Electron Beam Ionization Induced Oxidative Enzymatic Activities in Pepper (*Capsicum annuum* L.), Associated with Ultrastructure Cellular Damages

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Mature green pepper fruits (*Capsicum annuum* L.) were subjected to ionizing radiation, in the range of 1−7 kGy, with accelerated electrons. Ultrastructural changes by electron microscopy, and the activity of several oxidative metabolism-related enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaicol peroxidase (POX), and lipoxygenase (LOX), were determined in pericarp tissue just after the ionization treatment and during postionization storage at 7 °C followed by 3 days at 20 °C. Changes in oxidative stress during the ionization treatment was assessed by the accumulation of malondyaldehide (MDA), a lipid peroxidation product. The ionization induced modifications in the cell ultrastructure, a moderate separation of the plasma membrane from the cell wall being observed for all doses. At 5 and 7 kGy, peroxisomes were not detected and the structures of the chloroplast and vacuoles were seriously damaged. Lipid peroxidation and lipoxygenase activity increased with the ionization dose, staying constant and decreasing, respectively, during the storage period. Conversely, catalase, ascorbate peroxidase, and superoxide dismutase had lower values than in nonionized fruits and, in general, their values did not change or diminished slightly from the seventh day of storage. Peroxidase exhibited an increase in activity with the ionization dose, although these was not a linear relationship, with higher values at 3kGy. Ionization of pepper, especially at doses of 5 and 7 kGy, caused a significant oxidative damage in the fruit, since it increased oxidation and decreased the antioxidant enzymatic defense systems causing ultrastructural changes at cell level.

KEYWORDS: Pepper; ionization; electron beam; guaiacol peroxidase; ascorbate peroxidase; lipoxygenase; catalase; superoxide dismutase; lipidic peroxidation; ultrastructure; storage

INTRODUCTION

Food ionization has been used for treatments of disinfestation (*1*, *2*), inhibition of sprouting (*3*), delay of fruit ripening (*4*, *5*), and treatments of pasteurization and sterilization (*6*, *7*). In some cases, it can replace the use of chemical additives or be used in combination with them (*8*). The effects of the ionization of foods cannot be generalized if the food and the dose absorbed by it (*9*) are not specified.

The treatment of fruits and vegetables with ionizing radiation can act on the mechanisms responsible for ripening and aging, ensuring that both processes evolve slowly, to increase the commercial useful life of these products. When these products are ionized, they can suffer alterations in their chemical composition, affecting to a greater or lesser extent their nutritional and organoleptic properties. This has motivated the study of the processes involved in these alterations.

It is generally accepted that the mechanism of ionization damage involves oxidative process. Several studies have shown that the ionization treatment could cause an increase in the level of cellular reactive oxygen spices generating oxidative stress (*10*, *11*). During the process of ionization, and because of the radiolysis of water molecules, the free radicals are formed with an average life of 10^{-9} s. The effects of ionizing radiation on foodstuffs can be direct or indirect. In the first case, the radiation acts directly on the matter, causing the breakdown of molecules such as DNA and other food constituents. In the second case, the radiation acts through water radiolysis and, therefore, the different water radiolysis products will react with other compounds, causing significant chemical changes, like hydroperoxide formation (*12*, *13*).

Superoxide dismutase (SOD), peroxidase (POX), and catalase (CAT) are important enzymes that protect plants against oxidative damage. SOD catalyzes the dismutation of superoxide radical (O_2^-) to O_2 and H_2O_2; the different isoforms of this enzyme can be found in peroxisomes, chloroplasts, mitochon-

dria, and cytosol (*14*). POX and CAT then catabolize H_2O_2 to H_2O and O_2 and hence limit the potential for further free-radical production from H_2O_2. While catalases are mainly found in peroxisomes, the peroxidases are also found in chloroplasts, mitochondria, and the cell wall (*15*). Ascorbate peroxidase (APX) scavenge the H_2O_2 using ascorbate as an electron donor as part of a system for the protection of chloroplast and other cellular compartments against oxidative damage (*16*). At present, our knowledge concerning the role of the antioxidant system in protecting plants under ionization stress is limited because few data exist on this matter (*17, 18*).

Changes in oxidative stress during ionization treatment can be assessed by the increase of lipid peroxidation; membrane lipids and free fatty acids are highly susceptible to oxidation and increasing oxidative stress is thus indicated by the accumulation of lipid peroxidation products. Thiobarbituric acid-reactive substances (TBARS), like MDA, are produced from the spontaneous decomposition of lipid hydroperoxides and, thus, they are considered sensitive markers of peroxidative damage (*19*).

Lipoxygenase (LOX) plays a central role in senescence-induced membrane deterioration by peroxidizing free polyunsaturated fatty acids (PUFA) (*20*). Both soluble and membrane-associated LOXs have been identified in fruits and were capable of the specific oxygenation of PUFAs esterified to phospholipids in the membrane bilayer (*21*).

Information on ionization damage to cells analyzed at the structural level is also limited (*22, 23*), and the available data indicate considerable differences in the level of cell damage.

The aim of this work was to determine if the electron beam ionization causes oxidative stress on pepper fruit and, consequently, a cellular damage. The effect of the treatment on the antioxidant enzyme activities (CAT, APX, SOD, POX) was measured to obtain more information about the defense mechanisms occurring in the cell in response to the radiolytic compounds and, therefore, to develop treatment protocols to obtain more hygienic and better-preserved fruits during the storage period.

MATERIALS AND METHODS

Plant Material and Experiment Design. Pepper fruits (*Capsicum annuum* var. California) harvested in their green state were used in this study. The fruits were bought in Hortamira S. L. (Murcia, Spain), previously visually selected for size and green color. The fruits were distributed in five batches of 50 fruits; four of them were ionized at different doses, and the other one was separated as a control and was later stored as the treated fruits. After the ionization, fruits were stored at 7 ± 1 °C and periodic samples were taken after 1, 3, 7, 13, and 17 days. In each sampling, the fruits removed from the camera at 7 °C were kept for 3 additional days at 20 °C to simulate the chain of commercial distribution before carrying out the following analytical determinations.

Ionization Treatments. Ionization was carried out using a Rhodotron (I. B. A., Belgium) circular electron accelerator (Ionmed, Tarancón, Spain) at an energy level of 10 MeV. Treatment lots were deposited in a transporting tape leading to the electron beam; the samples were arranged in a monolayer. The programmed ionization doses were 1, 3, 5, and 7 kGy; nonionized samples were separated as control lots. The treatment protocol and the number of dosimeters per treatment batch, as well as the determination of the real dose absorbed by the fruits, were carried out in the Research and Development Department of IONMED. Radiochromic dosimeters (1×1 cm) FTW-60.0 (Far West Technology, U.S.) were employed, and the absorbed dose was measured at 600 nm in a spectrophotometer Genesys-5 (Espectronic, U.S.) with an uncertainty of $\Delta abs = 0.006$ for a level of confidence of 95%. Six dosimeters per each 50 fruits were randomly placed in both faces (three

Table 1. Conditions Applied during the Ionization Treatments and the Later Readings of the Dosimeters in Order To Verify the Real Absorbed Dose

applied dose (kGy)	speed of the conveyor belt (m/min)	beam current intensity (mA)	width of the electron beam (cm)	dosimeter measuring (kGy)	optic Abs	real absorbed dose (kGy)
1	5.00	0.64	103.00	1	0.20 ± 0.001	0.95 ± 0.01
3	4.99	2.197	103.51	3	0.37 ± 0.01	3.02 ± 0.07
5	4.99	3.55	103.49	7	0.52 ± 0.01	5.09 ± 0.09
7	4.69	4.430	103.51	10	0.69 ± 0.01	7.37 ± 0.11

in the upper side and three in the lower side) of the fruits to verify the real dose absorbed by the fruits and the studies of penetration of the radiations.

The variability of the real dose of ionization absorbed by the samples was less than 1% of the programmed dose applied. The dosimeters also verified the homogeneity of the dose and validated the ionization process. The treatment conditions and the later readings of the dosimeters are shown in **Table 1**.

Electron Microscopy. Observations of the ultrastructures of treated and control fruits were carried out with electron microscopy, according to Olmos and Hellín (*24*). Briefly, samples taken just after the treatments were fixed for 2.5 h at 4 °C, in a 0.1 M sodium phosphate buffer (pH 7.2), containing 2.5% glutaraldehyde and 4% paraformaldehyde. Tissue was postfixed with 1% osmium tetroxide prepared in the same buffer for 2 h. The samples were then dehydrated in a graded alcohol series and were embedded in Spurr's resin. Blocks were sectioned on a Reichert ultramicrotome. Thin sections for transmission electron microscopy (TEM) were placed on copper grids and were stained with uranyl acetate, followed by lead citrate. The ultrastructure of the tissue was observed with a Zeiss EM10 and a Zeiss EM109 electron microscope.

Enzyme Extraction and Assays. Fruits (10 g FW) were extracted by gentle grinding (mortar and pestle) at 2 °C, with 50 mM potassium phosphate buffer (pH 7.4), containing inert sand and 1 mM EDTA, 1% (w/v) poly(vinylpyrrolidone) PVP, 0.5% (w/v) Triton X-100, 5 mM cysteine, and 0.1 mM PMSF. The buffer was supplemented with 0.2 mM ascorbate, when used for extraction of ascorbate peroxidase (APX). The homogenates were filtered through a Miracloth and were centrifuged at 10000*g* for 15 min. The resulting supernatant was used as an enzyme extract for determining CAT, SOD, APX, POX, and LOX activities.

Catalase (CAT; EC 1.11.1.6) was assayed according to Aebi (*25*) in a reaction mixture (3 cm³) composed of 50 mM potassium phosphate buffer (pH 7.0) and 10 mM H_2O_2. The reaction started by adding an aliquot of enzyme extract, and the activity was followed by monitoring the decrease in absorbance at 240 nm as a consequence of H_2O_2 consumption. The enzyme activity was expressed in unit of activity (U) for which one unit represents the amount of enzyme responsible for decomposing 1 μmol H_2O_2 per minute at 25 °C.

Total superoxide dismutase (SOD; EC. 1.15.1.1) activity was analyzed spectrophotometrically at 550 nm by ferricytochrome *c* method using xanthine/xanthine oxidase as the source of superoxide radicals (*26*). The assay was performed at 25 °C in a 3-mL cuvette containing 50 mM potassium phosphate (pH 7.8), 0.1 mM EDTA, 50 mM cytochrome *c*, 1 mM xanthine, and an aliquot of enzyme extract. The reaction started adding the xanthine oxidase to the mixture. One unit of SOD was considered the amount of enzyme that inhibited cytochrome *c* reduction by 50%.

Ascorbate peroxidase (APX; EC 1.11.1.11) activity was determined in a mixture containing HEPES–KOH buffer (pH 7.2), 0.22 mM ascorbic acid, 1 mM EDTA, 1 mM H_2O_2, and enzyme extract. The activity was determined by monitoring the H_2O_2-dependent decomposition of ascorbate at 265 nm, according to Nakano and Asada (*27*). The unit of APX activity was defined as the amount of enzyme that oxidized 1 μmol of ascorbate per minute at 25 °C.

Guaiacol peroxidase (POX; EC 1.11.1.7) activity was determined in a reaction mixture composed of 50 mM potassium phosphate buffer (pH 7.0), 9 mM guaiacol, 10 mM H_2O_2, and enzyme extract (*28*). The enzyme activity was measured by monitoring the increase in absorbance

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at 470 nm as result of guaiacol oxidation. The unit of POX activity represents the quantity of enzyme that oxidized 1 μmol of guaiacol per minute at 25 °C (the extinction coefficient was 26.6 mM^{-1} cm^{-1}).

Lipoxygenase activity (LOX; EC 1.13.11.12) was measured at 30 °C using the method described by Minguez-Mosquera et al. (*29*). To 3 mL of phosphate buffer (200 mM and pH 6.5) was added an amount of crude enzymatic extract between 0 and 60 μL and a constant volume of linoleic acid solution of 20 μL. The increase in absorbance at 234 nm between 10 and 40 s, as result of the formation of conjugated dienes from linoleic acid, was used for the quantification of activity per unit of time. One unit was defined as the amount of enzyme that caused an increase of 0,01 of absorbance per minute.

Activities of all enzymes were expressed as specific activities (units per mg protein fresh weight). Protein was determined by the method of Bradford (*30*), using bovine serum albumin (BSA) as the standard.

Lipid Peroxidation Measurement. Malondialdehyde (MDA) was quantified in fruits as indices of lipid peroxidation using the thiobarbituric acid reactive substrates (TBARS) assay (*31*). Pepper tissue was homogenized in 0,1% trichloroacetic acid (TCA) solution with inert sand. The homogenate was centrifuged at 15000*g* for 10 min and 0.5 mL of the supernatant obtained was added to 1.5 mL 0.5% thiobarbituric acid (TBA) in 20% TCA. The mixture was incubated at 90 °C in a shaking water bath for 20 min, and the reaction was stopped by placing the reaction tubes in an ice−water bath. The samples were then centrifuged at 1000*g* for 5 min, and the absorbance of supernatant was read at 532 nm. The value for nonspecific absorption at 600 nm was subtracted. The amount of MDA−TBA complex (red pigment) was calculated from the extinction coefficient 155 m/M cm.

The data are the means of four determinations in three different assays.

Statistics. Tests for significant differences were carried out using the General Linear Model of the SPSS (version 11.0) statistical package. Analysis of variance (ANOVA) was conducted for ionization doses and storage as factors. When differences were significant, multiple comparisons were made using Tukey's test which compares the samples on the basis of the mean of the factor variances.

RESULTS

Effect on Cellular Ultrastructure. **Figure 1** shows the electron micrographs of the control fruits and of the fruits treated at all ionization doses. In control pepper fruits, epidermal and hypodermal cells of the pericarp contained normal chloroplasts, mitochondria, and peroxisomes (**Figure 1A,B**). However, after electron beam ionization (1−7 kGy), we observed a moderate separation of the plasma membrane from the cell wall. The number of peroxisomes was significantly reduced after electron beam ionization at 1−3 kGy doses (**Figure 1C,D**), and peroxisomes were not observed after the 5−7 kGy treatments (**Figure 1E,F**). Chloroplasts of pericarp cells treated with electron beam ionization over 5 kGy showed altered structure; they contained abundant plastoglobuli and altered thylakoid structure (**Figure 1E**).

Treatment with 7 kGy destroyed the chloroplasts' inner structure, however, it seemed to delay the degradative processes of starch grains (**Figure 1F**). After doses higher than 5 kGy, the vacuolar structure showed ruptures of the tonoplast and degradation of the cytoplasm could be observed also. The vacuoles contained abundant vesicles and osmiophilic materials, which may be a sign of autophagy (**Figure 1E,F**).

Effects on Antioxidant Enzymes. The enzymatic systems with antioxidant properties which are able to act in different organelles of the plant cell as a defense mechanism against free radicals, formed because of the treatment and because of the physiological mechanisms occurring during the storage period, were also studied.

Figure 2 shows the activity of CAT which acts on hydrogen peroxide and which, as can be observed, was reduced with the
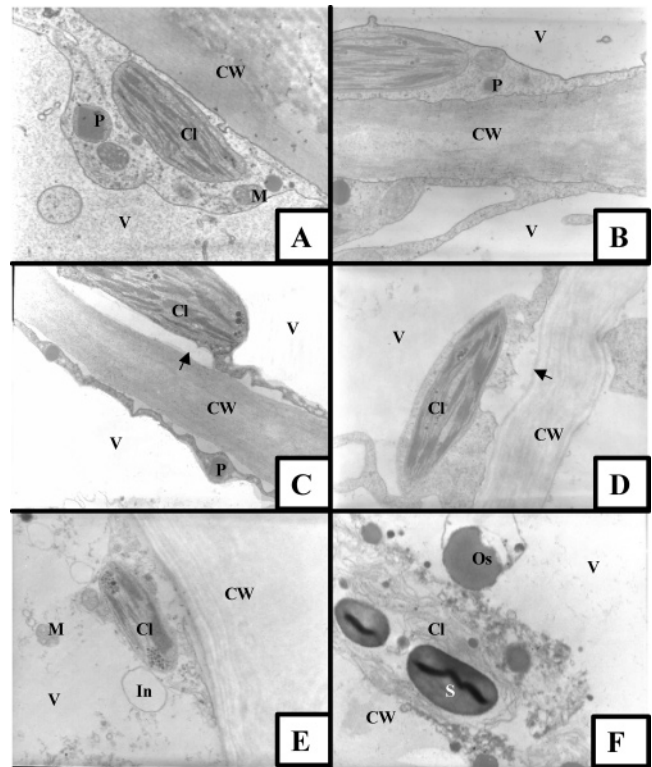

Figure 1. Effect of ionization on the cellular ultrastructure of pepper fruits. (**A**, **B**) Images of a control fruit. (**C**, **D**, **E**, **F**) Fruits ionized at 1, 3, 5, and 7 kGy, respectively. Magnifications = 10000×. CW, cell wall; V, vacuole; Cl, chloroplast; S, starch granules; P, peroxisomes; In, invagination; M, mitochondria; Os, osmiophilic materials; arrows, separation point of the plasma membrane from the cell wall.
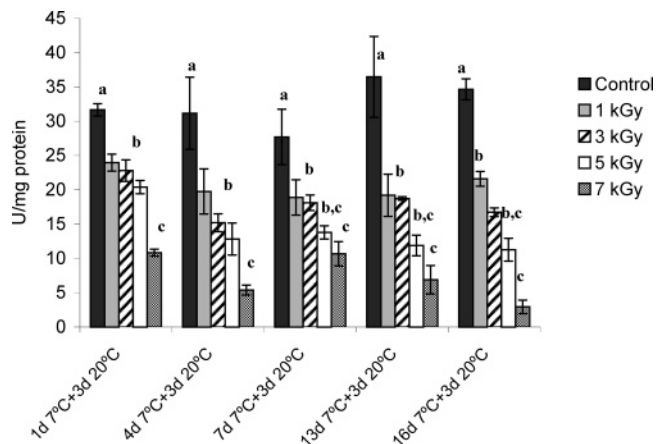

Figure 2. Influence of ionization dose on CAT activity and its evolution during storage period of pepper fruits. Statistical differences were analyzed by ANOVA (*p* < 0.05), subset *a* > *b* > *c*.

treatment dose, especially at 5 and 7 kGy, showing also a slight decrease in this activity during the storage period of treated samples, in contrast to control fruits whose activity remained practically constant during time and even slightly increased at the end of the storage.

Figure 3 shows the evolution of the SOD activity in relation to the storage period and the ionization doses applied. The ionization treatment caused a moderate and not statistically significant decrease in the SOD activity at 1 and 3 kGy. At higher doses (5 and 7 kGy), the activity was inhibited in nearly 70% regarding the activity shown by the control fruits. It can be observed that the activity in the control fruit, as well as in
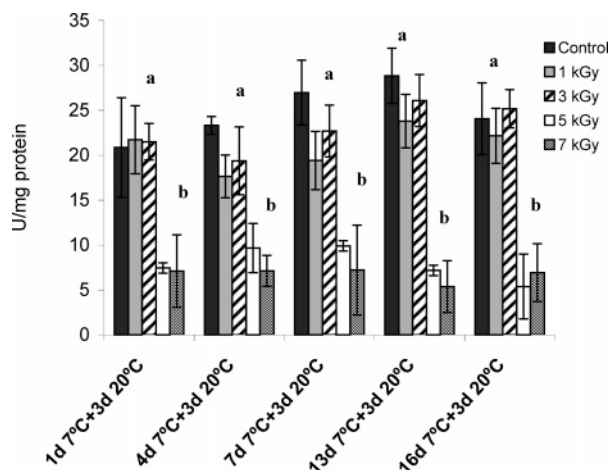
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Figure 3. Effect of ionization dose applied and storage period on SOD activity in pepper fruits. Statistical differences were analyzed by ANOVA ($p < 0.05$), subset $a > b > c$.
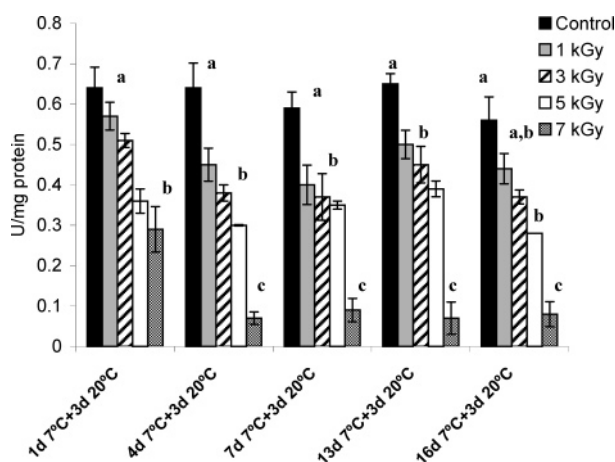

Figure 4. Influence of ionization dose on APX activity and evolution during storage period of pepper fruits. Statistical differences were analyzed by ANOVA ($p < 0.05$), subset $a > b > c$.

Figure 5. Effect of ionization dose on POX activity and evolution during storage period of pepper fruits. Statistical differences were analyzed by ANOVA ($p < 0.05$), subset $a > b > c$.

Figure 6. Influence of ionization dose applied and storage period on lipid peroxidation in pepper fruits, determined through the measurement of concentration of MDA. Statistical differences were analyzed by ANOVA ($p < 0.05$), subset $a > b > c$.

the 1 and 3 kGy treated ones, showed a trend toward increase as the storage period advanced and it slightly decreased at the end of this period, although these changes during storage were not statistically significant. The SOD activity in 5 and 7 kGy treated peppers stayed low during the whole storage period.

The ionization treatment caused a decrease in the APX activity (**Figure 4**) which was higher as the treatment dose increased. During the storage period, the APX activity in control fruits remained nearly constant, while in ionized fruits this activity decreased during the first 7 storage days (4 days at 7 °C plus 3 days at 20 °C), and then it became stable until the end of the experience. The decrease in the APX activity was significantly higher in fruits treated at 7 kGy than in the rest of the treated pepper.

In contrast to the behavior of APX, when the total POX (guaiacol-peroxidase) was measured (**Figure 5**), we observed an increased in all ionized fruits regarding control fruits, but this increase was not directly related to the ionization dose. The POX activity in fruits treated at 3 kGy was significantly higher than in the rest of the fruits, followed by the activity shown by fruits ionized at 1, 5, and 7 kGy. During the storage period, the POX activity at 1 and 7 kGy decreased, and it equaled the values of control fruits at the end of the study. Although peppers ionized at 3 and 5 kGy initially show a decreased in the activity, from day 10 (7 days 2 °C + 3 days 20 °C) onward, the activity
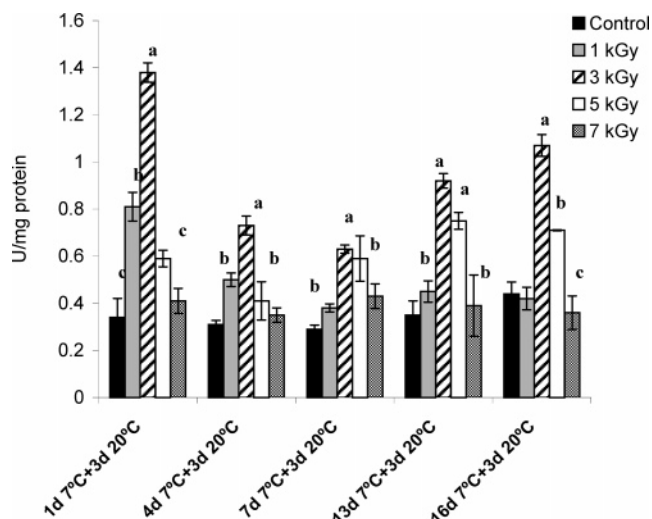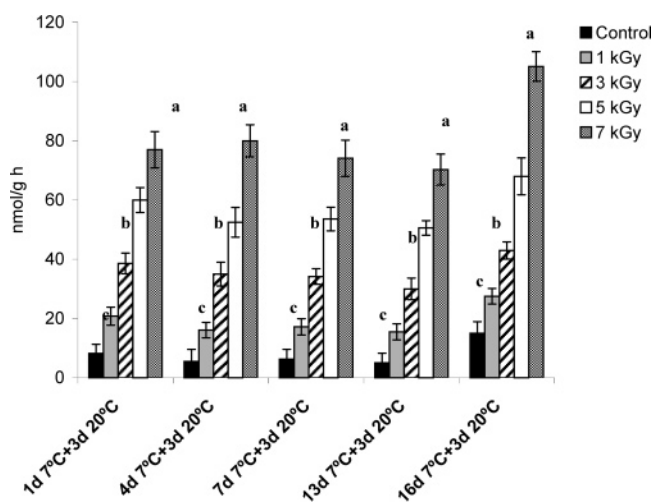
increases again showing values which are significantly higher than those of the rest of the fruits at the end of the storage period.

Induced-Lipid Peroxidation of Cellular Membranes. To elucidate the effect of these treatments on the integrity of the cellular membranes, the lipid peroxidation (**Figure 6**), using the accumulation of MDA as a sensitive marker of it, and lipoxygenase activity (**Figure 7**) have been studied.

The effect of the ionization treatment on the two parameters was similar, showing a gradual increase as the ionization doses increase in both cases, a fact showing a higher dose-dependent lipid peroxidation in treated samples. The concentration of MDA was practically constant during the storage period in all samples (treated and untreated ones) and only after 16 days of storage (13 days at 7 °C plus 3 days at 20 °C) could we detect a slight increase of it, more marked in 7 kGy ionizated samples. The lipoxygenase showed its maximum activity at day 7 of storage (4 days at 7 °C and 3 days at 20 °C), and then the activity of treated ones showed a downward trend until the end of the studied period, when the LOX activity was similar for all samples, except for the 7 kGy ionized ones, in which it was higher.
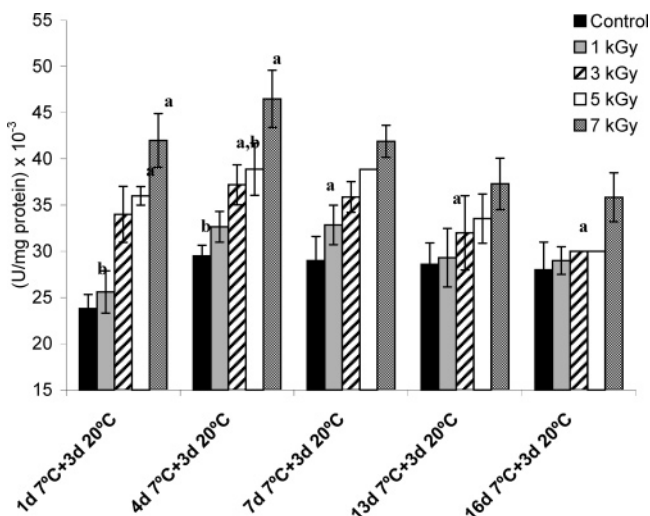
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Figure 7. Effect of ionization dose applied and storage period on LOX activity in pepper fruits. Statistical differences were analyzed by ANOVA ($p < 0.05$), subset *a > b > c*.

DISCUSSION

The electron microscopy analysis (**Figure 1**) showed an alteration of the cell ultrastructure in pepper caused by the ionization treatment, but few studies have reported results at the subcellular level concerning the effect of different doses of radiation on the ultrastructure of the treated plant tissues (*22, 23*). The observed separation of the cell wall and the plasma membrane is related to the results obtained by different studies that have determined for some fruits that using doses over 1 kGy results in a texture loss, higher as the doses increases (*12, 13, 32−36*). Today, it is accepted that the changes in polysaccharides of the cell wall are the main event responsible for the alteration of the fruit texture (*37*). The texture loss caused by the fruit ionization has been associated to the hydrolysis or partial degradation of the polysaccharide chain to pieces with a lower molecular weight (*32*). Therefore, in-vitro studies about the ionization of aqueous solutions of pectin confirm that OH• radicals produced in the water radiolysis can act as oxidants of specific sites causing the split of polysaccharide molecules by the breakdown of glycosidic links (*38*).

Ultrastructural studies on banana fruit peel revealed that gamma ionization at exposures above 0.2 kGy induced dilation between thylakoids and a loss of granal stacking; at 1 kGy, granal stacking persisted, although dilation of the chloroplast envelope was noted (*22*). Keresztes and Kovacs (*23*) have observed too that fruits of Hardentpont pear stored for two months, and previously gamma-ionized (1 kGy), showed altered chloroplast ultrastructure. Other situations causing oxidative stress in plants, like drought, saline stress, or UV−B radiations, have shown alterations in the chloroplast ultrastructure similar to those we found in the ionized pepper, showing a thylakoidal structure notably disorganized, an increase in the number and size of plastoglobulin, and large starch grains (*39−41*).

The delay of the degradative processes of starch grains at 7 kGy of pepper fruit may be due to the inhibition of synthesis of amylolytic enzymes (*42*). Similarly, Kovacs et al. (*43*) have observed that the ionization of apple and pear fruit at 1 kGy delayed starch degradation. The accumulation of starch in pea was interpreted by some authors as a consequence of disruption in the chloroplast envelope after the UV-B treatment (*40*).

The decrease in the number of peroxisomes, even their disappearance at doses over 5 kGy, may be due to the oxidative degradation of their lipid membrane since they are an extremely

fragile organelle bound by a single membrane (*44*). Although there are not specific studies about the oxidative degradation of peroxisomes because of the ionization treatment in plants, some studies about other organelles, like isolated mitochondria of bean hypocotyl and isolated microsomes of cauliflower, showed that O_2^-, OH•, and H_2O_2 radicals produced in the ionization at 5, 2, and 4 kGy, respectively, oxidized the lipid membranes causing the structural disintegration of the organelles (*17, 18*).

The decrease in the enzymatic activities (CAT, SOD, APX) observed in this study (**Figures 2**, **3**, and **4**, respectively) after the ionization treatment can be explained by the fact that these enzymes have been found in the peroxisomes and chloroplasts of pepper (*44, 45*), which are seriously affected after the ionization treatment. Some studies showed similarities between responses to ionization treatments and other oxidative stress such as UV-radiation, ozone treatment, or senescence on the behavior of this antioxidant (*19, 41, 46*).

Orendi et al. (*47*) showed that the decrease of CAT activity is an early symptom of stress damage. CAT is possibly involved in the signal mechanism of H_2O_2 (*48*), and its inactivation may be necessary to signal activity of hydrogen peroxide (*49*). CAT can be inactivated in vitro by superoxide anion, hydroxyl radical, and hydrogen peroxide (*50*).

On the other side, studies carried out on pea leafs have showed a decrease in the APX activity during processes of oxidative stress such as senescence probably because the H_2O_2 mediates oxidation of ascorbic. As suggested by Smirnoff and Pallanca (*51*), the ASC pool could be reduced by oxidative stress when the capacity of regenerative systems is exceeded, a fact that could also explain the decrease of the APX activity found in this study.

The increase observed in the total peroxidase activity (POX) (**Figure 5**) agrees with the chromatographic data shown by Martínez-Solano et al. (*45*) where they observed the influence of these treatments on the different isoenzymes of peroxidase. In the control fruit, only a band of pI < 6, which is repeated in all treatments, was found. However, in fruits treated at 3 and 5 kGy, three other moderately ionic isoenzymes with pI between 6 and 7 are induced and another of pI 9 corresponds to a cationic isoenzyme, probably related to the soluble fraction that is induced as a response to the stress of the treatments.

The increase in the MDA concentration (**Figure 6**) for all ionized fruits shows that the ionization treatments cause an oxidative stress in the fruit which is dose-dependent since the MDA content increases as the treatment dose increases. This suggests that the free radical which is produced by the ionization process can directly desterify the fatty acids from the lipids associated to the membranes (*52*). These results relate to the analysis of the cell structure where the loss of integrity of the cell membranes (**Figure 1**) could be observed. The decrease in antioxidant enzymes activities in pepper after ionization treatment could originate an increase in the ratio between free-radical generation and free-radical degrading enzymes resulting in an increase in lipid peroxidation (MDA) and membrane deterioration caused by an increased leakage of reactive oxygen spices.

On the other hand, free polyunsaturated fatty acids from the action of the free radicals on the lipid membrane serve as substrates for LOX which produces other oxy-free radicals and lipid hydroperoxides, which decompose later into TBARS. The increase in the LOX activity observed (**Figure 7**) with the ionization dose can be also related to the observed increase in MDA. Other authors observed an increase in the LOX activity

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after the ionization treatment which induced the senescence because of the deterioration of the lipid membranes (*17*, *18*).

This study has shown that the characteristics of pepper fruits that have been ionized, specially at high doses, are similar to those occurring in the physiological state of senescence, such as increase of the LOX activity and decrease of the CAT, APX and SOD activities, together with punctual increases of POX activity because of the induction of new isoenzymes, and the accumulation of lipid hydroperoxides and other oxygen reactive species (*19*). This unbalanced physiological situation cause a peroxidative stress (accumulation of MAD) in the fruit and, therefore, an ultrastructural alteration in the pepper cell. The conclusion is that the ionization of pepper at doses over 1 kGy induces a senescence-like peroxidation of membranes by free radicals generated during electron beam ionization and, therefore, the ionization treatment with accelerated electrons at the experimental doses and conditions studied cannot be considered acceptable for being used as an alternative storage technique in pepper (*Capsicum annum* var. California).

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