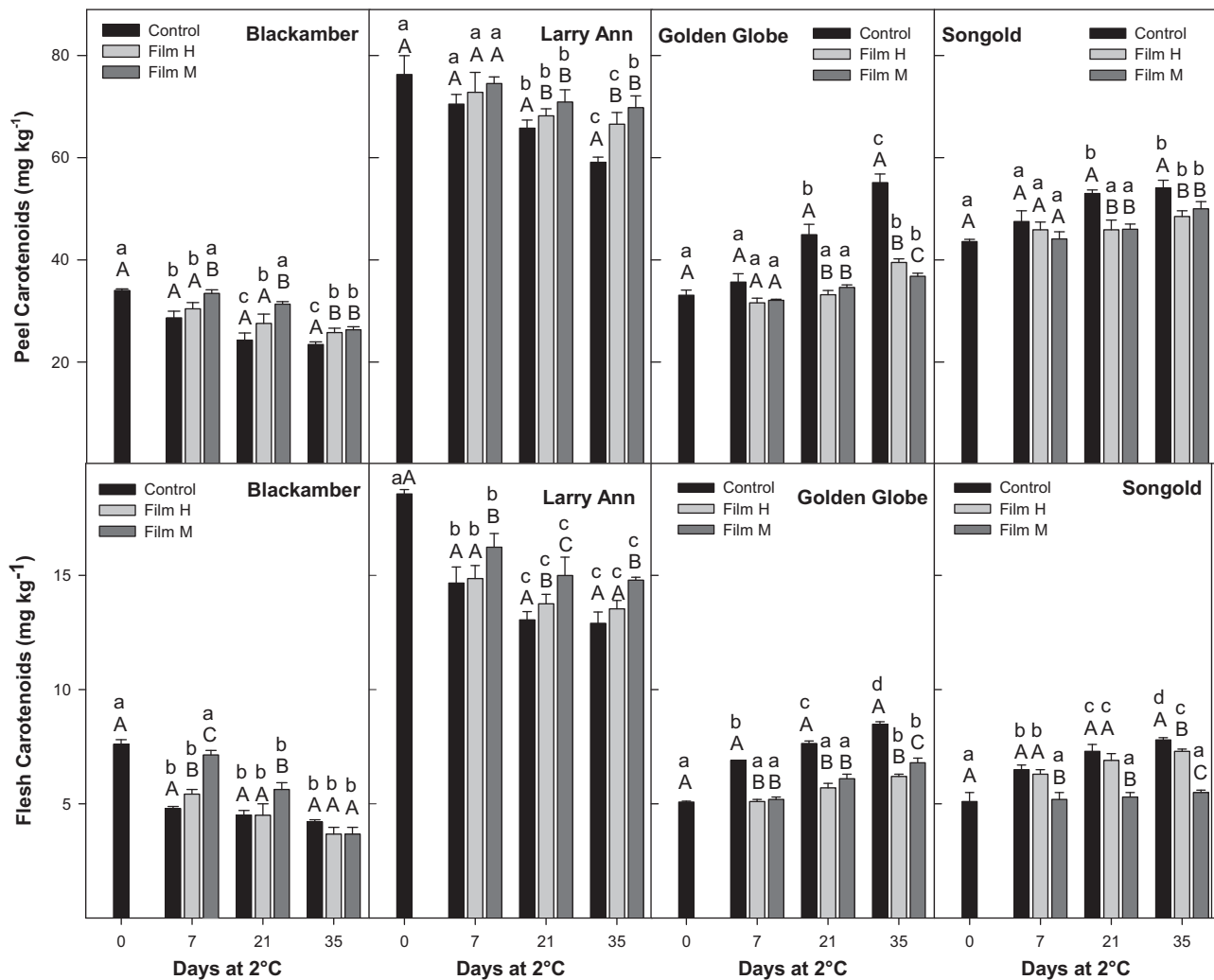


Fig. 2. Changes in total carotenoids (peel and flesh) during storage at 2°C of 4 plum cultivars under MAP conditions with 2 different films (M and H) or with a macroperforated film (control). Results are the mean \pm SE ($n=4$). Minor and capital letters show significant differences ($p < 0.05$) during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

1 mL from the extracts was filtered through 0.45 μ m Millipore filter and then injected into a C18 Supelco column (Supelco C-610H, 30 cm \times 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 \times 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 $\epsilon_{\text{cm}}^{1\%} = 2560$ and the results were the mean \pm 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, CO₂ concentrations increased and O₂ 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 CO₂ and O₂ concentrations at equilibrium of ≈ 6.5

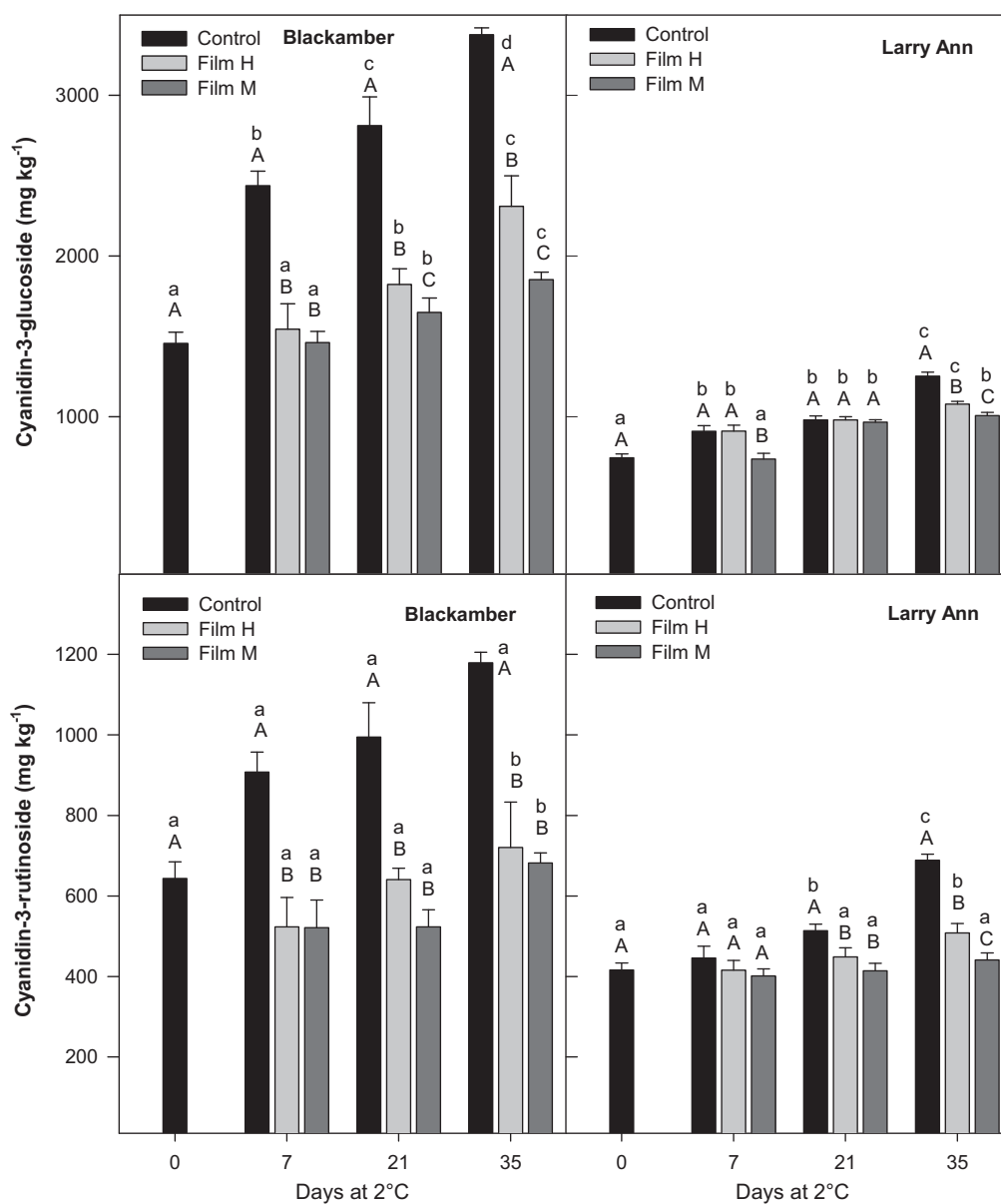


Fig. 3. Changes in anthocyanins (cyanidin-3-glucoside and cyanidin-3-rutinoside) in the peel of purple plum cultivars during storage at 2°C under MAP conditions with 2 different films (M and H) or with a macroperforated film (control). Results are the mean \pm SE ($n=4$). Minor and capital letters show 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 CO₂ and O₂, 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 cul-

tivars. 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 O₂ and high CO₂) 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 O₂ and high CO₂ 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

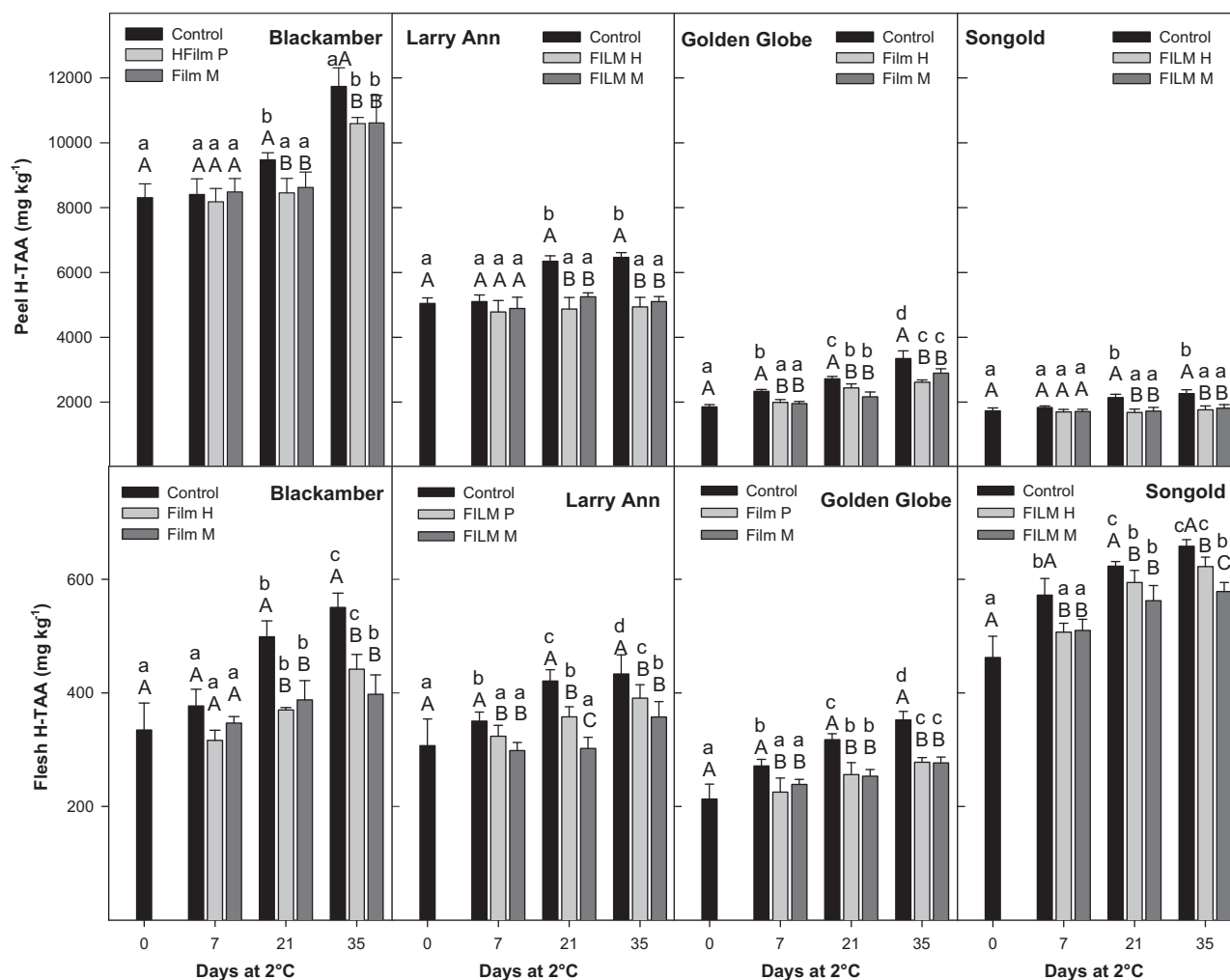


Fig. 4. Changes in total hydrophilic antioxidant activity (H-TAA) in the peel and flesh of 4 plum cultivars stored at 2 °C under MAP conditions with 2 different films (M and H) or with a macroperforated film (control). Results are the mean \pm SE ($n=4$). Minor and capital letters show significant differences ($p < 0.05$) 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

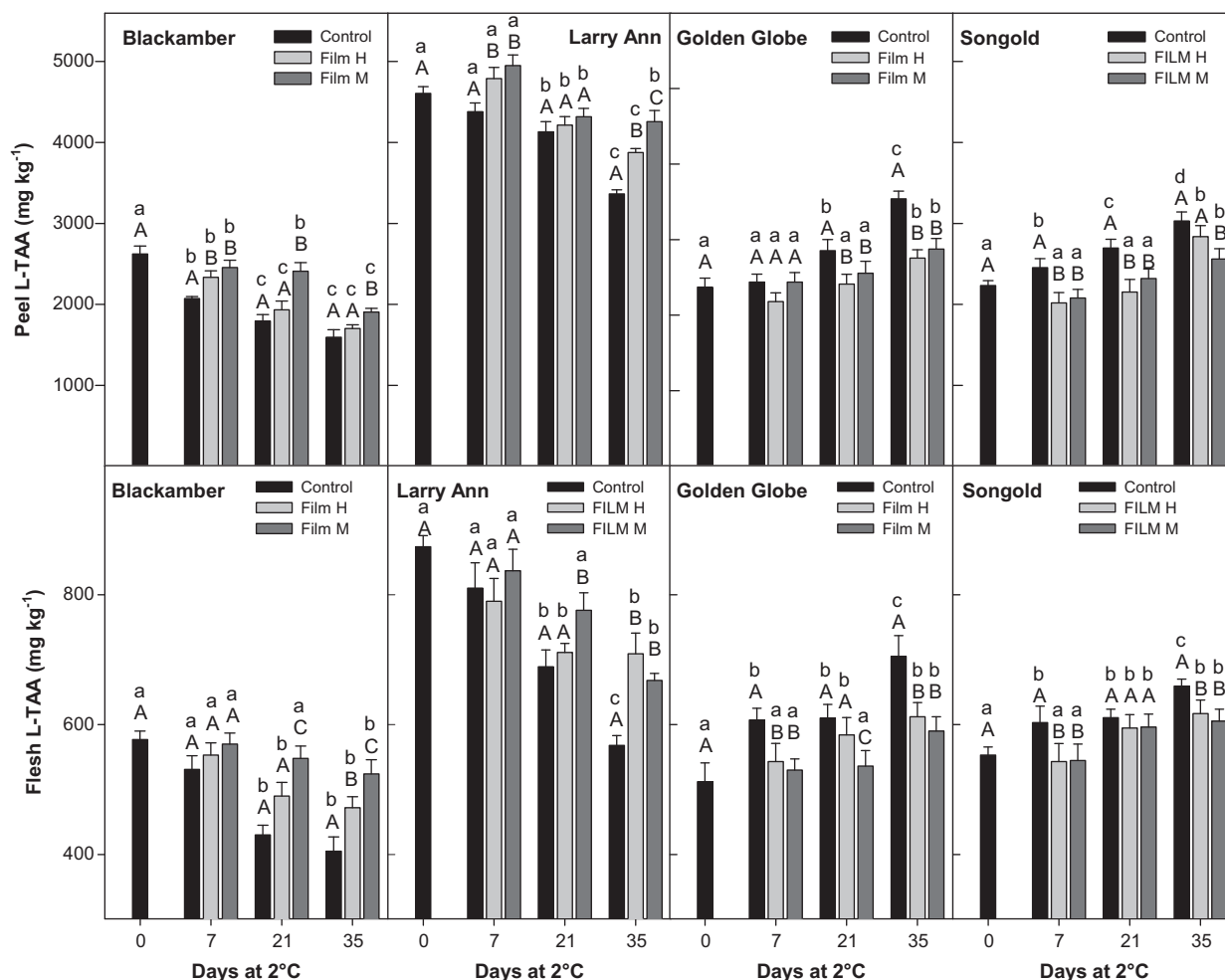


Fig. 5. Changes in total lipophilic antioxidant activity (L-TAA) in the peel and flesh of 4 plum cultivars stored at 2 °C under MAP conditions with 2 different films (M and H) or with a macroperforated film (control). Results are the mean \pm SE ($n=4$). Minor and capital letters show significant differences ($p < 0.05$) 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 ($8308 \pm 428 \text{ mg kg}^{-1}$) and SG the lowest ($1733 \pm 89 \text{ mg kg}^{-1}$) in the peel, while in the flesh the highest H-TAA was found in SG ($462 \pm 37 \text{ mg kg}^{-1}$) and the lowest in GG ($213 \pm 26 \text{ mg kg}^{-1}$). With respect to L-TAA, lower variations were found among cultivars at harvest, with levels of ≈ 2500 and $\approx 550 \text{ mg kg}^{-1}$ 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 $874 \pm 17 \text{ mg kg}^{-1}$ 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 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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