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## Improvement of postharvest keeping quality of white pepper fruits (*Capsicum annuum*, L.) by hydrogen peroxide treatment under storage conditions

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**ABSTRACT** Sweet pepper is one of the most important vegetable crops in the world, it has excellent nutritive value but it is susceptible to relatively fast quality changes after harvest time. The objective of the present research was to evaluate the effect of dipping pepper fruits in hydrogen peroxide solutions on postharvest keeping quality during storage time. Whole pepper fruits were soaked for 30 min in a solutions of hydrogen peroxide (0, 1, 5 and 15 mM) then, air dried and stored at room temperature (20°C) for 2 weeks and in fridge (10°C) for 4 weeks. Hydrogen peroxide treatments significantly reduced weight loss, rot rate index and nitrate content of fruits specially with 15 mM hydrogen peroxide as compared with control treatment (0 mM hydrogen peroxide). Moreover, hydrogen peroxide treatments significantly increased general appearance, ascorbic acid content and the activity of the antioxidant enzymes such as ascorbate peroxidase and dehydroascorbate reductase. For dry matter and TSS%, there are no significant differences among treatments. Therefore, the use of hydrogen peroxide in postharvest treatments have a good potential strategy to improve the postharvest quality, extend shelf life period and maintained some nutritional quality as well as inhibited decay development of white peppers which natural infected under storage conditions.

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**KEY WORDS**

pepper fruits  
hydrogen peroxide  
postharvest quality

Sweet pepper (*Capsicum annuum*, L.) is one of the most important commercial vegetable crops worldwide and a main component of the traditional human diet. It is one of the vegetables that has excellent nutritive value, higher content of ascorbic acid, which required for human nutrient, and there is now strong evidence to link dietary ascorbic acid with protective effects against various oxidative stress-related diseases (Davey et al. 2000). Nevertheless, it is a very perishable vegetable with a short shelf life and high susceptibility to fungal diseases (Hardenburg et al. 1990).

There are an increasingly demand from consumers side in the market for products with excellent quality and extended shelf life, so the preservation of the postharvest quality became more and more important together with the optimization of storage conditions. Pepper fruits after harvesting commonly encountered postharvest problems, such as strong physiological activities, quality degradation, shrivelling associated to rapid loss of weight, nutritive components as well as fast physical decay and rapid senescence. Therefore, maintaining freshness of pepper fruits has been a challenge in keeping its postharvest quality such as reducing water loss,

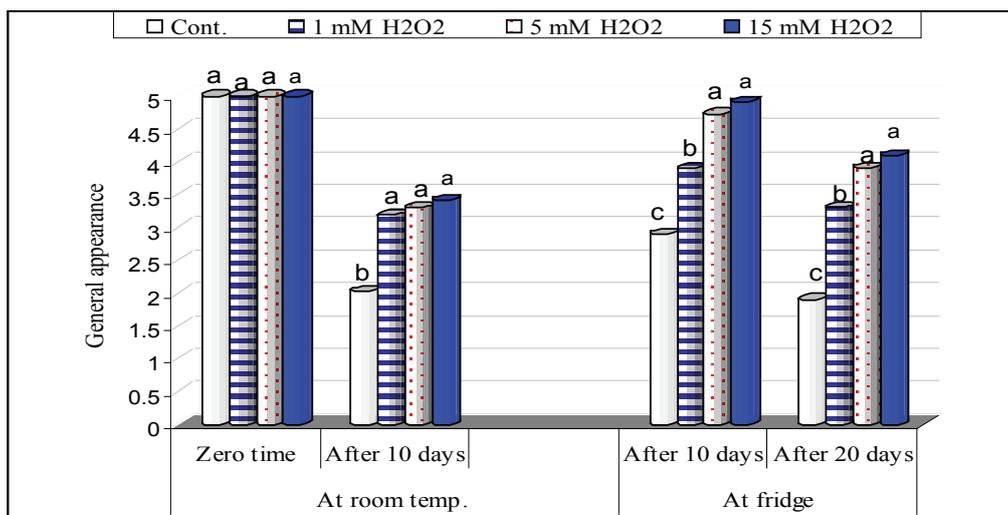
delaying softening and extending shelf life period (Gonzalez et al. 1999 and Xie et al. 2004).

Postharvest treatments with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) have been proposed as alternative to chemical treatments. It is a compound allowed for use in organic crop production according to National Organic Program (NOP, 2003). The USA considered H<sub>2</sub>O<sub>2</sub> as a GRAS (Generally Regarded As Safe) as alternative postharvest treatment to replace currently used chemicals for extending the storability and shelf life of fruits and vegetables as well as it allowed as a water, surface disinfectant and postharvest aides for organic crops (Suslow, 1997).

H<sub>2</sub>O<sub>2</sub> is an environment friendly compound whose activity is based on oxidation of fungi and bacteria, and it was successfully used to control vegetable pathogens during storage (Afeq et al. 1999). It is now clear that H<sub>2</sub>O<sub>2</sub> function as signalling molecules in plants, it is a form of reactive oxygen species (ROS) generated as a result of oxidative stress. Oxidative stress arises from an imbalance in the generation and metabolism of ROS, with more ROS (such as H<sub>2</sub>O<sub>2</sub>) being produced than are metabolized. H<sub>2</sub>O<sub>2</sub> is generated via superoxide, presumably in a non-controlled manner, during electron transport processes such as photosynthesis and mitochondrial respiration (Neill et al. 2002).

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**Figure 1.** General appearance in zero time (before storage) and after storage time (10 and 20 days) as influenced by hydrogen peroxide treatments under both room temperature (20 °C) and fridge (10 °C) conditions. Means designed by the same letter are not significantly difference at the 5% level according to Duncan’s test.

Washing fresh mushrooms with H<sub>2</sub>O<sub>2</sub> added to the wash water has been investigated for enhancing the quality and extending shelf life of postharvest mushrooms. The main improvements in the quality compared to control occurred after 9 days of chilled storage such as reduced bacterial populations and better retention of whiteness as well as extended shelf life period of whole mushrooms during storage time (Brennan et al. 2000). Chikhthimmah et al. (2005) examined utilization of H<sub>2</sub>O<sub>2</sub> in irrigation water to reduce microbial populations on fresh mushrooms and consequently enhance fresh mushroom quality. They also indicated that H<sub>2</sub>O<sub>2</sub> in irrigation water at the level of 0.5% improved point-of-sale quality and increased shelf life period of fresh mushrooms for 6 days during postharvest storage at 12°C compared to control (untreated with H<sub>2</sub>O<sub>2</sub>).

Therefore, use of H<sub>2</sub>O<sub>2</sub> as an alternative to chemical materials for disinfecting fresh-cut or whole fruits and vegetables appeared to reduce microbial populations on fresh products and extend the shelf life without leaving significant residues or causing loss of quality (Sapers and Simmons 1998 and Sapers et al. 2001). In this concern, Ukuku (2004) found that H<sub>2</sub>O<sub>2</sub> treatments of whole and fresh-cut cantaloup and honeydew melons resulted in significant improving general appearance of fruits, extended fruit shelf life and highly reduction of *Salmonella* spp. population on surface of cantaloup and melon fruits. Moreover, Bhagwat (2006) showed that, shelf life of melon fruits (fresh-cut) treated with H<sub>2</sub>O<sub>2</sub> was extended by 4 to 5 days compared to that of chlorine-treated melons.

On fresh cut-tomato, Kim et al. (2007) investigated the effect of H<sub>2</sub>O<sub>2</sub> treatments on nutritional quality during storage in fridge. They found that vitamin C content was decreased

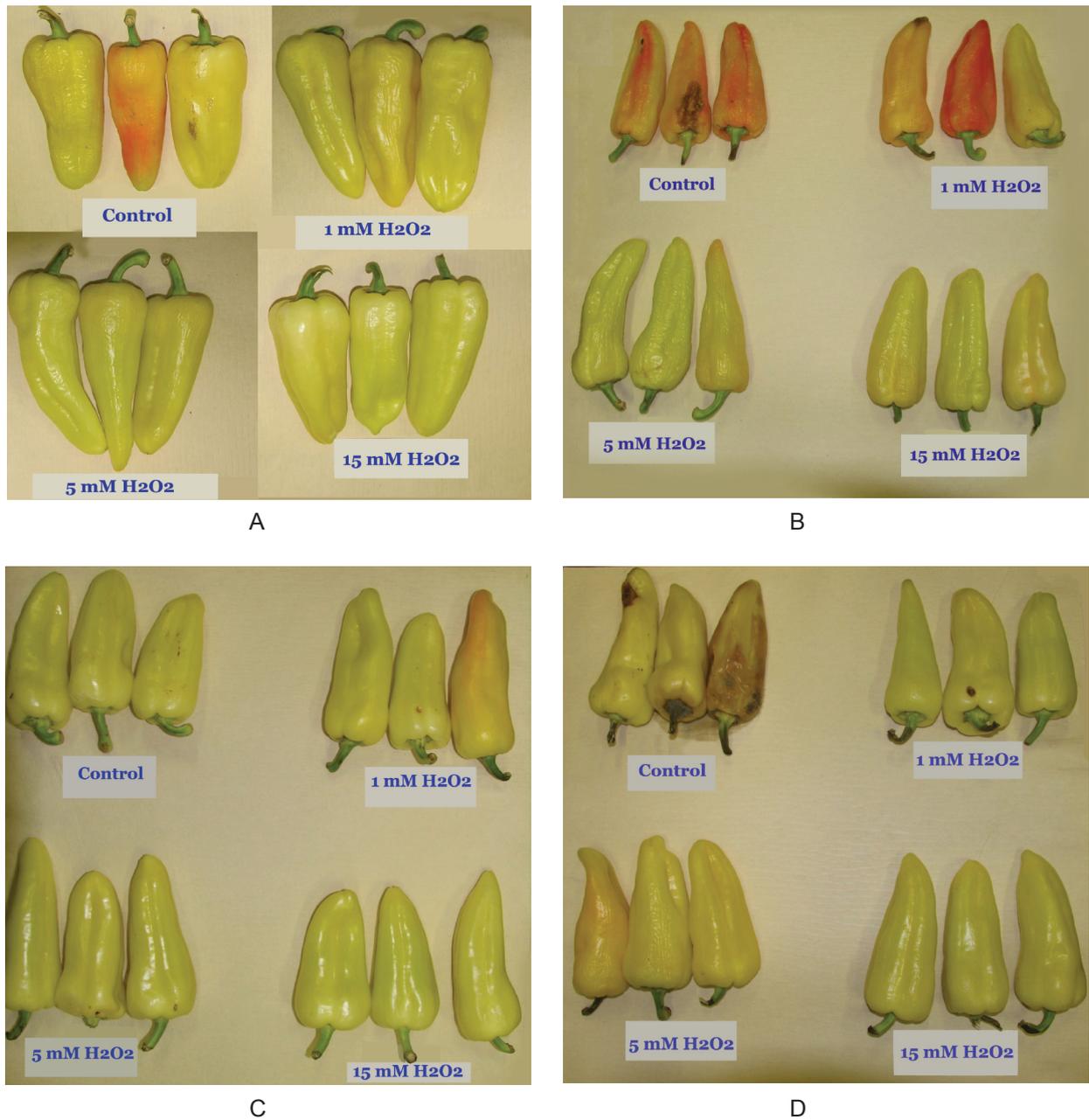
after 1 day from H<sub>2</sub>O<sub>2</sub> treatments, however it increased directly after 7 days specially with the rate of 0.2 and 0.4 M H<sub>2</sub>O<sub>2</sub> compared to control (untreated fruits).

Control of postharvest diseases of fruits is mostly dependent on controlled atmosphere storage, refrigeration and fungicides. Among these, fungicides are widely used to reduce postharvest decay and extend the shelf life of fruit. However, fungicides are becoming less effective because of the development of pathogen resistance, along with consumer concerns about possible risks associated with the use of chemicals (Wilson et al. 1994). Among a number of new strategies investigated to control postharvest decay without the pollution of the environment and risk to public health, induced resistance in harvested crops is promising (Terry and Joyce 2004).

The objective of this work was to investigate the effects of H<sub>2</sub>O<sub>2</sub> treatments (0, 1, 5 and 15 mM) on the quality maintenance of pepper fruits under two postharvest storage conditions (10°C and 20°C).

### Materials and Methods

This study was carried out at the Department of Vegetable and Mushroom Growing, Faculty of Horticulture, Corvinus University, Budapest, Hungary, using freshly pepper fruits (*Capsicum annuum*, L. white type, “H6” hybrid) during 2007 season. Pepper fruits obtained from plants grown under unheated plastic houses in the Experimental Farm of Corvinus University which produced conventionally. As an attempt we try to improve postharvest quality of pepper fruits by the application of hydrogen peroxide during storage time.



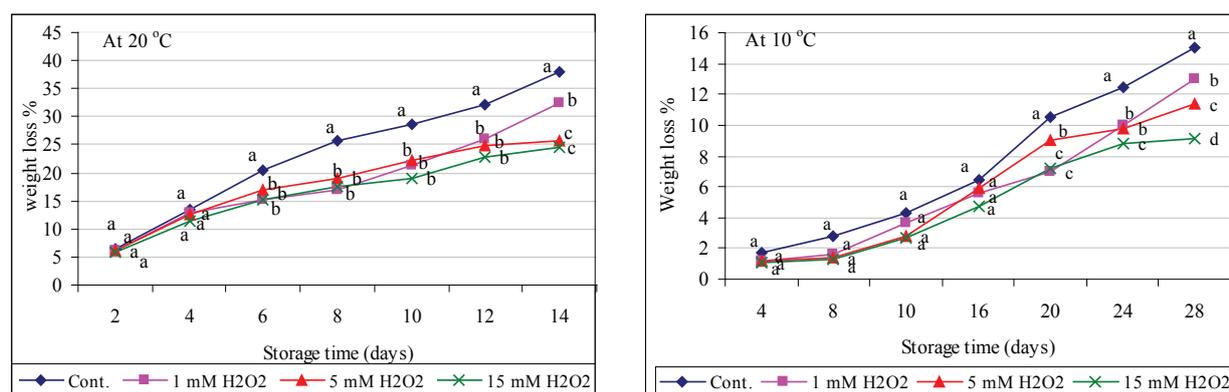
**Figure 2.** Effect of hydrogen peroxide treatments on the general appearance and delaying senescence of pepper fruits. A- Fruits after 1 week stored at 20°C, B- Fruits after 2 weeks stored at 20°C, C- Fruits after 2 weeks stored at 10°C and D- Fruits after 4 weeks stored at 10°C.

### Treatments

Pepper fruits were harvested at mature white stage, all fruits were selected for uniformity of shape, color and size while any blemished or diseased fruits were discarded.

The treatments which applied to pepper fruits were as follows: 1) Control: (0 mM  $H_2O_2$ ), dipping pepper fruits in normal tap water, 2) 1 mM  $H_2O_2$ , 3) 5 mM  $H_2O_2$ , 4) 15 mM

$H_2O_2$ . All pepper fruits were dipped in the solutions of  $H_2O_2$  in the four concentrations for 30 minutes after directly air-dried at room temperature. All treated fruits were divided into two groups, one stored at room temperature (20°C) for 14 days and the second one stored in fridge (10°C) for 28 days. All treatments were arranged in three replicates, each one contain 12 fruits.



**Figure 3.** Weight loss of pepper fruits as influenced by hydrogen peroxide treatments during storage time at both room temperature (20°C, every 2 days) and fridge (10°C, every 4 days). Means designed by the same letter (at the same storage time) are not significantly difference at the 5% level according to Duncan's test.

The treatments were repeated after 1 week for fruits stored at room temperature and after 2 weeks for which stored at fridge by spraying the solutions on fruits.

#### Initial and stored quality measurements

1- General appearance (GA): It was evaluated subjectively considering the fruit freshness, wilting and shriveling, according to the following scale: 1= extremely poor, 2= poor, 3= fair, 4= good and 5= excellent (Troncoso et al. 2005).

2- Weight loss: It was measured every 2 days during preservation for room temperature storage and every 4 days for cooling storage. Ratio of weight loss = (peppers weight before storage - peppers weight after storage) × peppers weight before storage<sup>-1</sup> × 100 %.

3- Index of rot: It was determined according to Xiong et al. (2003) where Index rot rate = [weight of rotten peppers (moldy and rotten) / original weight of peppers] × 100%.

4- Biochemical assays of enzyme activities: For enzymatic activity assay of ascorbate peroxidase (APX), 0.5 g pepper fruits material was homogenized on ice in 3 ml 50 mM Tris buffer (pH 7.8), containing 1 mM EDTA-Na<sub>2</sub> and 7.5% polyvinylpyrrolidone K-25. Homogenates were centrifuged (12 000 rpm, 20 min, 4°C) and total soluble enzyme activities in the supernatant were measured spectrophotometrically. After homogenized immediately 10µl of 0.3 M ascorbic acid was added. Activity of APX (E. C. 1.11.1.11) was determined according to Asada (1992). For enzymatic activity assays of dehydroascorbate reductase (DHAR), 0.5 g pepper fruits material was homogenized as described before. Assays were carried out at 25 uC, using a model UV-160A spectrophotometer (Shimadzu, Japan). Activities of DHAR (E. C. 1.8.5.1) was determined according to Klapheck et al. (1990).

5- Ascorbic acid content (mg/100 g f.wt.): It was determined by colorimetric method according to HCISA (1971).

6- Dry matter %: It was determined by drying samples

at 105°C till constant weight according to AOAC (1990) methods.

7- TSS (Brix): It was determined by digital hand-hold refractometer, PAL-1 according to AOAC (1990) methods.

8- Nitrate content (ppm): It was determined by nitration of disulphonic acid method using spectrophotometer at 420 nm according to Becker (1965).

All measurements were estimated immediately after harvest and before use treatments, after 1 & 2 weeks for room temperature storage and after 2 & 4 weeks for cooling storage. Except for enzyme activities determined once after 10 days from treatments at 20°C. The whole experiment was conducted twice and the results which included were the average of the two experiments.

#### Statistical analysis

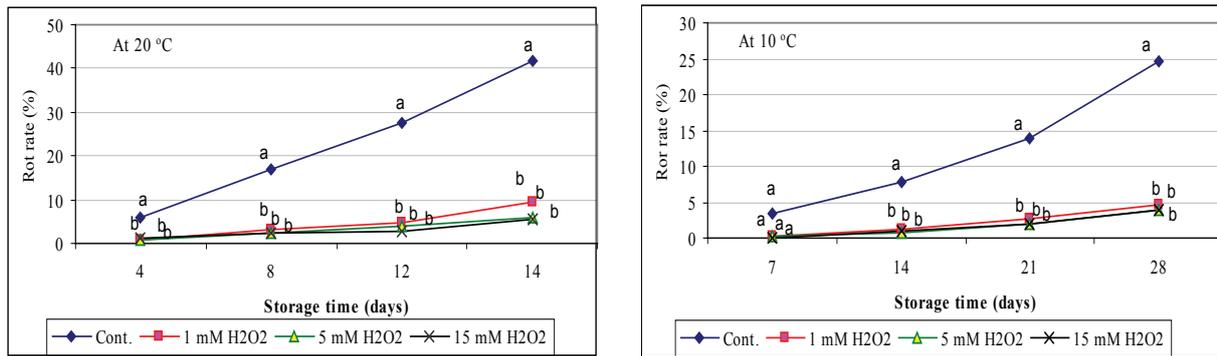
All experiments were conducted in a completely randomized design with three replicates for each treatment. Data were analyzed by one-way analysis of variance (Anova) using statistical software SPSS 14.0 for windows. Duncan's multiple range test was used for comparison among the treatment means.

## Results and Discussion

### Effect of hydrogen peroxide treatments

#### General appearance

The effect of H<sub>2</sub>O<sub>2</sub> on general appearance (GA) of pepper fruits under both storage conditions is shown in Figures 1 & 2. After 10 days, GA was significantly improved by H<sub>2</sub>O<sub>2</sub>, however the control showed the worst values. The differences among H<sub>2</sub>O<sub>2</sub> concentrations were not significant in most cases. Similar results were obtained after 20 days in the fridge condition, therefore H<sub>2</sub>O<sub>2</sub> treatments gave the best



**Figure 4.** The role of hydrogen peroxide in reducing rot rate index of pepper fruits during storage time under both 20°C (room temperature) and 10°C (fridge) conditions. Means designed by the same letter (at the same storage time) are not significantly difference at the 5% level according to Duncan's test.

appearance at 5 and 15 mM concentrations. The keeping quality of general appearance was improved by using H<sub>2</sub>O<sub>2</sub> attributed to the effect of H<sub>2</sub>O<sub>2</sub> on the reduction of weight loss and rot rate of pepper fruits (Figs. 3 & 4). H<sub>2</sub>O<sub>2</sub> treatments have beneficial effects on fruit physiology such as delaying ripening of peppers (Fig. 2) by the increasing antioxidants content in fruits (Saltveit and Sharaf, 1992). In the same time, ethylene production by fruits can be reduced by H<sub>2</sub>O<sub>2</sub> and this reduction keeps the appearance of fruits in the best state. In this concern, Gane (1937) stated that ethylene can be removed or reduced by both of ozone and H<sub>2</sub>O<sub>2</sub> treatments on banana fruits. Moreover, Gomez et al. (2008) stated that spinach leaves received heat shock treatments showed an early increase of H<sub>2</sub>O<sub>2</sub> production which improved the appearance and extended the postharvest life of spinach leaves.

#### Weight loss %

Data presented in Figure 3 show that, H<sub>2</sub>O<sub>2</sub> treatments significantly reduced fresh weight loss of pepper fruits during storage time under room temperature and fridge conditions. Therefore, the best reduction of weight loss was less than 25% at 20°C after 2 weeks and about 9% at 10°C after 4 weeks obtained by using 15 mM H<sub>2</sub>O<sub>2</sub>. Untreated fruits (control) produced the highest values of weight loss under both storage conditions (38 and 15%), respectively. In this concern, Afek et al. (2000) mentioned that hydrogen peroxide reduce the weight loss of potatoes during storage time by reducing the sprouting rate.

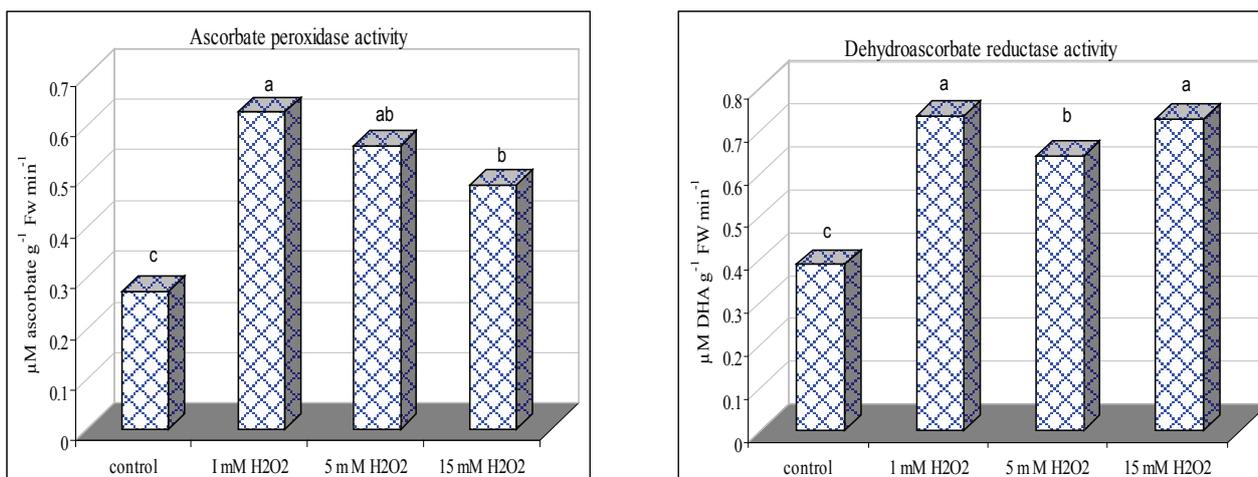
The reduction of weight loss rate may be attributed to reducing the respiration process rates during postharvest storage, this agreement with Du et al. (2007). In this concern, Gane (1937) observed that using both of ozone and H<sub>2</sub>O<sub>2</sub> for banana postharvest treatment retarded the fruit ripening. This retardation is probably due to decreasing the respiration rate and plugging of the stomata of the fruit by the decomposition products formed by the action of ozone and H<sub>2</sub>O<sub>2</sub>. Similarly,

Neil et al. (2002) and Desikan et al. (2004) demonstrated that abscisic acid (ABA) induced stomatal closure of guard cells in *Arabidopsis* and it requires H<sub>2</sub>O<sub>2</sub> to induced stomatal closure. Therefore, this reduction is important for peppers storage and transports to consumer markets because H<sub>2</sub>O<sub>2</sub> treatments reduced this loss by about 10%. It means that H<sub>2</sub>O<sub>2</sub> can save about 10 tons per every 100 tons fruits during storage time compared to control treatment (0 mM H<sub>2</sub>O<sub>2</sub>).

#### The rot rate index

When pepper fruits stored at 20°C the decay of the control appeared on the 5<sup>th</sup> day of storage time (about 10% rotted) and the rot rate reached more than 40% after 2 weeks which were significantly greater than those of peppers treated with H<sub>2</sub>O<sub>2</sub> (Fig. 4). Similar results were obtained when pepper fruits stored at 10 °C, the decay of untreated fruits (control) appeared on the 10<sup>th</sup> day of storage time (5% rotted), the maximum rot rate was less than 25% after 4 weeks. The decay of fruits treated with 1, 5 and 15 mM H<sub>2</sub>O<sub>2</sub> was delayed and less than those of the control. The differences among H<sub>2</sub>O<sub>2</sub> treatments were not significant in both storage conditions. The greatest inhibition of fruit rot rate was obtained by 15 mM H<sub>2</sub>O<sub>2</sub> treatment which was 5% after 2 weeks at 20°C and 3.5% after 4 weeks at 10°C storage conditions.

In this study, it was found that H<sub>2</sub>O<sub>2</sub> treatments highly decreased the extension of rot in pepper fruits and thereby extended their shelf life. Similar results were obtained by Sapers and Simmons (1998), Chikthimmah et al. (2005) and Fan et al. (2008), they showed that treatments of H<sub>2</sub>O<sub>2</sub> had been reduce populations of *Pseudomonas*, *Botrytis*, *Cladosporium* and others of pathogens populations as well as extended the shelf life of whole and fresh-cut vegetables and mushrooms. The reduction of rot rate by using H<sub>2</sub>O<sub>2</sub> attributed to that H<sub>2</sub>O<sub>2</sub> as a reactive oxygen species (ROS) plays an important and manifold role in plant disease resistance to infection with pathogens. Also, Malolepsza and Rozalska (2005) stated that H<sub>2</sub>O<sub>2</sub> activate the disease resistance reaction of tomatoes to



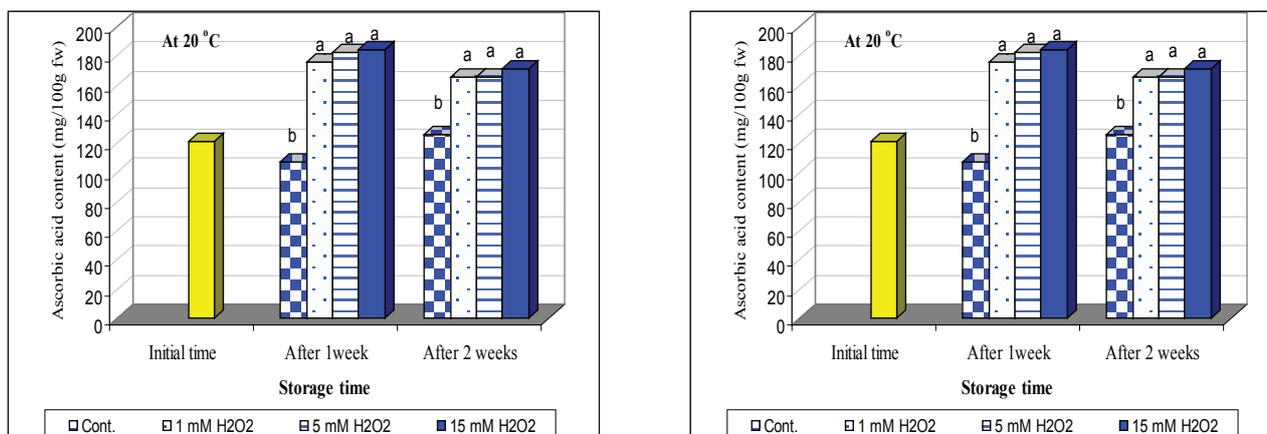
**Figure 5.** Activities of both ascorbate peroxidase and dehydroascorbate reductase in pepper fruits during postharvest storage time (at the 10<sup>th</sup> day) as influenced by H<sub>2</sub>O<sub>2</sub> treatments at 20 °C (room temperature) condition. Means designed by the same letter are not significantly difference at the 5% level according to Duncan’s test.

infection with a pathogen (*Botrytis cinerea*) and may act as a direct antimicrobial agent delaying *B. cinerea* spore germination and giving the plant time to mobilize further defence reactions. These results agreed with Lu and Higgins (1999) and Mellersh et al. (2002).

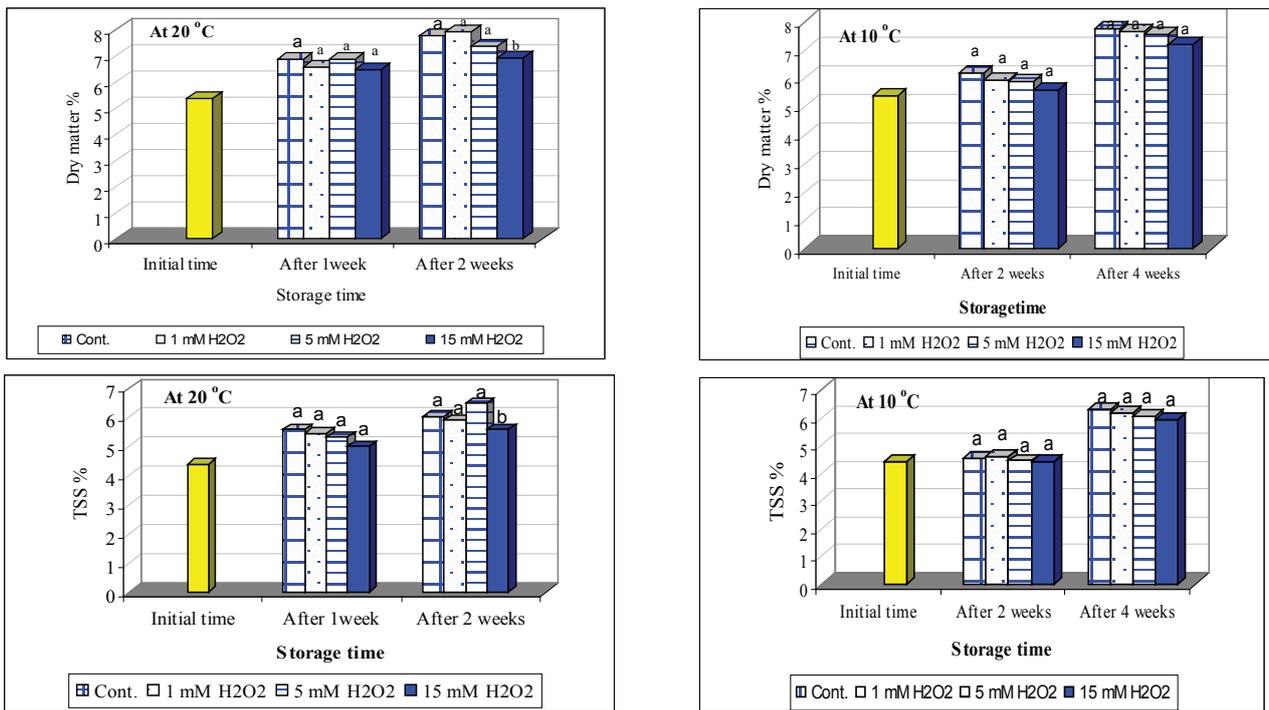
*Enzyme activities*

The relation between H<sub>2</sub>O<sub>2</sub> treatments and enzyme activities of pepper fruits were shown in Figure 5. Both of ascorbate peroxidase and dehydroascorbate reductase activities were significantly increased in fruit treated with H<sub>2</sub>O<sub>2</sub> compared to untreated fruits (control). Therefore, the higher values

were resulted from fruits treated with the lower concentration (1mM H<sub>2</sub>O<sub>2</sub>) comparing with the other two concentrations. These results are agreement with Hafez and Király (2003) and Hafez et al. (2004), they stated that it is possible to immunize tobacco leaves against the infection with *Tobacco mosaic virus*, *Pseudomonas syringae* pv. *phaseolicola* and *Botrytis cinerea*, when leaves pre-treated with H<sub>2</sub>O<sub>2</sub> at 5-7 mM which elevated the level and the activity of the antioxidant enzymes. This increment may be also due to increasing the fruit content from ascorbic acid (Fig. 5) which considered important for nutritional value and antioxidants properties of pepper fruits.



**Figure 6.** Changes of ascorbic acid content in pepper fruits during postharvest storage time at both room temperature (20°C) and fridge (10°C) conditions as influenced by H<sub>2</sub>O<sub>2</sub> treatments. Means designed by the same letter (at the same storage time) are not significantly difference at the 5% level according to Duncan’s test.

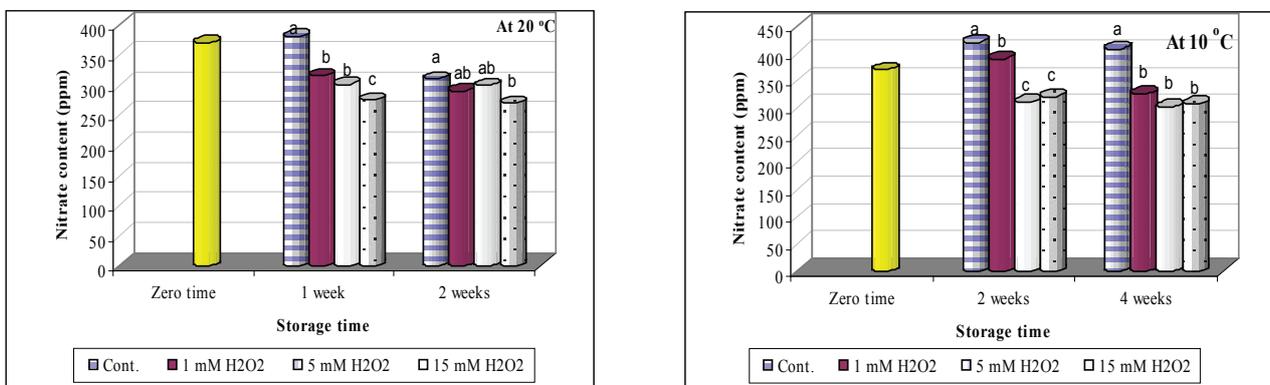


**Figure 7.** Effect of H<sub>2</sub>O<sub>2</sub> treatments on dry matter and TSS (%) of pepper fruits during storage time under room temperature (20°C) and fridge (10°C) conditions. Means designed by the same letter (at the same storage time) are not significantly difference at the 5% level according to Duncan's test.

*Ascorbic acid content*

Data presented in Figure 6 show that ascorbic acid content was increased during storage time at both room temperature and fridge in most cases. The differences between H<sub>2</sub>O<sub>2</sub> treatments and control were highly significant. The highest values were obtained from fruits treated with H<sub>2</sub>O<sub>2</sub> at 15 mM, however the lowest value was resulted from untreated fruits with

H<sub>2</sub>O<sub>2</sub> (control). The differences among H<sub>2</sub>O<sub>2</sub> concentrations were not significant under both storage conditions. Raffa et al. (2007) showed that ascorbic acid content was increased with increasing storage time. The increment of ascorbic acid content by H<sub>2</sub>O<sub>2</sub> treatments may be due to increasing the enzyme activities in fruits (Fig. 5). The stability of ascorbic acid directly increased in the presence of H<sub>2</sub>O<sub>2</sub> at low concentration (0.5 ppm) during storage of orange, grape and pomegranate



**Figure 8.** Effect of H<sub>2</sub>O<sub>2</sub> treatments on nitrate content of pepper fruits during storage time under room temperature (20°C) and fridge (10°C) conditions. Means designed by the same letter (at the same storage time) are not significantly difference at the 5% level according to Duncan's test.

fruit juices (Özkan et al. 2004). These increasing in ascorbic acid content related to H<sub>2</sub>O<sub>2</sub> treatments because it can be regenerated by two enzymes namely monodehydroascorbate reductase and dehydroascorbate reductase (Nishikawa et al. 2003), which could explain the increase by H<sub>2</sub>O<sub>2</sub> treatments during storage time.

#### Dry matter and TSS (%)

Data in Figure 7 show that, both of dry matter and TSS % of pepper fruits were increased directly with increasing storage time at both conditions, however the differences among treatments were not significant in most cases. The highest values of dry matter and TSS % were resulted in untreated fruits with H<sub>2</sub>O<sub>2</sub> (control) in most cases. This increment may be due to the increasing of water loss of untreated fruits (control) as shown in Figure 3. and these led to increasing both of dry matter and TSS % (Troncoso et al. 2005). In this concern, Sanchez et al. (2006) mentioned that, the association between postharvest time and dry matter content of cassava roots was significant and positive effect.

#### Nitrate content (ppm)

The content of nitrate was significantly influenced by H<sub>2</sub>O<sub>2</sub> treatments as shown in Figure 8. The highest nitrate content was obtained from untreated fruits (control) compared with the H<sub>2</sub>O<sub>2</sub> treatments. The lowest values were produced from fruits treated with H<sub>2</sub>O<sub>2</sub> at 15 mM and the differences among H<sub>2</sub>O<sub>2</sub> concentrations were not significant under both storage conditions. Changing in nitrate content under room temperature storage was higher than under fridge storage, the values were decreased with storage time. Similar results were obtained by Lin and Yen (1980) and Rogozinska et al. (2005), they observed that nitrate content was decreased in potato tubers with storage time especially in the first two months from storage period at 4°C. Increasing nitrate content in untreated fruits (control) attributed to increasing water loss (Fig. 3) and dry matter % (Fig. 7).

## Conclusion

Results of the experiments indicate that using H<sub>2</sub>O<sub>2</sub> for postharvest treatment is a practical strategy to reduce weight loss, rot rate and nitrate content as well as improve both of general appearance and fruit shelf life. That is means more profitable for producers, marketers and finally consumers alike. Moreover, it improved nutritional quality especially antioxidants content which are playing an important role in foods as health-protecting factor. It has been recognized as being beneficial for prevention of widespread human diseases, including cancer and cardiovascular diseases, when taken daily in adequate amounts. In the same time, H<sub>2</sub>O<sub>2</sub> considered as a GRAS (Generally Regarded As Safe) and allowed for use in organic agriculture system.

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