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ORIGINAL PAPER

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Effects of combinational use of controlled atmosphere, cold storage and edible coating applications on shelf life and quality attributes of green peppers

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Abstract The effects of combinational use of controlled atmosphere (CA), Semperfresh edible coating (sucrose polyester base coating) and cold storage on shelf life, quality attributes and post storage life of green slender peppers (*Capsicum annuum* L.) were investigated. Changes in weight, quality, pH, total titratable acidity, ascorbic acid, soluble solids, respiration rate and total chlorophyll content were recorded periodically for comparing the effects of the applied conditions. In the study, application of CA storage was determined to give the best results in terms of the analyzed parameters. Use of Semperfresh in combination with cold storage was significantly effective in retaining higher contents of vitamin C, and total chlorophyll. However, it didn't provide a synergetic benefit for improving the quality of the peppers under CA and normal air conditions.

Keywords Controlled Atmosphere · Cold storage · Edible coating · Green peppers

Introduction

Green slender peppers (*Capsicum annuum L.*) have both nutritional and commercial importance in terms of their high vitamin content and consumption demand. However, they are liable to spoilage like all fruits and vegetables under improper storage conditions. The most commonly encountered post harvest problems for green peppers are shriveling, wilting and pathogenic disorders. Moreover, chilling injury may occur below 7 °C and results in deterioration of the calyx and sheet pitting [1]. Therefore, relatively higher temperatures are recommended for the handling of peppers. On the other hand, as increasing the temperature brings forth the problem of higher water losses, proper temperature selection emerges as the most important storage factor for the peppers. The optimum storage temperature is reported to be 12 °C [2].

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Controlled atmosphere (CA) storage may markedly contribute to preservation of post harvest quality of fresh fruits and vegetables by maintaining an atmosphere with increased CO_2 and decreased O_2 relative to normal air. Stevens and Herchel [3] showed that 3.0–5.0% O_2 had beneficial effects while, increasing the CO_2 level did not make sense in terms of the quality attributes of peppers. Similarly, 3.0% O_2 was found to be the most effective rate in the case of storage at 7 °C for 3 weeks [4]. Almasi and Balla [5] stated the optimum gaseous compositions for the green peppers as 2.0–8.0% O_2 and 3.0% CO_2.

The use of edible coating is an alternative post harvest preservation method for fresh fruits and vegetables that has been gaining an increasing interest because of the ecological considerations and the trends toward convenience foods. Successful applications of edible coatings have been reported in the cases of various fruits and vegetables [6, 7, 8].

The primary purpose of the present study is to prolong the shelf life of pepper fruits by alternative techniques, each with their optimum combinational application conditions, and to investigate their combinational effects on respiration rate, quality, pH, total titratable acidity, ascorbic acid, soluble solids, total chlorophyll content and weight of the fruits during the storage.

Materials and methods

Fruit

Green peppers were harvested in July 1997 from Aksehir village in Konya, Turkey and immediately transported to the laboratory for the experiments. They were washed and dried, and defective ones were eliminated before treatments.

Experimental procedure

A total of 2640 pepper fruits (≅50 kg) were used for all treatments. The peppers were divided into two lots of 375 and 505 each. The first lot of 375 fruits were coated with 1.0% Semperfresh edible coating and further divided into three groups of 125 fruits each.

These groups were placed into CA (3.0% CO_2/3.0% O_2, 12 °C, ≅90–95% RH), cold storage (12 °C, ≅90–95% RH) and ambient air conditions (0.0% CO_2/21.0% O_2, 23±2 °C, ≅40–50% RH) separately. The second lot of 505 fruits left was also placed into the same conditions without being coated. However in this case, an additional 65 peppers were placed into CA and cold storage chambers each in order to search for post storage effects of these conditions by removing the fruits from the chambers after 21 days and exposing them to ambient air. The reason for the 21 day selection was based on the observations obtained from a preliminary test prior to experiment, to check on the CA chamber conditions with a control group in ambient air conditions, where the peppers were recorded to start showing significant quality changes. In the study, three replications of each analysis were performed.

CA storage

A CO_2 incubator (Forma Scientific, model 3165) was used for providing CA conditions in the study. A refrigeration system was installed on it to maintain the desired temperature (12 °C) within the chamber, as the incubator originally hadn't been able to provide temperatures below 25 °C. The chamber had a vented system to provide uniform distribution of the gases throughout the chamber. CO_2 and nitrogen gases were used to increase the CO_2 level and decrease the O_2 level within the chamber respectively. The incubator had the capability to maintain the desired O_2 (3.0%) and CO_2 (3.0%) level within the tolerance range of ±0.1%. The required RH (≅90–95%) was provided by means of a reservoir initially placed in the bottom of the chamber, which was filled with water.

Cold storage

A storage chamber (Nuve, type ES 500) was used for providing the desired temperature (12 °C) in the study. The required RH (≅90–95%) was provided by pans filled with water and put in different parts of the chamber. The chamber had a ventilation system and the capability of maintaining the temperature within the range 10–75±1.0 °C.

Semperfresh fruit coating

The Semperfresh edible coating solution, composed of sucrose esters of fatty acids, sodium carboxymethyl cellulose and monodiglycerides of fatty acids, was obtained from the ACC group (Ankara) in concentrated liquid form (50.0%, w/v). It was diluted with water to obtain the recommended concentration for the green peppers (1.0% w/v). The diluted solution was left for 30–45 min with occasional stirring, and then applied to the peppers.

Analytical methods

Respiration. Five pepper fruits from each application were randomly selected per replication and placed into glass jars. The jars were sealed and left for 24 h to allow the formation of its own atmosphere within the jar. Then, respiration was measured with an oxygen analyzer (Oxycheck Oxygen Monitor, Critikon, Model 2000) by tearing the seal and inserting the syringe of the analyzer immediately into the jar.

Titratable acidity. The method described by Lees [9] was used for the determination of titratable acidity and the results were expressed as percentage citric acid.

pH measurement. pH was measured from the pressed fruit juice by a pH-meter (Nel, Model 842).

Soluble solids. Soluble solids were measured from the pressed juice of the pepper fruits by means of a refractometer (Carl Zeis, Jena refractometer, DDR 818408) and the results were expressed as percentage by weight.

Ascorbic acid. The 2, 6-dichloroindophenol titrimetric method [10](AOAC) was used to determine the ascorbic acid content of pressed fruit juice. Results were expressed as milligrams of ascorbic acid/100 g sample.

Weight loss. Ten fruits for each specific condition were randomly selected and the fruits were weighed during the study with a laboratory weight balance (Avery Berkel, Model CB 062) per replication. Results were expressed as percentage weight loss.

Quality change. Five panelists were asked to rate the peppers subjectively for their flavor and appearance with a 5-point scale per replication. 5 represented the most liked score, 3 for the marketable level and 1 corresponded to the least liked score.

Chlorophyll. A spectrophotometric method was used for determination of total chlorophyll as described by Holden [11]. The results were expressed in micrograms/100 cm^2

Statistical analysis. The analysis of variance method was applied to the data obtained from each treatment to detect significance of differences at the 5% level (or P=0.05) and Duncan's New Multiple Comparison method was used to make a statistical comparison between the different storage techniques.

Results and discussion

Weight loss

In the study, peppers in all the conditions exhibited a general reduction in weight as expected (Fig. 1). Generally, both groups of coated and uncoated peppers in CA showed significantly better retention of weight (Fig. 1a) compared to other storage combinations. The use of Semperfresh in CA, in cold storage and in ambient air conditions gave conflicting results in the study. While its use resulted in a 0.4% decrease in weight loss in cold storage compared to uncoated ones, an opposite effect was observed in CA and ambient air conditions, where use of the coating resulted in higher amounts of loss from the peppers compared to those without coating. These types of conflicting results about application of sucrose ester-based coatings on some produce have been reported before [6, 12, 13].

Generally, the positive effects of Semperfresh and similar edible coatings have been based on their hygroscopic characteristic, which enables formation of a water barrier between the fruit and the environment [8]. This barrier, through which O_2 molecules move very slowly and the passage of CO_2 molecules is slightly restricted, gives the selective permeability property to the coating [14].

In the case of the present study, coated peppers in cold storage were observed to benefit from the prevailing high RH, the low temperature conditions and from the modified atmosphere formed around the fruits of peppers, which slowed down transpirational losses. However, as for CA, a less favorable gaseous composition seemed to appear around the coated peppers due to accumulation of CO_2 around the skin of fruits resulting from both CO_2 produced during transpiration by fruits and CO_2 diffusing from the CA chamber. The increase in percentage water loss in normal air upon the use of the coating can be accounted to accelerated evaporation

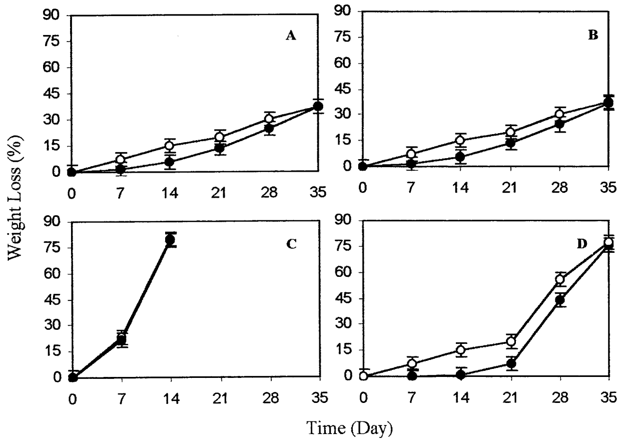

Fig. 1 Weight loss of green peppers. **a** *Black circle* Coated in controlled atmosphere (CA)^{a}, *white circle* uncoated in CA^{a}. **b** *Black circle* Coated in cold storage^{b}, *white circle* uncoated in cold storage^{bc}. **c** *Black circle* Coated in ambient air^{d}, *white circle* uncoated in ambient air^{d}. **d** *Black circle* Coated after 21 days in CA^{c}, *white circle* coated after 21 days in cold storage^{c}. *Error bars* indicate standard errors for a mean of three replicates of ten fruits each at each sampling date. The superscript letters shows statistical significance at P=0.05

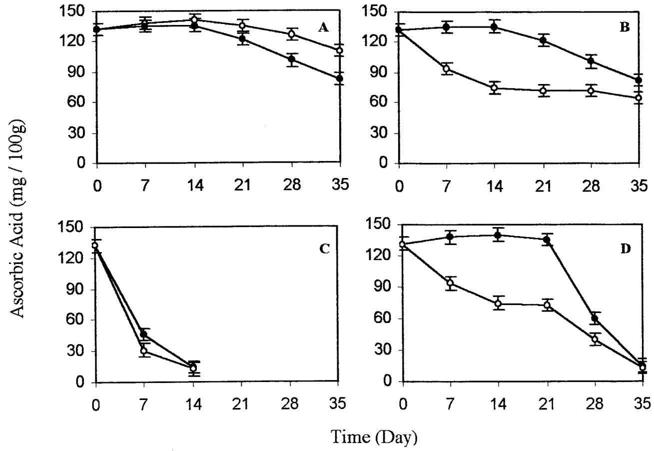

Fig. 2 Ascorbic acid content of green peppers **a** *Black circle* Coated in CA^{a}, *white circle* uncoated in CA^{a}. **b** *Black circle* Coated in cold storage^{a}, *white circle* uncoated in cold storage^{b}. **c** *Black circle* Coated in ambient air^{c}, *white circle* uncoated in ambient air^{c}. **d** *Black circle* Coated after 21 days in CA^{a}, *white circle* coated after 21 days in cold storage^{b}. *Error bars* indicate standard errors for a mean of three replicates of ten fruits each at each sampling date. The superscript letters shows statistical significance at P=0.05

from the outer surface drawing water to the surface by mass flow from the epidermal cells owing to low humidity (≅40–50%) and high temperature (23±2 °C). Hygroscopic components in the coating seem to increase this loss, particularly in adverse conditions of ambient air, similar to the findings of Elson et al. [15].

Ascorbic acid

In the study, both coated and uncoated pepper groups in CA were observed to give the best results in terms of vitamin C retention; they managed to keep 77.6% and 83.0% of their original values respectively. Peppers coated with Semperfresh in cold storage were also recorded to have high vitamin C retention, enough to be classified with the same statistical significance with the ones in CA and to be significantly higher in retention than those without coating in cold storage (Fig. 2).

However, the fruits coated with Semperfresh and exposed to normal air after 21 days in CA and cold storage were detected to be inefficient in preserving the superior effects of the these applications.

Soluble solids

Initial soluble solids content of all peppers stored under different conditions decreased at the end of the storage period (Fig. 3). The loss of soluble solids during the storage period is natural, as sugars, which are the primary constituent of the soluble solids content of a produce, are consumed by respiration and used for the metabolic activities of the peppers.

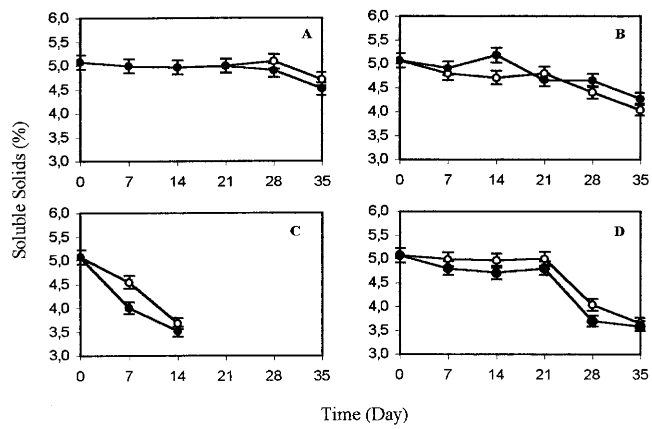

Fig. 3 Soluble solids content of green peppers. **a** *Black circle* Coated in CA^{bc}, *white circle* uncoated in CA^{a}. **b** *Black circle* Coated in cold storage^{b}, *white circle* uncoated in cold storage^{bc}. **c** *Black circle* Coated in ambient air^{d}, *white circle* uncoated in ambient air^{d}. **d** *Black circle* Coated after 21 days in CA^{bc}, *white circle* coated after 21 days in cold storage^{c}. *Error bars* indicate standard errors for a mean of three replicates of ten fruits each at each sampling date. The superscript letters shows statistical significance at P=0.05

In the study, CA storage exhibited a significantly superior effect in retention of the soluble solids content of peppers compared to all other applications. Similar retention of higher sugar contents in the CA-stored fruits than to those in conventional storage have been reported [16, 17]. Exceptional rises (Fig. 3) were observed in most of the applied conditions, which conflicted with the general decrease, and can be accounted to increased concentrations of soluble solids due to the water loss during the storage period.

Respiration

Rate of respiration is often a good index for the storage life of fresh fruits and vegetables; that is, the higher the rate, the shorter the life, and the lower the rate, the longer the life. Accordingly, in the study, peppers spoiled in a shorter period under conditions in which they respired more rapidly. This fact was especially apparent in the case of peppers subjected to normal air. However, the use of reduced O_2 (3.0%) and elevated CO_2 (3.0%) were very efficient in slowing down the respiration rate and thus extending the shelf life of the peppers. In fact, all coated peppers except the ones in CA respired more slowly compared to uncoated ones (Fig. 4a, b, c).

In the study, although the edible coating reduced the respiration rates in cold storage and normal air, this was not the case for CA, where the uncoated peppers showed significantly lower respiration rates compared to all other applications. This result seemed to be parallel to the findings of other analyses of the study for combinational use of CA and edible coating, in which the CO_2 rate around the fruit was estimated to increase unfavorably compared to uncoated ones. Generally, controlled and modified atmosphere conditions have been known to be very efficient in slowing down the respiration rate of the peppers, and the coated peppers in the study were seen to benefit from the gaseous environment formed in which CO_2 was increased and O_2 was reduced. The effects of reduced O_2 levels have been stated to be due to the suppression of activity of cytochrome oxidase, which mediates the basal metabolism and is very sensitive to O_2 [18, 19]. However, Solomos [20] has reported that such reduction results from the diminution of activity of other oxidases (such as polyphenol oxidase, ascorbic acid oxidase, and glycolic acid oxidase), whose affinity for O_2 may be 5–6 times lower than that of cytochrome oxidase. High CO_2 levels have also been reported to reduce the respiration rate of fresh fruits and vegetables. Studies on Krebs cycle intermediates and enzymes have shown accumulation of succinic acid due to inhibition of succinic dehydrogenase activity, which cause the retardation of a produce's respiration [21, 22]. In the study, generally, it was observed that peppers did not show climacteric characteristics, as they didn't exhibit a distinct increase in their respiration rates that could be considered a climacteric peak.

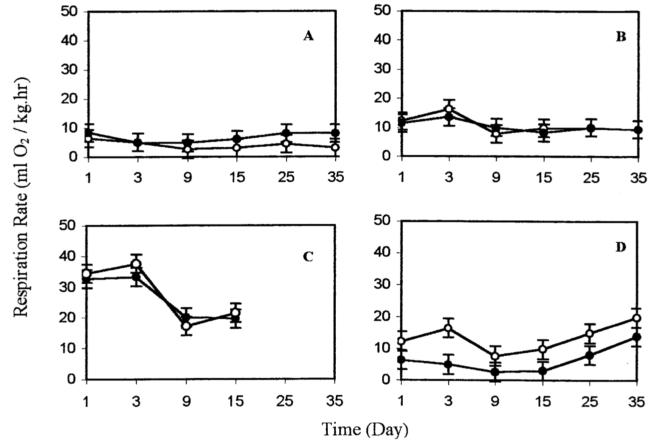

Fig. 4 Respiration rate of green peppers **a** *Black circle* Coated in CA[b], *white circle* uncoated in CA[a]. **b** *Black circle* Coated in cold storage[c], *white circle* uncoated in cold storage[c]. **c** *Black circle* Coated in ambient air[d], *white circle* uncoated in ambient air[d]. **d** *Black circle* Coated after 21 days in CA[b], *white circle* coated after 21 days in cold storage[c]. *Error bars* indicate standard errors for a mean of three replicates of ten fruits each at each sampling date. The superscript letters shows statistical significance at P=0.05

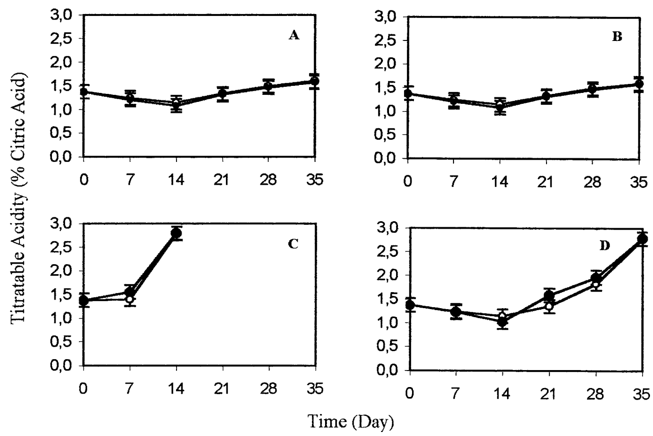

Fig. 5 Titratable acidity of green peppers. **a** *Black circle* Coated in CA[a], *white circle* uncoated in CA[a]. **b** *Black circle* Coated in cold storage[ab], *white circle* uncoated in cold storage[b]. **c** *Black circle* Coated in ambient air[c], *white circle* uncoated in ambient air[c]. **d** *Black circle* Coated after 21 days in CA[b], *white circle* coated after 21 days in cold storage[b]. *Error bars* indicate standard errors for a mean of three replicates of ten fruits each at each sampling date. The superscript letters shows statistical significance at P=0.05

Total titratable acidity

In the study, the commonly encountered case of decreasing total titratable acidity content in fresh fruits and vegetables after harvest didn't occur in the case of these peppers. Generally, all peppers exhibited an increase, when their initial titratable acidity contents (expressed as citric acid) were compared to final ones at the end of storage period, to varying extents, depending on the applied specific treatment (Fig. 5). However, the use of CA and edible coating in CA were recorded to manage to keep this change in a significantly narrower range (Fig. 5a) compared to all other applications. This reduced range of change in citric acid content in CA can be accounted to lower respiratory activity, which is the indicator of retardation in overall metabolic activities.

In addition, increased CO_2 fixation or the presence of a less active enzyme due to the high CO_2 levels in CA conditions can contribute to the low citric acid content. It was reported that as the CO_2 concentrations in the atmosphere are increased, CO_2 fixation also increases [17].

In the study, the use of Semperfresh in cold storage, normal air and in CA resulted in slightly higher retention values. However, that was not sufficient to be considered under a different statistical classification from the uncoated ones at P=0.05. The findings of Hulme and Arthington [23] were similar to the present study in the case of comparison of CA and normal air conditions, in the way that citric acid was found to be higher in fruit from air storage than those from CA storage (Fig. 5a, c). This result indicates that the production of citric acid may be oxygen dependent. It has been reported that quinic acid might be converted to citric acid upon oxidation in fresh fruits and vegetables [23].

pH

In this study, while relatively reduced changes in pH value of all peppers in cold storage and in CA were recorded, more rapid and sharper ones in the case of normal air condition were observed.

In the case of CA-stored vegetables like green beans and spinach, generally decreased acidity (increased pH) has been reported and in these studies an increase in pH values were accompanied by decreases in titratable acidity [24]. However, these findings were found to be inconsistent with the results of the present study as all conditions applied to peppers resulted in decreases in pH (Fig. 6). In this study, although the use of edible coating in CA was found to be the most efficient storage application, there was no significant difference detected between coated and uncoated peppers under all conditions (Fig. 6a, b, c).

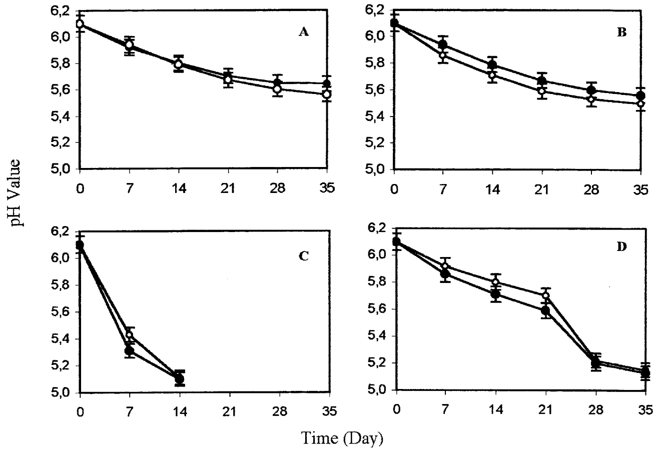

Fig. 6 Changes in pH value of green peppers. **a** *Black circle* Coated in CA^{a}, *white circle* uncoated in CA^{ab}. **b** *Black circle* Coated in cold storage^{bc}, *white circle* uncoated in cold storage^{cd}. **c** *Black circle* Coated in ambient air^{f}, *white circle* uncoated in ambient air^{f}. **d** *Black circle* Coated after 21 days in CA^{de}, *white circle* coated after 21 days in cold storage^{e}. Error bars indicate standard errors for a mean of three replicates of ten fruits each at each sampling date. The superscript letters shows statistical significance at P=0.05

Total chlorophyll content

In this study, chlorophyll, the primary pigment responsible for the green color of the peppers, decreased in all conditions during the storage period, similar to all green plants subjected to processing or storage (Fig. 7). The peppers stored in cold storage and in normal air exhibited particularly remarkable color changes during the storage period of 35 days, such that the initial dark-green color of peppers became olive-green in cold storage and red in normal air. The overall conversion period of the color involved stages that were ranked as green, breaking, red with some green, and finally red. The peppers under normal air started to degreen after 3 days and they obtained the full red color after 10 days exposure to ambient conditions.

The main reason for this color conversion from green to red can be accounted to the decrease in chlorophyll that gives the green color and increase in the carotenoid pigment that is responsible for the red color. The carotenes became visible as the chlorophyll decreased during storage. On the other hand, there was a definite retardation of color development in the CA chamber. The use of Semperfresh did not cause any significant difference for the peppers in all conditions in terms of total chlorophyll content except for those under cold storage. Higher retention of total chlorophyll was observed at 12 °C by

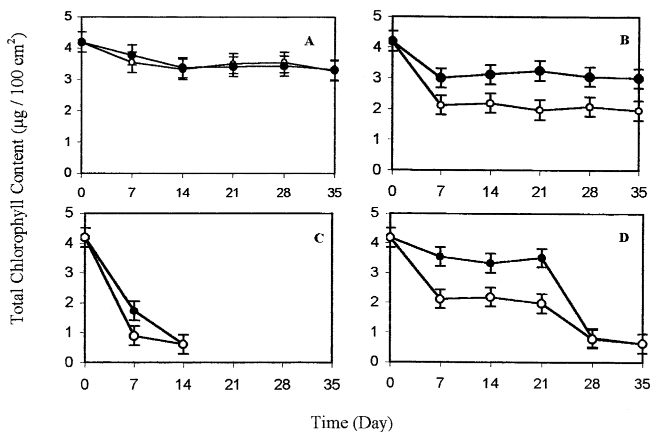

Fig. 7 Total chlorophyll content of green peppers. **a** *Black circle* Coated in CA^{a}, *white circle* uncoated in CA^{a}. **b** *Black circle* Coated in cold storage^{ab}, *white circle* uncoated in cold storage^{c}. **c** *Black circle* Coated in ambient air^{d}, *white circle* uncoated in ambient air^{d}. **d** *Black circle* Coated after 21 days in CA^{bc}, *white circle* coated after 21 days in cold storage^{c}. *Error bars* indicate standard errors for a mean of three replicates of ten fruits each at each sampling date. The superscript letters shows statistical significance at P=0.05

means of the coating treatment (Fig. 7a, b, c). Coated peppers exposed to normal air after 21 days storage period under CA and at 12 °C lost their total chlorophyll content immediately in a similar manner to those initially placed in normal air (Fig. 7d).

Quality

According to sensory evaluations based on color, shape, size, maturity, freedom from defects (sunscald, freezing injury, hail, scars) and decay, a general decrease in the quality of peppers in all conditions was detected in the study (Fig. 8). Peppers, both coated and uncoated groups in CA, managed to maintain their above-mentioned quality parameters successfully, even at the end of the 35th day of the storage period. The subjective quality scores were observed to correlate with the changes in other analyzed compositional quality attributes i.e. changes in chlorophyll, titratable acidity, pH, soluble solids, respiration and weight loss. There was no significant difference detected between coated and uncoated peppers in terms of quality change under all conditions.

However, the use of edible coating in CA and cold storage gave different results, such that higher quality scores were given to the uncoated peppers in cold storage compared to ones in CA (Fig. 8a, b, c).

In conclusion, CA, cold storage and edible coating have been used as alternative techniques for prolonging shelf life of various fruits and vegetables. In this study, combinational uses of these different storage methods were applied to green slender peppers and their effects were observed both separately and together. The CA (3.0% CO_2/3.0% O_2, 12 °C, ≅90–95% RH*)* technique was detected to provide the best storage conditions with minimal losses in terms of the analyzed parameters. The groups of peppers, both coated and uncoated, under CA were still in better form at the end of storage period of 35 days than those in other storage combinations. On the other hand, one important point in the study was that use of edible coating in CA storage unfavorably resulted in significantly higher rates of respiration rates and lower rates of soluble solids contents during the experiments.

The use of edible coating in cold storage (12 °C, ≅90–95% RH) was observed to be another effective storage technique, especially in retaining significantly higher contents of vitamin C and total chlorophyll contents compared to application of cold storage alone. This particular combination of edible coating and cold storage gave significantly similar results with the ones in CA for most of the quality attributes of peppers.

Although edible coating application in ambient conditions (0.0% CO_2/21.0% O_2, 23 ±2 °C, ≅40–50% RH) didn't provide any significant difference for the peppers, use of it after 21 days in CA and cold storage resulted in significant differences for respiration rate and ascorbic acid retention of peppers.

According to the above considerations, recommending the most applicable storage condition for the harvested green peppers is not a practical thing to do. The satisfaction of consumers by providing higher quality produce for wider range of periods throughout the year and economic gains due to the reduced losses were the two main expected results upon successful application of different post harvest storage techniques. In fact, the decision whether to store in one or another post harvest storage application actually depended on economic considerations whatever the technological advantages or superior effects on prolonging the shelf life of the produce were. However, about a fivefold extension in the shelf life of the peppers in CA and threefold in cold storage, as in the case of the present study, are certain to make a sense in terms of economic considerations.

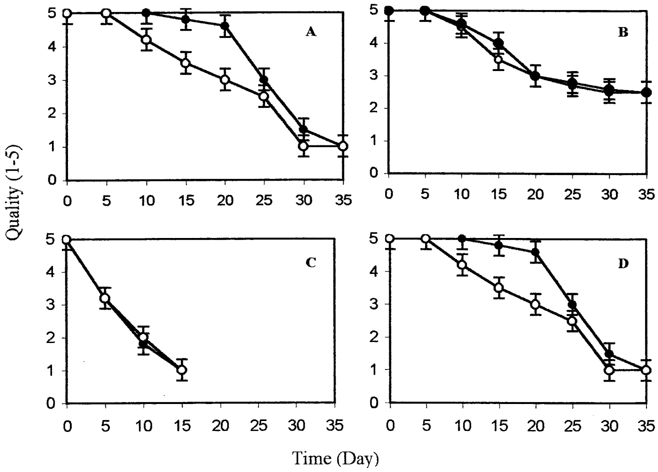

Fig. 8 Changes in quality of green peppers. **a** *Black circle* Coated in CA[ab], *white circle* uncoated in CA[a]. **b** *Black circle* Coated in cold storage[bc], *white circle* uncoated in cold storage[c]. **c** *Black circle* Coated in ambient air[d], *white circle* uncoated in ambient air[d]. **d** *Black circle* Coated after 21 days in CA[bc], *white circle* coated after 21 days in cold storage[c]. *Error bars* indicate standard errors for a mean of three replicates of ten fruits each at each sampling date. The superscript letters shows statistical significance at *P*=0.05

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