JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Antioxidant Systems and Their Relationship with the Response of Pepper Fruits to Storage at 20 °C

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Fresh peppers (*Capsicum annuum* L., variety California) in their green and red ripe stages were stored at 20 °C for 7 and 19 days to determine the effects of storage on whole fruit antioxidant capacity (TAA) and ascorbate (ASC) content, as well as on some antioxidant enzyme activities, such as catalase (CAT), superoxide dismutase (SOD), and those of the ASC−glutathione cycle. At least one Mn-SOD, two Fe-SODs, and three CuZn-SODs were detected in the fruit extract after native polyacrylamide gel electrophoresis. All of the SOD isozymes and glutathione reductase had higher activity levels in the red control fruits than in the green fruits, whereas the activities of monodehydroascorbate and dehydroascorbate reductase were higher in green fruits. Ascorbate peroxidase (APX) was found to be similar in both fruits. SODs, CAT, and APX seem to be involved in pepper fruit ripening and senescence during storage at 20 °C, perhaps influencing the active oxygen species levels in the fruit. TAA, as well as the ASC content, was higher in red peppers than in green, and storage increased the ASC in both green and red fruits.

KEYWORDS: Antioxidants; *Capsicum annuum*; pepper; ripening; storage

INTRODUCTION

AOS, such as superoxide radicals ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\bullet}OH$), are produced as a result of many biochemical reactions and are considered to be the prime causes of oxidative damage, including protein denaturation, mutagenesis, and lipid peroxidation in aerobic cells. Many conditions that limit productivity, including ozone exposure, metal toxicity, exposure to radiation, wounding, chilling, drought, salinity, heat stress, pathogens, and senescence, result in the enhanced production of AOS (*1*−*7*). Plant cells are protected against the effects of AOS by a complex antioxidant system that involves the water soluble reductants, ASC and GSH, lipid soluble antioxidants (α-tocopherol and β-carotene), and enzymes such as SOD (EC 1.15.1.1), CAT (EC 1.11.1.6), and enzymes of the ASC−GSH cycle: APX (EC 1.11.1.11), MDHAR (EC 1.6.5.4), DHAR (EC 1.8.5.1), and GR (EC 1.6.4.2) (*8*, *9*). An important feature of these protective mechanisms is that their activity is enhanced when plants are exposed to conditions that increase free radical production (*10*, *11*). Thus, these protective antioxidant mechanisms play important roles in tolerance and/or acclimation of plants to environmental stress.

Reactions involving AOS are an intrinsic feature of senescence and fruit ripening. Increases in hydroperoxides (H_2O_2 and lipid hydroperoxides) have been reported during banana, pear, pepper, and tomato ripening (*12*−*14*) as well as during the senescence of pea leaves (*2*, *11*, *15*, *16*). Hydrogen peroxide also produces a response of the defense system (*7*, *10*). It is likely, therefore, that the antioxidant system will play an important role in both senescence and fruit ripening.

Fruits are a good source of antioxidants (*17*), but little is known about the effects of storage on the retention of dietary antioxidants by fresh fruits, although it is clear that the metabolism of vegetable crops continues to function despite separation from the plant (*19*). AOS are involved potentially in many aspects of the storage process, being associated with accelerated symptoms of breakdown and sugar accumulation, but little information is available regarding the effects of storage on the activities of different antioxidant enzymes, and any results usually refer only to one or two of these activities. This aspect is important since specific responses have been observed for different antioxidant enzymes in relation to senescence and ripening (*2*, *11*, *20*), processes that can take place during storage.

The following study describes the response of pepper fruits in two ripening stages (green and red) to storage at 20 °C. The ASC content, TAA, and several antioxidant enzyme activities, including SOD, CAT, and the enzymes of the ASC−GSH cycle, were studied in order to ascertain their possible involvement in the changes that occur in both fruits during storage. The results show that storage at 20 °C induces ripening- and senescence-associated symptoms in the fruits of both stages, which were accompanied by differential changes in SOD, CAT, and APX, depending on the particular stage. There was a particularly

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10.1021/jf030052i CCC: $25.00
Published on Web 09/09/2003

strong increase in ASC during storage, while increased TAA capacity was observed in green peppers, suggesting that storage at 20 °C might actually enhance the nutritional value of pepper fruits.

MATERIALS AND METHODS

Fruit Samples. Recently collected (controls) green and red fruits of pepper (*Capsicum annuum* L.) cv. California were maintained in darkness at 20 °C, in a controlled temperature and humidity (65−70%) chamber for 7 or 19 days. Such storage of pepper fruits without waxing at 20 °C is a normal practice used in ripening studies. Ethylene production, respiration rates, color, and ion leakage were measured immediately after storage. The pericarp of fruits (without seeds) was frozen in liquid nitrogen and stored at −80 °C until further analysis. The experiment was repeated twice.

Ethylene and CO_2 Production. The fruits were placed in hermetically sealed jars, and ethylene and CO_2 were analyzed according to the protocols described by Pretel et al. (*21*) and Serrano et al. (*22*), respectively.

Color Measurement. Fruit color was determined by reflection using the Hunter Lab system in a Minolta colorimeter, as described by Flores et al. (*23*).

Ion Leakage. Ion leakage was measured using a conductivity method as described by Serek et al. (*24*), with modifications as described by Ben-Amor et al. (*25*).

TAA. All operations were conducted at 4 °C. One gram of frozen tissue was homogenized in 2 mL of cold 50 mM potassium phosphate buffer (pH 7.8), filtered through a nylon mesh, and centrifuged at 12 000*g* for 5 min. The supernatant was immediately used to measure TAA capacity. This assay will measure all of the fruit compounds able to eliminate ABTS radicals; the end point or decrease in absorbance method, as described by Cano et al. (*26*), was used. The reaction mixture, in a final volume of 1 mL, contained 0.1 mM ABTS, 0.01 mM H_2O_2, and 0.25 μM horseradish peroxidase in 50 mM glycine−HCl buffer, pH 4.5. The samples were added when absorbance at 730 nm was stable, after which the decrease in absorbance at 730 nm, due to the disappearance of the ABTS radicals formed by the peroxidase/H_2O_2 system, was measured at 25 °C. Different amounts of L-ascorbic acid were used as a standard, and the TAA values were expressed as L-ASC equivalents, defined as the amount of L-ASC producing the same absorbance decrease as the sample assayed.

ASC Determination. Fruit ASC content was determined as follows: frozen tissue (0.5 g) was ground in 1 mL of cold 5% metaphosphoric acid, and the extract was kept on ice, in the dark, for 30 min. After centrifugation at 20 000*g* for 10 min, the supernatant was passed through a C18 column (Waters) and a 0.2 μm filter, before analysis by high-performance liquid chromatography (Shimadzu LC10), while reduced ASC was determined as described by Castillo and Greppin (*27*). DHA was separated from ASC, as described by Jiménez et al. (*28*), by incubating the samples for 24 h, at room temperature, with 1 mM dithiothreitol (final concentration). The DHA concentration was measured as ASC following rechromatography. The system was calibrated with different concentrations of ascorbic acid as a standard.

Enzyme Activity Assays. For the extraction of CAT, SOD, GR, MDHAR, and DHAR activities, 1 g of frozen fruit tissue was ground in 2 mL of 50 mM Tris-HCl buffer (pH 7.2) containing 0.1 mM ethylenediaminetetraacetic acid (EDTA), 5 mM cysteine, 0.2% (v/v) Triton X-100, 4% (w/v) soluble PVP, and 0.1 mM PMSF, using a mortar and pestle.The homogenate was filtered through one layer of nylon. For APX extraction, sodium ASC (20 mM) was included in the extraction buffer and EDTA was omitted. After centrifugation at 15 000*g* for 10 min, the supernatant was immediately frozen at −20 °C, except in the case of APX activity, which was determined immediately. All of the operations were carried out at 4 °C, and the enzyme activities were measured as previously described (*28*). Enzyme activities were corrected for nonenzymatic rates and for interfering oxidations (*28*).

Nondenaturing PAGE was performed to separate the SOD isozymes using 12% (w/v) acrylamide gels, with 7.5% (w/v) acrylamide stacking gel and a mini-protean III dual slab Cell (Bio-Rad). Each lane of the gel contained the same amount of protein. SOD isozymes were localized photochemically, and isozyme identification was performed using the selective inhibitiors, KCN and H_2O_2, according to Gómez et al. (*29*). The percentage of activity for the different SODs was quantified by recording the transmittance of gels in a CS-9000 densitometer (Shimadzu, Kyoto).

Protein Content. Protein was determined by the Coomasie Brilliant Blue dye binding method of Bradford (*30*), using bovine serum albumin as a standard.

Statistical Evaluation. Data were analyzed and compared by the Student's *t*-test. All of the changes described were relative to the values found in the freshly picked control pepper fruits.

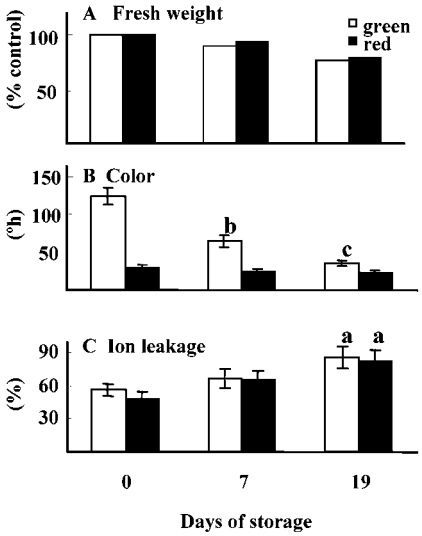

Figure 1. Fresh weight (A), color (B), and ion leakage (C) in green (white squares) and red (black squares) pepper fruits during storage at 20 °C. Values are means ± SE of at least four samples. Values relative to the controls were significant at $p < 0.05$ (a), $p < 0.01$ (b), and $p < 0.001$ (c).

RESULTS AND DISCUSSION

Weight, Color, and Ion Leakage. Both green and red peppers lost around 10 and 30% of their fresh weight after 7 and 19 days of storage at 20 °C, respectively (**Figure 1A**). We consider that both green and red peppers ripened until senescence, as can be seen by the changes in fruit color and by the similar increase in ion leakage for both fruits (**Figure 1B**,**C**). It is known that fruit ripening is accompanied by the deterioration of cell membranes (*31*), pigment accumulation, and changes in the cell walls that result in softening. We observed these changes in the color parameters and in the softening of the pepper fruits after storage; the color change was greater in green fruits than in red ones. It was reported that AOS mediate the induction of genes involved in carotenoid synthesis, in the transformation of chloroplasts into chromoplasts (*32*). Such a transition seems to have taken place in green peppers after 19 days of storage, when the results for color were similar to those for the freshly picked control red peppers (**Figure 1B**). The observed increase in ion leakage with storage (**Figure 1C**) could be due, at least partly, to the described loss of cellular membrane integrity, as was reported for microsomal membranes during the senescence of bell pepper (*33*) and the ripening of tomato fruit (*15*). During the senescence process, a large increase in the generation of AOS, mainly $O_2^{\bullet -}$ and H_2O_2, has been described in different cell compartments, including mitochondria and peroxisomes, and such generation results in, among other things, a dramatic increase in lipid peroxidation and membrane leakiness (*2*, *16*). In the case of our green and red pepper fruits, this could mean that both ripening and senescence processes occurred during

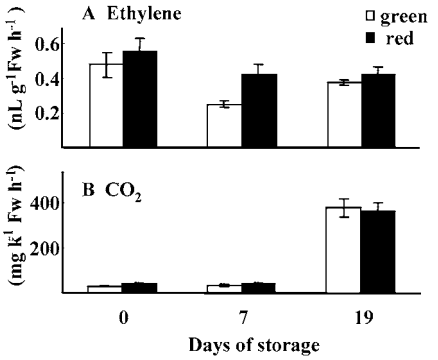

Figure 2. Ethylene (A) and CO_2 (B) emission in green (white squares) and red (black squares) pepper fruits during storage at 20 °C.

Table 1. Effect of Storage at 20 °C on ASC Content, ASC Redox State, and Total Antioxidant Activity of Green and Red Pepper Fruits[a]

		control	7 days	19 days
ASC (μmol g^{-1} FW)	green	2.4 ± 0.1	3.8 ± 0.5 a	3.9 ± 0.2 b
	red	3.6 ± 0.2	3.6 ± 0.01	5.6 ± 0.08 c
redox (ASC/total)	green	0.99	0.99	0.96
	red	0.9	1	0.95
TAA (μmol equiv ASC g^{-1} FW)	green	4.42 ± 0.04	6.90 ± 0.36 b	6.74 ± 0.46 b
	red	7.32 ± 0.16	4.02 ± 0.22 b	7.38 ± 0.36

[a] Values are means ± SE of at least four samples. Values relative to the controls were significant at $p < 0.05$ (a), $p < 0.01$ (b), and $p < 0.001$ (c).

storage at 20 °C. Green peppers ripen to red, and red fruits senesce, but in this case, green peppers detached from the plant were also senescing.

Ethylene and CO_2 Emission. Some fruits exhibit respiratory increases during ripening, although it has been described that pepper fruits show a nonclimateric type ripening physiology (*21*, *34*). We only detected an increase in respiration at 19 days of storage of both green and red pepper fruits (**Figure 2A**,**B**), which may be related with the senescence process that was also occurring in both fruits at the end of the storage period (*21*). Increased O_2 consumption has been observed in the dark-induced senescence of pea leaves (*35*).

Ascorbic Acid Content. Levels of ascorbic acid in the pepper fruits (45 and 65 mg/100 g in freshly picked control green and red peppers, respectively; **Table 1**) are in the lower part of the range reported for ASC in several pepper types (*36*), 60−200 mg/100 g. The ASC content was higher in red control peppers than in green ones, and in both fruits, ASC increased after 7 and 19 days of storage at 20 °C; as the fruit ripened and senesced, the increase was around 60% after 19 days (**Table 1**). The percentage of the oxidized form, DHA, was found to be higher in control red fruits, as reflected by the redox state value (ASC/total ratio) (**Table 1**). After 19 days of storage, DHA had increased in green fruits but decreased in red peppers and the redox state was found to be similar for both fruits.

TAA. Contributors to TAA in peppers are numerous and include ascorbic acid, flavonoids, capsaicinoids, and phenolic acids. As Howard et al. (*36*) described, the TAA in different pepper cultivars significantly increases with maturation as do the phenolics, and we observed the antioxidant capacity of pepper fruits to be higher in red peppers than in green (**Table 1**), although the levels in green fruits after 19 days of storage reached similar values to those of the red control fruits, perhaps due to the ripening process that occurred. Similar increases in antioxidant capacity have been described by Kalt et al. (*19*) in strawberries and raspberries during storage at 10, 20, and 30 °C for up to 8 days. In our study, however, the TAA of red fruits did not follow this pattern since this antioxidant activity was similar in both red control fruits and those stored for 19 days (**Table 1**).

Table 2. Effect of Storage at 20 °C on Protein Content and the Activities of SOD and CAT in Green and Red Pepper Fruits[a]

		control	7 days	19 days
protein (mg g^{-1} FW)	green	0.96 ± 0.30	0.22 ± 0.08	0.38 ± 0.04 a
	red	0.42 ± 0.10	0.24 ± 0.02	0.30 ± 0.08
SOD (U g^{-1} FW)	green	485.8 ± 20.6	806.0 ± 54.1 a	752.0 ± 122.1
	red	805.2 ± 52.4	860.6 ± 125.2	616.6 ± 25.2 a
CAT (μmol min^{-1} g^{-1} FW)	green	58.0 ± 10.6	38.6 ± 3.8	26.2 ± 5.8 a
	red	25.6 ± 1.4	16.2 ± 2.6 a	23.6 ± 2.4

[a] Values are means ± SE of at least four samples. Values relative to the controls were significant at $p < 0.05$ (a).

Conflicting results have been reported concerning the possible correlation between ASC content and TAA (*19*, *37*). In our study, the ASC content of green pepper fruits followed the same pattern as TAA during storage, suggesting that it is one of the main compounds responsible for TAA in these pepper fruits under these conditions, in agreement with the results described by Gao et al. (*37*). In contrast, it seems that ASC was not the main antioxidant contributing to the TAA of the red pepper fruits during storage, since the ASC level was unchanged after 7 days and had increased in fruits after 19 days of storage, while their TAA decreased and/or was maintained. This indicates that antioxidant compounds that contribute to TAA in red pepper fruits, other than ASC, could be decreasing during storage of red fruits only.

Protein Content. Changes in proteins are common features of fruit ripening (*14*). We found that green fruits had about a 2.3-fold greater protein content than red fruits, although this fell by up to 77% after 7 days of storage and it slightly increased after 19 days to represent 39.5% of the protein content in control fruits. However, in red fruits, the loss of proteins during storage was less pronounced and they showed a similar protein content to green fruits after both 7 and 19 days of storage (**Table 2**). As we found in peppers, losses of proteins have been described during the first stages of tomato fruit ripening as well as increases at the end of the process (*20*).

Because of the different protein contents found in control green and red pepper fruits, as well as the decrease produced by storage at 20 °C (**Table 2**), we decided to express the enzyme activities on a fresh weight (FW) basis, which was very similar in green and red fruits and was much less affected by the storage in both fruit types.

Antioxidant Enzymes. In both green and red peppers, the activities of the antioxidant enzymes CAT and SOD, and those enzymes involved in the ASC−GSH cycle, APX, MDHAR, DHAR, and GR, were also measured after 7 and 19 days of storage at 20 °C (**Tables 2** and **3**). Different responses of the activities of these enzymes were observed during this time. As regards SOD, this enzyme seems to be involved in pepper fruit ripening, since, in both green and red peppers, SOD activity had increased with ripening after 7 days. After 19 days of storage, this activity had decreased markedly in overripe red fruits, whereas green fruits showed higher SOD activity levels than control fruits after 19 days. As regards such changes in SOD, there are conflicting data in the literature dealing with ripening and senescence (*2*, *20*, *38*−*40*). In pepper fruits, SOD has been shown to increase from the green to the yellow/green stage (*41*) but to decline at the end of ripening as in saskatoon fruits (*14*), behavior similar to our results.

Table 3. Effect of Storage at 20 °C on the Activities of the Enzymes Involved in the ASC–GSH Cycle in Green and Red Pepper Fruits[a]

		control	7 days	19 days
APX (μmol min^{-1} g^{-1} FW)	green	6.20 ± 0.34	6.40 ± 0.26	6.90 ± 0.44
	red	5.92 ± 0.06	3.58 ± 0.30 b	0.42 ± 0.16 c
MDHAR (nmol min^{-1} g^{-1} FW)	green	257.2 ± 26.0	94.2 ± 10.0 b	15.8 ± 4.0 c
	red	180.0 ± 29.0	104.6 ± 9.2	79.2 ± 15.0 a
DHAR (nmol min^{-1} g^{-1} FW)	green	984.6 ± 101.2	779.4 ± 85.0	597.4 ± 67.4 a
	red	602.0 ± 14.0	438.6 ± 67.4	494.6 ± 46.0 a
GR (nmol min^{-1} g^{-1} FW)	green	126.2 ± 14.6	166.6 ± 12.6	184.0 ± 11.6 a
	red	222.6 ± 24.1	250.6 ± 27.8	260.6 ± 19.0

[a] Values are means ± SE of at least four samples. Values relative to the controls were significant at $p < 0.05$ (a), $p < 0.01$ (b), and $p < 0.001$ (c).

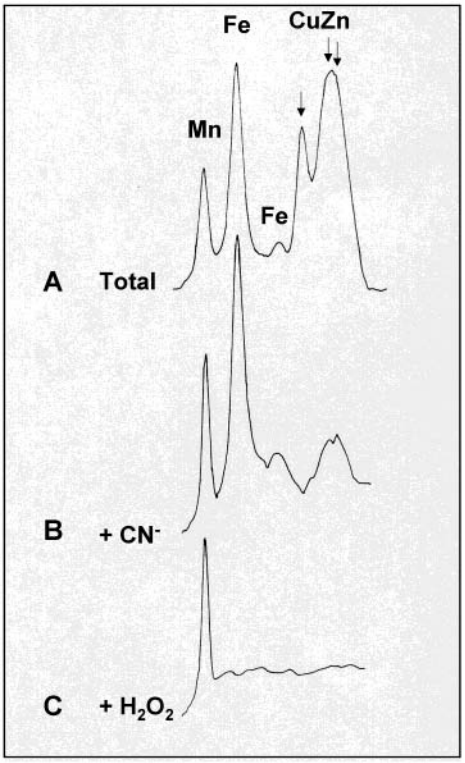

Figure 3. SOD isozyme identification after native PAGE on 12% (w/v) acrylamide gels for crude extracts of pepper fruits. (A) Total activity (no inhibitors), (B) stained in the presence of 2 mM KCN, and (C) stained in the presence of 5 mM H_2O_2.

Together with the analysis of total SOD activity, an analysis of the SOD isozyme pattern in both fruits was carried out. In pepper fruit extracts, native PAGE detected at least six SOD isoforms: one Mn-SOD, two Fe-SODs, and three CuZn-SODs, judging by their sensitivity to 2 mM KCN and 5 mM H_2O_2, respectively (**Figure 3**). Both green (**Figure 3**) and red (data not shown) control fruits had a similar qualitative composition of SOD isozymes. The densitometric quantification of the different isozymes showed that CuZn-SODs represented the major isozymes in the fruits (around 60% of the total activity), followed by Fe-SOD and Mn-SOD, which accounted for around 25 and 10−15% of the total activity, respectively (**Figure 4A**,**B**). All of the SOD isozymes seem to be involved in both the observed higher activity of the red control fruits, as compared with the green ones, and the significant increase of total SOD activity produced in green fruits at 7 days (**Table 2**). In the same green fruits, only CuZn-SOD isozymes continued to increase up to 19 days of storage (**Figure 4A**), reaching similar values to those of control red fruits, while Fe-SOD activity was

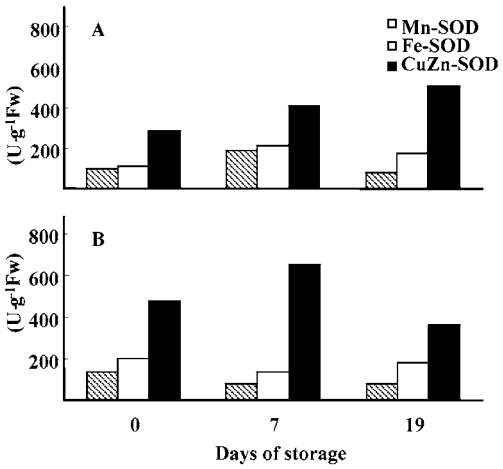

Figure 4. Relative enzyme activity of the different isoforms of SOD in green (A) and red (B) pepper fruits, based on densitometric measurements of the SOD activity bands.

the most reduced. This was not the case for red fruits, where the increase of the CuZn isozyme was accompanied by a loss in Fe- and Mn-SODs at 7 days, while at 19 days of storage, when the fruits were overripe and senescent, the loss in total activity was due mainly to an important decrease in CuZn-SOD while Mn-SOD did not change and Fe-SOD increased slightly. The differential responses found in these SOD isozyme activities could reflect changes in oxidative metabolism in the different cell compartments during the ripening and senescence of pepper fruits. Changes in the different SOD isozymes have been reported also during the ripening of tomato fruit (*42*, *43*). In pea leaves too, an increase in the peroxisomal Mn-SOD, as well as an induced new CuZn-SOD, has been reported during senescence, which resulted in an enhanced H_2O_2 concentration and in the conversion of peroxisomes into specialized glyoxysomes, which contain the fatty acid β-oxidation and glyoxylate cycle enzymes (*11*). In contrast, mitochondrial Mn-SOD activity levels decreased under the same senescent conditions (*2*). In our study, CAT and total SOD followed inverse patterns in the two fruit types as storage continued, the former being 2.3-fold higher in green than in red fruits. At the end of storage, the activity in green fruits was less than half that of the control fruits, reaching values similar to those in control red fruits in which the reduction in CAT activity was significant at 7 days. There are also conflicting data on CAT activity during ripening, since both increases and decreases in this enzyme have been reported in different fruits (*20*, *44*), differences that could be due to different ways of expressing activity and to the experimental conditions in which ripening occurred. In pepper fruits picked at different maturation stages, CAT specific activity has been found to remain fairly constant (*41*). In our green pepper fruits, the increase in SOD, which was accompanied by decreased CAT activity during storage, meant that the SOD/CAT ratio changed from 8.4 to 28.7 after 19 days at 20 °C. This situation involving the induction of an H_2O_2-generating enzyme and the loss of an H_2O_2-scavenging enzyme may have resulted in an increased H_2O_2 content in the cells of green fruits during storage, but this peroxide would not be sufficient to inhibit the CuZn isozyme. Similarly, in red fruits, the SOD/CAT ratio increased from 31.4 to 53.1 after 7 days of storage, whereas at 19 days this ratio was similar to that in freshly picked control fruits, due to the increase in CAT activity. An increase in H_2O_2 levels and lipid hydroperoxides at the onset of maturation and in mature banana and pear fruits has been reported (*12*, *13*). The suggested increase in H_2O_2 concentration

at the end of storage of red fruits, when peppers are overripe and senescent, could be inhibiting CuZn-SOD, which is sensitive to this compound. However, we cannot rule out the fact that in these conditions, another mechanism could control the activity of CuZn-SOD, acting at the transcriptional or transductional level, as has been described in other situations that induce oxidative stress (*42*, *45*, *46*). The decrease in CAT, mainly in green fruits, suggests a role for the peroxisomes in the storage process. Peroxisomes are known to be involved in the senescence process of pea leaves, related to AOS (*11*, *16*, *35*), but their role during fruit ripening is still unknown.

We also studied the effect of storage on the ASC−GSH cycle enzymes of green and red peppers (**Table 3**). In general, the activities of the ASC−GSH cycle enzymes involved in ASC reduction, such as MDHAR and DHAR, were higher in green than in red control fruits, while the levels of APX involved in ASC oxidation remained similar in both fruits (**Table 3**). As we previously described, the ASC content was found to be higher in red fruits, which, however, showed a lower redox state of ASC than green fruits, suggesting that the synthesis of ASC in red fruits was greater than its enzymatic reduction from MDHA and/or DHA. A similar pattern was found during storage, when green and red fruits ripened and became senescent. The ASC content increased in both fruits, and the enzyme activity involved in its regeneration from oxidized forms decreased, particularly in green fruits (40% for DHAR and 94% for MDHAR, as compared with 20 and 60% for DHAR and MDHAR in red fruits). These results suggest that the synthesis of ASC seems to be important during the ripening and senescence of green and red pepper fruits, allowing ASC levels not just to be maintained but even increased, although in red fruits the substantial decrease in APX activity observed after both 7 and 19 days may also have contributed to the decreased rate of ASC oxidation in red fruits during storage. The ASC content and activities of the ASC−GSH cycle enzymes have been reported to increase during maturation of sweet pepper, and while Imahori et al. (*41*) reported that these processes are correlated, Grantz et al. (*47*) showed that in tomato fruit, there was an inverse correlation between MDHAR and ASC levels, similar to that which we have described (**Tables 1** and **3**). The only enzyme that we found to behave differently was APX, the activity of which did not change in green fruits but decreased substantially in red fruits in an advanced stage of ripeness and senescence. One possibility is that reduced ASC is synthesized and its high level at the end of pepper fruit ripening could perhaps be destined for, among other processes, loosening of the cell wall, which leads to fruit softening, possibly by means of a mechanism similar to that described by Fry (*48*), implying the oxidative breakdown of polysaccharide chains and the involvement of AOS. However, a deeper study of ASC localization and transport in these fruits, particularly its possible presence and increase in the apoplast, is needed, because the effect on wall degradation must occur in this compartment, where ASC could act as a prooxidant.

As regards GR, this activity maintains the antioxidant GSH in its reduced form (*10*), and this compound plays an essential role in the antioxidant defense, particularly as a reductant for DHA. It is also involved in the redox balance of the cell, including the redox state of the sulfydryl proteins. Römer et al. (*49*) reported increased GR activity and an accumulation of GSH during the ripening of sweet pepper fruit. In our peppers, too, high GR activity was found in red fruits. This activity increased with storage in both green and red peppers, although the increase in overripe red fruits was not significant. Thus, GSH could also contribute to the TAA increase observed in our pepper fruits during storage. However, it is also important to note that all of the results regarding antioxidant enzyme activities correspond to those in total fruit extracts, and as has been indicated previously, these may not necessarily reflect the changes taking place in a specific organelle. In Yolo Wonders peppers, for example, a large increase in organelle APX activity was found during the transition from chloroplast to chromoplast, whereas only a slight difference was detected in the APX activity measured in the total fruit extracts (*50*), similar to that reported here during the storage and ripening of green pepper fruits. Moreover, we have reported recently that mitochondria from red pepper fruits showed higher APX and Mn-SOD than those from green fruits, whereas the content of ASC and the activity of GLDH were similar in the mitochondria from both fruits (*51*). These results, together with those for the activities of the other enzymes of the ASC−GSH cycle in mitochondria, suggest that ASC biosynthesis does not decrease as fruit mature and that GLDH, a mitochondrial membrane-bound protein that is responsible for the last step of ascorbic acid synthesis (*52*), is present in excess of requirements in both fruits. All of these results are in agreement with the total fruit ASC content found in controls and during the storage of both green and red pepper fruits. With respect to the postharvest aspect of this study, it is important to point out that green peppers have more than double the protein content, per gram of FW, and that there is a protein loss during storage, more important in green than in red fruits. Also, the large increase in ASC content of peppers, even after 19 days of storage at 20 °C, is important. Vitamin C content in green and red peppers was increased by around 60% during the storage, and the ASC in red fruits after 19 days of storage was more than double that in control green fruits. Related to TAA, green fruits increased their antioxidant properties during storage more than the red peppers, but control red peppers had almost double the capacity than the control green fruits; so, in terms of nutritional value, red peppers are better than green, and these properties are not lost but are even improved after storage at 20 °C. These fruits, at the end of the storage period, could be interesting for the food processing industry because of their added nutritional value.

ABBREVIATIONS USED

ABTS, ferrylmyoglobin/2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid); AOS, activated oxygen species; APX, ascorbate peroxidase; ASC, ascorbate: reduced form; CAT, catalase; CuZn-SOD, copper zinc-containing superoxide dismutase; DHA, ascorbate: oxidized form (dehydroascorbate); DHAR, dehydroascorbate reductase; GLDH, L-galactono-γ-lactone dehydrogenase; GR, glutathione reductase; GSH, glutathione: reduced form; Fe-SOD, iron-containing superoxide dismutase; MDHA, monodehydroascorbate; MDHAR, monodehydroascorbate reductase; Mn-SOD, manganese-containing superoxide dismutase; PAGE, polyacrylamide gel electrophoresis; SOD, superoxide dismutase; TAA, total antioxidant capacity.

ACKNOWLEDGMENT

We are grateful to Mrs. D. Lapaz for technical assistance.

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Received for review January 23, 2003. Accepted July 10, 2003. This work was supported by grants DGES: PB 98-0493-C02-02 and FEDER: IFD97-0889-C02-01 and by the SENECA Foundation (through a grant to A.J.).

JF030052I