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Food Chemistry 100 (2007) 1011–1018
Food Chemistry
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# Peroxidation of membrane lipids in minimally processed cucumbers packaged under modified atmospheres

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Received 14 February 2005; received in revised form 5 October 2005; accepted 5 October 2005

**Abstract**

The effect of storage conditions and physical tissue damage on membrane peroxidation in minimally processed cucumber tissue was investigated. Lipid peroxidation in samples stored under modified atmospheric packaging (MAP) and in covered Petri dishes (non-MAP) were determined by the FOX2 and TBARS assays. The initial level of lipid hydroperoxides and TBARS were found as 1.44–2.00 and 0.11–0.20 nmol/g, respectively. The levels of lipid hydroperoxides increased by five- to sixfold in chilled non-MAP tissues over 10 days. MAP with higher levels of oxygen was generally more effective in reducing the generation of lipid hydroperoxides and TBARS. The effect of dipping treatments using solutions containing $CaCl_2$ and/or ascorbic acid on peroxidation were also investigated. Tissue hardness of MAP sealed samples increased for the first 3 days for all tissues but chilled tissues started to soften on the sixth day.


*Keywords:* Lipid peroxidation; Minimal processing; Cucumber; Modified atmospheres

## 1. Introduction

Cucumbers (*Cucumis Sativus*, L.) are one of the common ingredients used in salad mixes. Washed waxed intact cucumbers have been tentatively excluded from the classification of minimally processed refrigerated fruits and vegetables. Whereas, precut or sliced cucumbers which have a relatively short shelf-life are considered MPR foods (Wiley, 1994).

Since fresh cuts have been subjected to severe physical stress and are more perishable than intact products, they should probably be stored at temperatures lower than that recommended for the intact commodities (Watada, Ko, & Minott, 1996). Refrigeration and freezing are major tools used by food scientists to maintain fruit and vegetable quality but, unfortunately, some tissues are sensitive to low temperatures. Decreasing temperature lowers metabolism, thus prolonging shelf-life.

Chilling injury (CI) is a physiological disorder induced by low, but not freezing temperatures. CI could be considered as an accelerated senescence process, since the process observed in chill-injured fruits (ion leakage, lipid peroxidation, loss of phospholipids and increased saturation) are the same as those observed for senescence (Marangoni, Palma, & Stanley, 1996). Cucumbers are chilling sensitive and are injured if held at temperatures of less than 10 °C for more than 3 days. Pitting and increased decay are the two visible symptoms of CI in whole cucumber fruit (Cabrera & Saltveit, 1990). It has been demonstrated that MAP increases the chilling tolerance of cucumber fruits (Wang & Qi, 1997).

Low relative humidity, high temperature, high $CO_2$, low $O_2$ or excess light are other environmental stress factors which cause a response of the plant tissues. Much of the stress induced by the storage environment is the result of attempts to prevent or retard microbial decay and the ultimate storage disorder – senescence (Shewfelt & del Rosario, 2000).

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doi:10.1016/j.foodchem.2005.10.055

The onset and continuation of senescence is believed to proceed via the phospholipase-mediated degradation of phospholipids, followed by further hydrolysis of diacyl glycerol and oxidation of polyunsaturated fatty acids (Collins & Marangoni, 2000). Increased lipid peroxidation (Hariyadi & Parkin, 1993) and reduced antioxidant activity (Kang & Saltveit, 2002) has been observed during the development of CI in cucumber seedlings. In continuously chilled whole cucumbers, lipid peroxidation, as indicated by ethane evolution, commenced between days 12–13, and 7–10 days of chilling was required to impart irreversible CI (Kuo & Parkin, 1989). Lipid peroxidation of plant membranes is a natural consequence of metabolic processes and may be a mediator of critical events during postharvest storage.

The main objective of this study was to determine the effect of different storage conditions (temperature, package atmosphere) and treatments (pre-storage dipping, tissue bruising) on the development of lipid peroxidation products in cucumber mesocarp tissue. The hardness values of MAP cucumber slices were also determined as an indicator of the cell wall integrity of the tissues.

## 2. Materials and methods

### 2.1. Fruit

Greenhouse cucumbers (*Cucumis sativus*, L.) harvested within 24 h, in mature condition, were purchased from a local supplier to be used in the experiments. The fruits were sorted to obtain a lot of similar size and shape, and free of deformities. The cucumber fruits were stored at 10 °C prior to the experiments. The cucumbers were washed with cold water (6–8 °C), peeled with a fruit peeler and cut into slices of 1 cm thickness. Tissue samples, which were to be used in the extract preparation step subsequent to storage, were excised from the mesocarp section of the slices. All experiments other than the determination of hardness value were done in triplicate and excised tissue samples for each replicate were obtained from the same cucumber.

### 2.2. Non-MAP storage

Excised mesocarp tissues, 0.50–1.00 g each, were placed in Petri dishes after their exact weights were recorded. Enough sample pieces were prepared from each cucumber to allow for extract preparation every 2 days of the storage period at 4–20 °C.

The effects of three dipping solutions on lipid peroxidation for samples stored at 4 °C were also investigated. The first dipping solution consisted of 1% (w/v) ascorbic acid, second solution contained 0.2% $CaCl_2$, and the third solution contained both 1% ascorbic acid and 0.2% $CaCl_2$. The control fruits were not dipped into any solution. The samples were placed in Petri dishes so that each dish contained three replicates of each treatment and sufficient number of samples to allow for extract preparation every 2 days of storage at 4 °C.

### 2.3. Storage under MAP

The processed fruit was weighed ($99 \pm 0.5$ g sliced cucumbers and 0.5–1.0 g tissue sample) and sealed in glass jars (720 ml) with hermetic lids, previously fitted with septa for gas sampling. The treatment of the fruit for bruised samples was achieved by dropping a weight from the same height on to each slice (800 g from 10 cm height, perpendicular to the plane of the slice). This application resulted in a bruised surface with visible change of appearance of the tissue up to ca 1 mm depth. Atmospheric packaging treatments included normal atmosphere packaging, where the jars were sealed directly, or super atmospheric oxygen packaging, achieved by the flushing of the jars with oxygen previous to sealing. The sample jars were then stored at 4 or 20 °C.

Hardness values of the cucumber slices were obtained prior to packaging and subsequent to opening of the jars every 3 days. A fruit hardness tester (Everwell Corporation, Model FT011, Japan) with a 10-mm conical probe was used for the determinations. Five hardness values (one from each slice), for each of the samples were determined by positioning the tester perpendicular to the slice plane at the centerline of the mesocarp tissue.

Analysis of the headspace gas composition was carried out on a gas chromatograph (Model CRA4, Shimadzu, Japan) equipped with a computerized integrator (Chromatopac, Shimadzu, Japan) and a thermal conductivity detector. A gas-tight syringe was used for the withdrawal of 0.2 ml gas samples from the self sealing septa on the lids of the jars. A Propak Q column was used for the detection of $CO_2$ and $N_2$ in the gas samples. A Molecular Sieve 5A column was used to determine the $O_2$ and $CO_2$ in the samples.

The respiration rate (RR) was determined by the glass jar technique (Fonseca, Oliviera, & Brecht, 2002). After peeling and slicing, 300 g of cucumber fruit was enclosed in 720 ml hermetically sealed jars with septa fitted to the lids for gas sampling. Approximately 400 ml of headspace remained in the jars. Sampling and gas composition determination was carried out after the first 6 and 18 h of packaging. RRs were calculated using the equations below (respiration rate as consumption of $O_2$ or production of $CO_2$, $R_{O_2}$ and $R_{CO_2}$ in $m^3\,kg^{-1}\,s^{-1}$; volumetric concentration, $Y$ as% v/v; initial and final time, $t_i$ and $t_f$ in s)

$$R_{O_2} = \frac{\left(Y_{O_2}^{t_i} - Y_{O_2}^{t_i}\right) \times V}{100 \times M \times (t_f - t_i)}; \quad R_{CO_2} = \frac{\left(Y_{CO_2}^{t_i} - Y_{CO_2}^{t_i}\right) \times V}{100 \times M \times (t_f - t_i)}$$

The fermentative index, which is the $CO_2$ production rate ($R_{CO_2}$) of the tissues under anaerobic conditions, for the characterization of the tissues was determined by the glass jar technique described above. The headspaces of the jars were initially flushed with $N_2$ gas and the gas com-

position within the jars were then determined with gas chromatography. The initial concentration of the $N_2$ gas was at least 99%.

### 2.4. Preparation of cucumber extracts

Previously weighed cucumber flesh was hand ground with a pre-cooled mortar and pestle on ice using 0.5 g of inert sand in 8 ml of 95% ethanol containing 0.1% (w/v) BHT to arrest any further oxidation, and 1 ml of pure water. Weight loss of the flesh due to dehydration was made up for with water. Each sample was then centrifuged (Model K-30, Sigma Laboratory Centrifuges, Germany) at 4 °C and 3000*g* for 10 min with supernatant aliquots being used for the TBARS and FOX2 assays. The blanks were processed identically except for the presence of the tissue segments.

### 2.5. Determination of lipid peroxidation

Lipid hydroperoxides were determined using the FOX version II (FOX2) assay (DeLong, Prange, Hodges, Forney, & Bishop, 2002) with some modifications. For each 1000 ml volume, the FOX reagent consisted of 90% HPLC grade methanol (v/v), 10% 250 mM $H_2SO_4$ (v/v) (25 mM final concentration), 880 mg of butylated hydroxytoluene (BHT, 4 mM), 98 mg of ferrous ammonium sulfate hexahydrate (250 μM), and 76 mg of xylenol orange (100 μM). A working reagent was prepared by mixing a solution of ammonium ferrous sulfate and xylenol orange in an aqueous solution of sulfuric acid and subsequently adding the methanolic solution of BHT. This solution is stable for 1 mon at 4 °C in the dark (Nourooz-Zadeh, 1999). The pure, double distilled water was generated by a Millipore water purification system (Millipore, Danvers, MA). All glassware and plastic tubes used for the chemical assays were rinsed with double distilled water.

For some samples, 100 μl of the plant extract was combined with 100 μl of 10 mM triphenylphosphine (TPP) in methanol. The mixture was momentarily stirred with a vortex stirrer and then incubated for 30 min to allow for the complete reduction of any present hydroxides by TPP (+TPP). Samples without TPP (−TPP) addition were treated identically, except that the TPP aliquot was substituted with methanol. Following the 30 min TPP incubation, 2000 μl of FOX2 reagent was added to each sample with the absorbance at 560 nm being recorded exactly 10 min after reagent addition on a UV–Vis Spectrophotometer (Model UV-1202, Shimadzu, Japan). The absorbance difference between the samples without and with TPP indicated the presence of lipid hydroperoxides. Hydroperoxide values were then expressed as micromolar $H_2O_2$ equivalents using a standard curve spanning 0–20 μM $H_2O_2$ range.

A modified TBARS assay (Hodges, DeLong, Forney, & Prange, 1999) was used as an alternative assessment of lipid oxidation in the non-MAP samples. The TBARS reagent consisted of 20% (w/v) trichloracetic acid (TCA), 0.65% (w/v) thiobarbituric acid (TBA) and 0.01% (w/v) BHT in double distilled $H_2O$. Two hundred microliters of the tissue extract were combined with 800 μl of water and either 1000 μl of reagent with TBA added (+TBA) or 1000 μl of reagent without TBA (−TBA). The samples were then mixed vigorously, incubated at 95 °C for 30 min, cooled under running tap water and centrifuged at 3000*g* for 10 min. Sample absorbances were measured spectrophotometrically at 532, 600 and 440 nm ($A532$, $A600$ and $A440$). The MDA equivalents are calculated using the following equations

$$[(A532_{+\mathrm{TBA}} - A600_{+\mathrm{TBA}}) - (A532_{-\mathrm{TBA}} - A600_{-\mathrm{TBA}})] = A$$

$$[(A440_{+\mathrm{TBA}} - A600_{+\mathrm{TBA}}) \times 0.0571] = B$$

$$\text{MDA equivalents (nmol ml}^{-1}) = ((A - B)/1,57,000) \times 10^6$$

### 2.6. Statistical analysis

Correlation analysis was performed on the FOX2 and TBARS results. Analysis of variance (ANOVA) was performed by the statistical data analysis tool-pack of MS Excel and followed by the least significant difference (LSD) where appropriate. Significance of results was defined as $p \leqslant 0.05$. The lipid peroxidation assays were performed on extracts prepared from three replicate samples. Each replicate prepared for the determinations were from the same cucumber to minimize inherent variability.

## 3. Results and discussion

### 3.1. Lipid peroxidation under non-MAP storage

The degree of lipid peroxidation was monitored by assaying lipid hydroperoxides (primary end products) and TBARS (secondary end products). Fig. 1A shows the results of the FOX assay used to determine μM $H_2O_2$ equivalents of lipid hydroperoxides per gram of cucumber tissue. The results of the TBARS assays have been depicted in Fig. 1B.

The two methods employed detect different groups of lipid peroxidation products, but it is evident that the trends are similar for the two methods. Correlation analysis revealed that the two assays correlated significantly for normal samples at 4 and 20 °C and bruised samples at 4 °C ($p \leqslant 0.05$). The storage temperature had a significant effect on lipid peroxidation and chilling at 4 °C resulted in increased peroxidation. By the end of the tenth day, lipid hydroperoxides increased by over sixfold in chilled tissue and over fivefold in chilled and bruised tissue.

Bruising is a mechanical damage imposed on the tissues which disrupts cellular structure and damages membrane systems, causing decompartmentation of enzymes and substrates, enhanced rates of reactions, and accumulation of secondary metabolites (Rolle & Chism, 1987). Thus,

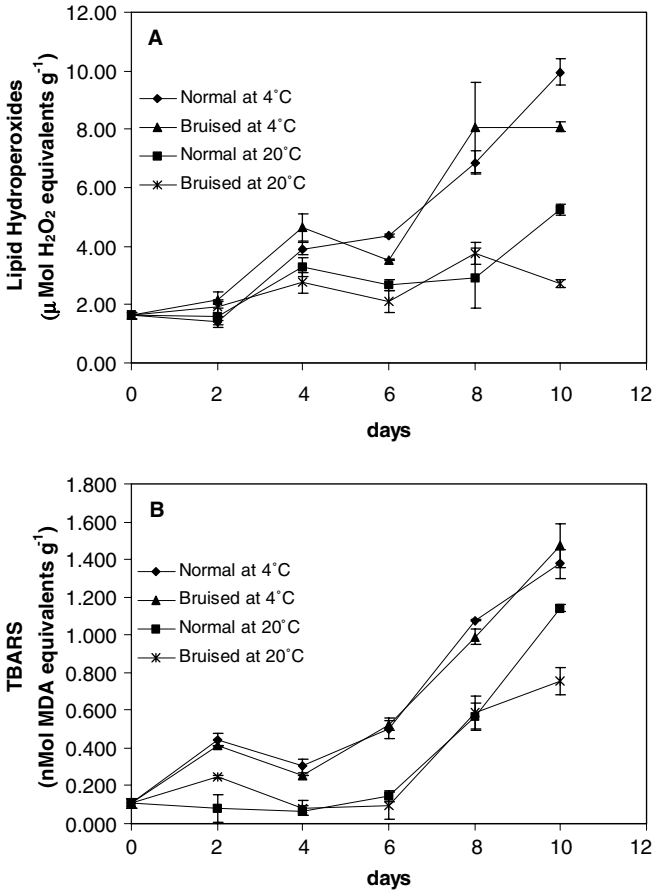


Fig. 1. Effect of chilling on the development of lipid peroxidation products in bruised and non-bruised cucumber mesocarp tissue. (A) Lipid hydroperoxides as detected by the FOXII assay. (B) TBARS levels.

increased peroxidation could be expected. However, no significant differences in lipid peroxidation were observed in the bruised and non-bruised samples. Wound respiration in some plant tissues is thought to be related to the α-oxidation of fatty acids, which oxidizes fatty acids to $CO_2$ (Brecht, 1995). The depletion of lipid components could be the reason why lipid peroxidation products are not higher in bruised tissues.

Besides the overall increase in lipid peroxidation during the 10-day storage period, less obvious trends may also be considered during the first few days. Looking at the TBARS results of the tissue stressed by chilling and/or bruising for the first 4 days of the experiment indicates an increase followed by a slight decrease. These ''peaks'' could be explained as a reaction of the tissue to the stress imposed on it. During the first 2 days the stress factor causes an increase in TBARS accumulation and in the following 2 days the tissue tries to acclimatise to the situation resulting in the decreasing of the amount of secondary lipid peroxidation products formed. Enzymic repair systems can remove peroxidized fatty acids from the membrane, thus reducing opportunities for propagation (Shewfelt & del Rosario, 2000). Another possible method of controlling lipid peroxidation could be increasing of the saturation index (SI) of the lipids (Marangoni et al., 1996). Previously proposed models for the mechanism of chilling injury based on phase transition in membranes have been discredited (Saltveit, 2002).

### 3.2. Effect of several dipping solutions on lipid peroxidation

Application of calcium salts are used extensively to maintain tissue firmness in fruits (Garcia & Barrett, 1995). Calcium is essential in maintaining the structural integrity of membranes and cell walls (Poovaiah, 1986). Ascorbic acid is one of the key antioxidants which plays an important role in the regeneration of α-tocopherol embedded in the membranes. It also functions as a non-specific scavenger of free radicals (Shewfelt & Purvis, 1995). Combinations of ascorbic acid and calcium dips have been used for browning inhibition of minimally processed apples (Ponting, Jackson, & Watters, 1972) and pears (Sapers & Miller, 1998).

Three different dipping treatments were applied to cucumber mesocarp pieces before they were placed in storage. The control tissue samples were not subjected to dipping. The effect of these treatments on the generation of lipid hydroperoxides in refrigerated cucumber tissue for the duration of 6 days is illustrated in Fig. 2.

Dipping into the solution containing only $CaCl_2$ was not effective in reducing lipid hydroperoxide formation during the first 2 days, but lipid hydroperoxide levels were much lower when compared with those of other treatments in the days that followed. Ascorbic acid, on the other hand, effectively reduced lipid hydroperoxide development during the first 4 days. Although the levels of peroxides increased for other treatments, the samples dipped in the solution containing both ascorbic acid and $CaCl_2$ did not show a sharp rise in the second day. Therefore, ascorbic acid and calcium dips may be applicable to minimally processed cucumber to reduce CI. Further research may be carried

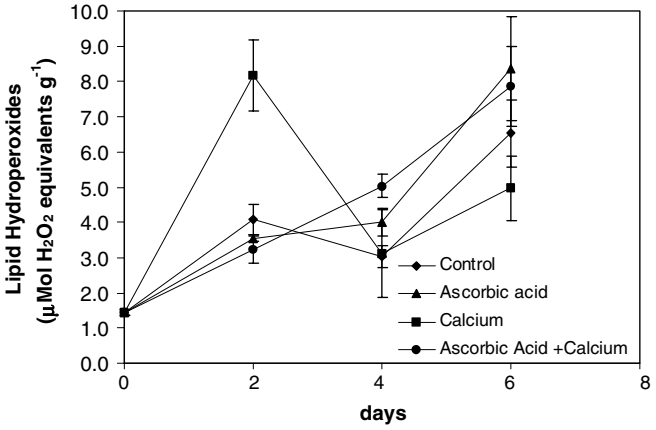


Fig. 2. Effect of various dipping solutions on the levels of lipid hydroperoxides in cucumber tissue stored at 4 °C. Control, no dipping; ascorbic acid, solution containing 1.0% ascorbic acid; calcium, solution containing 0.2% $CaCl_2$; ascorbic acid + calcium, solution containing 1.0% ascorbic acid and 0.2% $CaCl_2$.

out with dips composed of various concentrations applied at different temperatures. The $CaCl_2$ used in this study was a non-chelated form and possibly chelated $CaCl_2$ or calcium lactate could effect the degree of peroxide generation differently. However, it should also be noted that dipping solutions containing calcium resulted in hardening of the tissues. This effect is due to the interaction of calcium ions with the cell wall components. Calcium reacts with pectic acid to form calcium pectate. Calcium ions also affect tissue firmness by contributing to an increased membrane integrity and the consequent maintenance or increase of cell turgor pressure (Luna-Guzman & Barett, 2000).

### 3.3. Effect of MAP

The effect of modified atmosphere packaging was investigated in relation to the development of lipid peroxidation in cucumber mesocarp tissue. For this purpose, model modified atmospheres were created passively within hermetically sealed jars. Direct packaging of the fruit, bruising of the fruit, storage at different temperatures and $O_2$ flush sealing allowed for different atmospheric conditions to develop within the packages. Package atmospheric composition and fruit respiration rates were determined. Hardness values and drained weight were also determined to observe some of the physical changes that occurred during storage of the product.

The headspace composition of the normal atmospheric packaging treatment was the same as ambient air at the instant the jars containing processed fruit were sealed. The initial composition of oxygen flushed packages were about 70% oxygen (v/v). At the end of the fifth day, the compositions changed due to the respiration of the product. Fig. 3 shows the headspace compositions after a storage period of 5 days.

Cucumbers have been classified as immature fruit with low respiration with an intensity of 10–20 mg $CO_2$ $kg^{-1}$ $h^{-1}$ (43.1–86.2 ml $CO_2$ $kg^{-1}$ $h^{-1}$) at 10 °C (Collins & Marangoni, 2000). More precise respiration rates have been presented as 4.3 and 15 mg $CO_2$ $kg^{-1}$ $h^{-1}$ (18.5 and 64.7 ml $CO_2$ $kg^{-1}$ $h^{-1}$) at 5 and 20 °C, respectively (Watada et al., 1996). Respiration rates of fresh cuts are generally higher than intact products. Watada et al. (1996) report that slicing increased RR of cucumbers by 30% at 5 °C and 200% at 20 °C determined using a flow through system. The results of our RR determinations in using

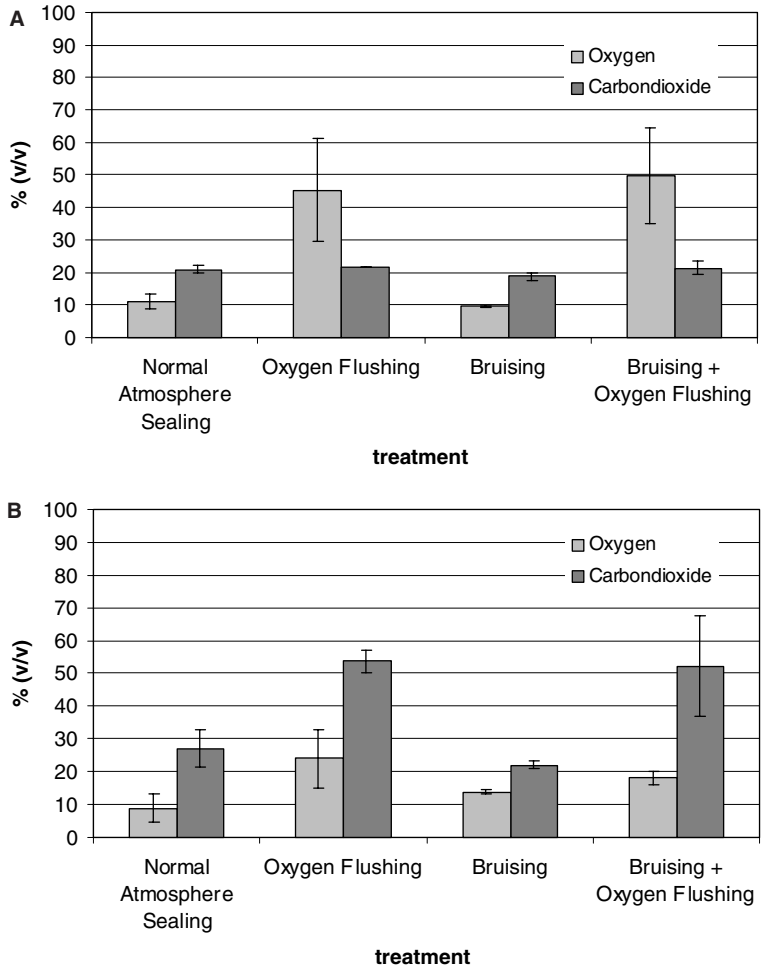


Fig. 3. Headspace oxygen and carbon dioxide concentrations at the end of a 5-day storage period: (A) at 4 °C and (B) at 20 °C.

the closed system of measurement are in accordance with these previous reports and are presented in Fig. 4.

The results of the lipid hydroperoxides assay for the samples stored under MAP are shown in Fig. 5. At 4 °C storage (Fig. 5A), levels of lipid hydroperoxides increased continuously for bruised samples. Whereas, in the non-bruised samples at 4 °C, lipid hydroperoxides increased in the first 3 days and then decreased in the days that followed. At 20 °C (Fig. 5B), bruised samples showed high levels of lipid hydroperoxides. These samples, when stored for longer than 3 days, showed significant decay due to the proliferation of microorganisms and liquid collected at the base of the jars (up to 11–12% of their weight). In the non-bruised samples at 20 °C, lipid hydroperoxides increased for the first 6 days of storage, but levels were considerably lower than those detected for the respective samples stored at 4 °C. Oxygen flushing had a reducing effect on lipid hydroperoxide accumulation in the tissues.

Unlike the determinations made for non-MAP stored samples, the results of the FOX2 and TBARS assays did not correlate for the samples under MAP. Fig. 6 shows the results of the TBARS assay performed on the MAP samples.

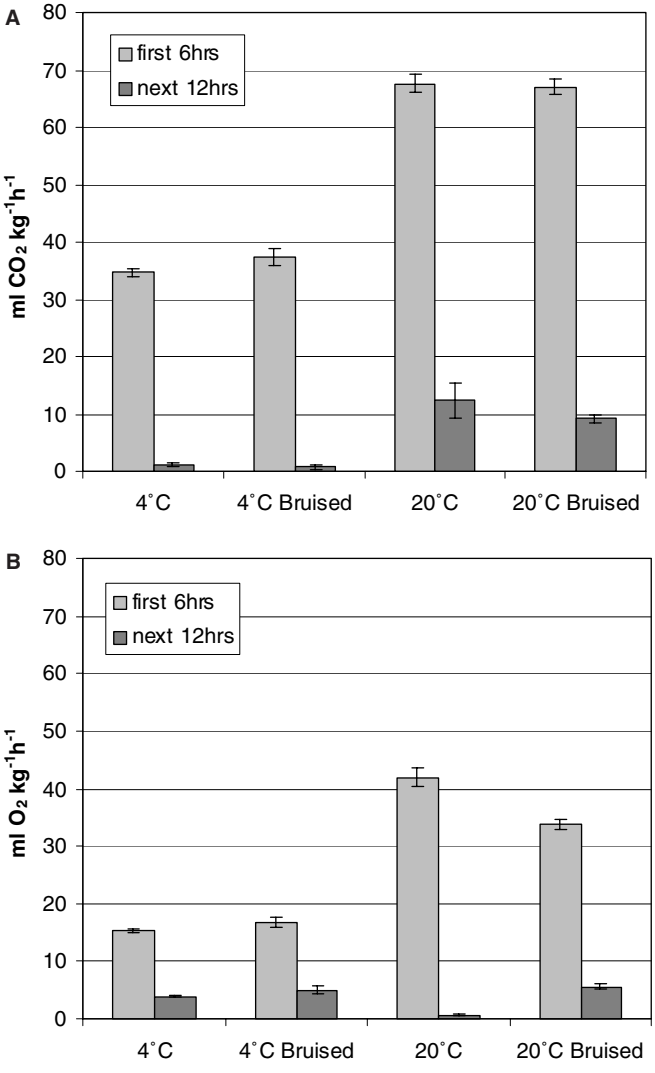


Fig. 4. Respiration rates expressed as: (A) rate of $CO_2$ production and (B) rate of $O_2$ consumption.

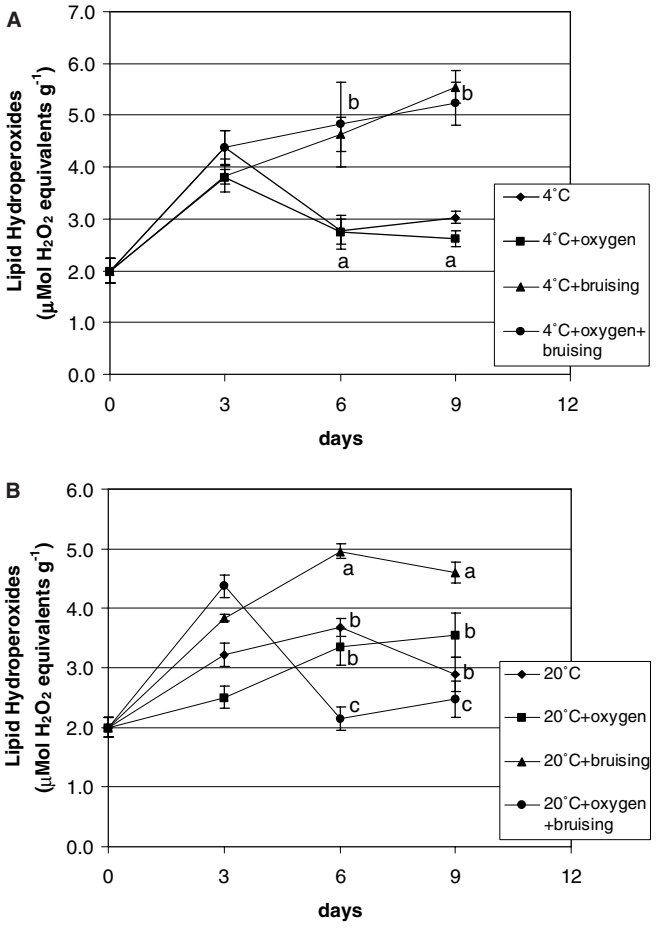


Fig. 5. Lipid hydroperoxides in cucumber tissue stored under model MAP: (A) at 4 °C and (B) at 20 °C. Means having different letters are significantly different ($p \leqslant 0.05$).

A detailed study of wounding responses in cucumber fruit has been provided by Walter, Randall-Schadel, and Schadel (1990). The initial response of cucumber to wounding is the production of a clear, sticky exudate that covers the entire wound surface, eventually drying to form a protective layer over the wound. This exudate appears to provide a moisture barrier and facilitate rapid cork initiation. Cork formation has been attributed to deposition of lignin and suberin in the cell layers beneath the wound surface (Walter et al., 1990).

In this study, the wounding of the tissues in the course of preparation resulted in exudate formation. Upon storage, non-bruised tissue became lighter in color, and obtained a dry corky texture. The hardness values of the fruit are presented in Fig. 7. All samples showed significant increase in hardness values during the first few days of storage. Considering that the rate of firming is similar for all treatments and that the overall weight loss throughout the assay was less than 1.4% in all samples (data not shown), firming can be attributed to the lignification/suberization of the

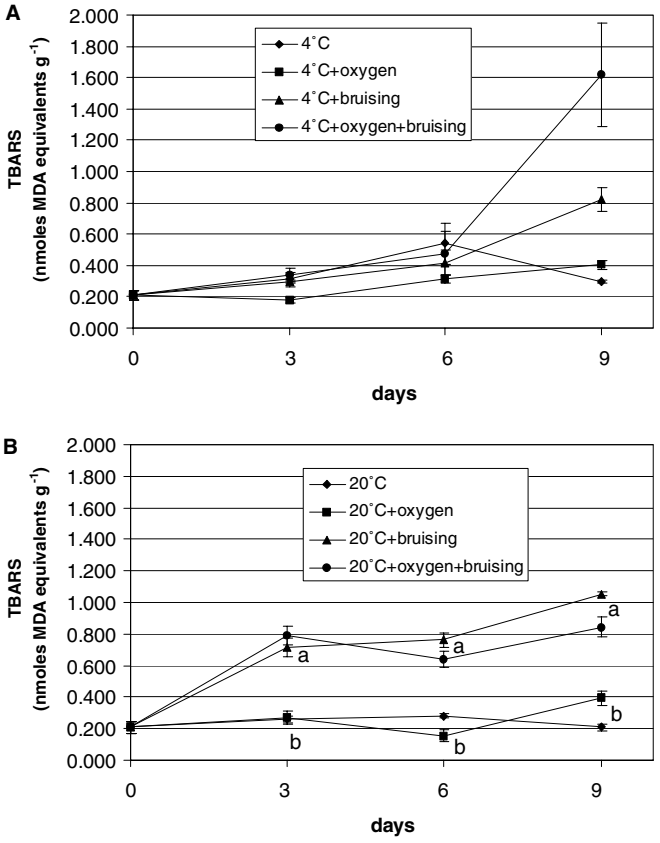


Fig. 6. TBARS values for the samples stored under MAP: (A) at 4 °C and (B) at 20 °C.

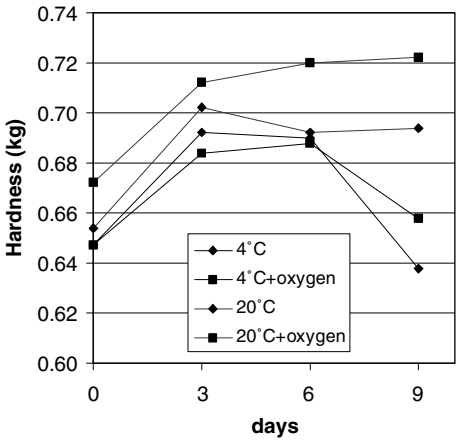


Fig. 7. Hardness of non-bruised cucumber mesocarp tissue stored under different temperatures and MAP applications.

tissues. Samples stored at 4 °C softened following the sixth day of storage.

## 4. Conclusions

Chilling is a stress factor in sensitive produce resulting in increased levels of lipid peroxidation. Minimally processed products must be stored at chilled temperatures. Dipping treatments which are regularly used in postharvest processing to improve other quality factors can potentially also be formulated to minimize lipid peroxidation. Modified atmosphere packaging with higher levels of oxygen generally reduces lipid peroxidation. Cucumbers are one of the major components of salads in Turkey. Improving the shelf-life of minimally processed cucumbers or salad mixes containing cucumbers is important for the fresh-cut market in Turkey and abroad. Thus more research in this field is recommended.

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