Effects of modified atmosphere packaging conditions and ethylene absorber on the quality of red bell pepper

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Summary
The objective of this study was to determine the effect of active and passive modified atmosphere packaging along with ethylene absorber on physico-chemical quality of red bell pepper (*Capsicum annuum* L.). Low-density-polyethylene films for packaging and 5 g of $KMnO_4$ pockets as ethylene absorber were used for storage of pepper under modified atmoshere packaging (MAP) conditions at 10 ± 1 °C and 95 ± 5 % relative humidity. The gas mixture (5 % O_2 + 5 % CO_2) was filled inside the bags for active MAP. The bags for passive MAP were sealed in ambient atmosphere. The final O_2 levels were 8.6 % and 7.9 % in active packages, while 6.8 % and 2.2 % in passive packages with and without ethylene absorber, respectively. The levels of CO_2 in all packages were at desired values. Weight loss was below 1 % in MAP samples, while it was 15.1 % in control samples. Both MAP versions maintained the physico-chemical quality better than that of control samples. However, passive MAP was more effective on antioxidant content (except for total phenolic and cysteine contents) and on antioxidant activity. In addition, ethylene absorbers showed positive effects on the bioactive content of pepper.

Keywords
modified atmosphere packaging; ethylene absorber; pepper; antioxidant

Pepper belongs to *Capsicum* genus of Solanaceae family. *Capsicum annuum* L. is the most common of the 5 pepper species [1]. It is grown especially in tropical and subtropical parts of the world as an annual herbaceous crop. Turkey is the third in the world production of pepper, contributing by 6.9 % to the fresh pepper production. In 2016, Turkey produced approximately 2.5 million tonnes of pepper, the varieties being commonly long, bell and capia pepper. Exports of fresh pepper from Turkey were approximately 97 000 tonnes in 2016 [2]. It is thought that preservation of quality and extension of shelf life using advanced technology can increase the exportation of pepper.

Peppers have become popular for the abundance and the kind of phytochemicals they contain. Among the antioxidant phytochemicals, peppers are rich in phenolic compounds and vitamins, in particular A and C. [3]. In addition, thiols are significant phytochemicals contained in pepper. These are known to protect body cells against various kinds of oxidative damage. Glutathione (GSH), cysteine (CYS), homocysteine (HCYS), *N*-acetylcysteine (NAC) and captopril (CAP) are thiols. GSH is the most abundant thiol, which is mostly found in fruits and vegetables. In one study, it was shown that red pepper contained 42 $\mu mol \cdot kg^{-1}$ GSH and 349 $\mu mol \cdot kg^{-1}$ CYS. However, studies measuring the content of thiols have been limited [4, 5].

Modified atmospheric packaging (MAP) is a method based on changing the ambient atmosphere composition to extend the shelf life and protect the quality of food. Low O_2 and elevated CO_2 concentrations in MAP slow down respiration of fruits and vegetables [6]. Two types of MAP are used, namely, passive and active. In passive MAP, appropriate atmosphere can be passively achieved within a sealed package by consumption of O_2 and production of CO_2. Gas permeability of the selected packaging film should let more CO_2 exit than O_2 enter, because high levels of CO_2 can cause damage to most fruits and vegetables. Therefore, permeability of the film and respiration rate of the

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food should be well known [7]. In active MAP, the package atmosphere is initially replaced by the desired gas mixture or is adjusted using O_2, CO_2 and ethylene absorbers. Ethylene is a compound active as a plant hormone, which is produced during ripening of fruits and vegetables. In general, pepper is classified as a non-climacteric fruit based on ethylene production. However, this is limited to certain cultivars of *Capsicum*. Several studies showed that some cultivars of pepper could be climacteric [8–10]. Using potassium permanganate as ethylene absorber in packages is a commercial method to absorb the ethylene produced to the atmosphere surrounding the fruits and vegetables during their shelf life. Potassium permanganate removes the exogenous ethylene by oxidizing it to ethylene glycol, which later decays to carbon dioxide and water. Since this compound is not volatile, there is no chemical interaction with the product [11].

Prevention of water loss and chilling injury are the benefits of MAP and cold storage. Evaporation from the product can rapidly occur if it is not packaged or if the package material does not provide an adequate water vapour barrier. Shriveling, tissue softening and physiological disorders happen as a result of water loss, which decreases the quality and marketability of fruits and vegetables. Peppers are subjected to chilling injury when stored below 7 °C, which is associated with sunken, water soaked sheet pitting and seed discoloration near the calyx [12, 13].

According to the literature, MAP was used to prevent peppers from the described adverse effects. SALTVEIT [14] recommended the optimum O_2 and CO_2 concentration in the range of 2–5 % at 8 °C to provide quality for peppers. In another study, various CO_2 (2.5; 5; 10 and 15 kPa) and O_2 (2.5 and 5 kPa) combinations were tried and it was found that 5 kPa CO_2 and 5 kPa O_2 were highly effective to maintain quality attributes of fresh-cut peppers [15]. The effect of passive MAP on the shelf life of green chillies was studied and it was reported that MAP application extended the shelf life of green chillies to 28 days compared to 15 days in the case of untreated samples [16]. In the study of SINGH et al. [17], MAP was applied to green bell pepper with a moisture absorbent under ambient atmosphere, which prolonged the shelf life of capsicum by 7 days compared to MAP alone.

To the best of our knowledge, no study has dealt thoroughly with active and passive MAP of red pepper using ethylene absorbers. In this study, red peppers were packed with low density polyethylene (LDPE) film and active (5 % CO_2 and 5 % O_2) and passive MAP (ambient atmosphere) were compared throughout 21 days of shelf life. In addition, ethylene absorber ($KMnO_4$) was used in the packages. Headspace gas analysis, weight loss, antioxidants (total phenolic content, ascorbic acid, glutathione and cysteine), 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity, ferric ion reducing antioxidant power (FRAP) and cupric ion reducing antioxidant capacity (CUPRAC) values were determined.

MATERIALS AND METHODS

Materials

Red bell peppers were obtained from local markets in Sakarya, Turkey in July 2016 and December 2016. The LDPE film (thickness 50 μm, 5 000 $cm^3 \cdot m^{-2} \cdot d^{-1}$ (23 °C), 24 000 $m^3 \cdot m^{-2} \cdot d^{-1}$ (23 °C) and 13.50 $g \cdot m^{-2} \cdot d^{-1}$ (38 °C)) of oxygen, carbon dioxide and water transmission rates, respectively) were provided by Korozo (Istanbul, Turkey). Ethylene absorbers were obtained from Sedef (Istanbul, Turkey). *N*-(1-pyrenyl)-maleimide (NPM), DPPH, neocuproine, copper(II) chloride, ammonium acetate, *T*ris(hydroxymethyl) aminomethane hydrochloride (Tris-HCl), L-serine, diethylenetriaminepentaacetic acid (DETAPAC), 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ), iron(II) sulfate heptahydrate (≥99.0%), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox, 97%), L-ascorbic acid (≥99.0%), GSH (≥98.0%) and CYS (97%) were supplied by Sigma Aldrich (St. Louis, Missouri, USA). Hydrochloric acid, boric acid, methanol (HPLC grade), Folin-Ciocalteu phenol reagent, gallic acid (≥98.0%), sodium carbonate, iron(III) chloride hexahydrate, sodium acetate, metaphosphoric acid, potassium phosphate, acetonitrile (HPLC grade), acetic acid (HPLC grade) and orthophosphoric acid (HPLC grade) were obtained from Merck (Darmstadt, Germany).

Processing and packaging

Red bell peppers were washed with sodium hypochlorite solution (1.3 $mmol \cdot l^{-1}$) for 1 min, rinsed with tap water and their surface was dried at room temperature. The surface-sanitized peppers were divided into three groups for different packaging, namely, active packaging with LDPE (5 % O_2 and 5 % CO_2), passive packaging with LDPE (ambient atmosphere) and control (unpackaged samples). Each MAP group was also divided into two, namely, with and without ethylene absorber. An amount of 5 g of potassium permanganate was sealed with polyethylene film and placed in each

package that contained 500 ± 50 g of peppers. All samples were stored at 10 ± 1 °C and 95 ± 5 % relative humidity for 21 days in an air-conditioning cabinet (Mikrotest, Ankara, Turkey). All analyses were made in triplicate for 3 different bags. Each replicate was prepared with a mixture of three cutlets from whole treated peppers because the antioxidant contents and antioxidant activity may vary significantly between samples. In this way, standard deviations decreased and better results were obtained from the analysis.

Headspace gas analysis

Oxygen and carbon dioxide were measured with a headspace gas analyser (Oxybaby M+; Elit Electronic, Istanbul, Turkey). Gas analysis was performed with needle attached to the gas analyser through a septum pasted on the packaging film. The packages were opened and used for physical and chemical analysis after the gas analysis.

Weight loss

Packaged and unpackaged samples were weighed initially and weekly during storage. The results were expressed as percent weight loss [15].

Determination of antioxidants

Total phenolic content (*TPC*) was determined using a Folin-Ciocalteu reagent [18]. Three grams of fresh red pepper were cut and homogenized with 10 ml of methanol:water (75:25) solution. After keeping at ambient temperature in an ultrasonic water bath for 15 min, the tubes were centrifuged at 13 130 ×*g* at 4 °C for 10 min and then the supernatants were separated for further analysis. One hundred microlitres of the extract were mixed with 0.2 ml of the Folin-Ciocalteu reagent and with 2 ml of distilled water. After 3 min, 1 ml of sodium carbonate solution (0.2 g·ml^{-1}) was added. Following incubation for 1 h in the dark at ambient temperature, absorbance of the sample was measured at 765 nm in UV-Vis spectrophotometer UV-1240 (Shimadzu, Kyoto, Japan). Quantification was done by using a standard curve of gallic acid (r^2 = 0.991). The results were expressed as grams of gallic acid equivalents per kilogram dry weight.

Ascorbic acid content was determined using a chromatographic method [19]. Five grams of pepper were transferred into a tube and 5 ml of metaphosphoric acid solution (0.025 g·ml^{-1}) was added. After homogenization, the tubes were centrifuged at 13 000 ×*g* at 4 °C for 10 min. Half millilitre of the supernatant was taken and it was added metaphosphoric acid solution to a final volume of 10 ml. Samples were filtered through nylon filters (pore size 0.45 μm). Then they were injected to a reverse-phase high-performance liquid chromatography (HPLC) system. The HPLC system (Hitachi, Tokyo, Japan) consisted of L-2130 pump, L-2200 autosampler, L-2455 diode array detector and L-2300 oven. Reliasil ODS-1 C18 column (5 μm, 250 mm × 4.6 mm; Orochem, Naperville, Illinois, USA) was utilized for separation. The diode array detector was operated at a wavelength of 244 nm. The mobile phase comprised potassium phosphate solution (0.02 g·ml^{-1}) adjusted to pH 2.4 by addition of orthophosphoric acid. A calibration curve was constructed using an ascorbic acid standard (r^2 = 0.999).

Limit of detection (*LOD*) for the method was determined from repeated analyses of standard ascorbic acid solution at low concentrations. The lowest concentration was determined and *LOD* was reported at three times the standard deviaton of replicates (99% confidence). Limit of quantification (*LOQ*) was calculated as ten times the standard deviation of replicates. *LOD* and *LOQ* were found to be 0.62 mg·l^{-1} and 1.86 mg·l^{-1}, respectively.

Thiol content of the extracts was determined using the chromatographic method to analyse γ-glutamyl cycle intermediates as developed by Winters et al. [20] and modified by Demirkol et al. [5]. The samples (0.1 kg·l^{-1} each) were mixed with a serine borate buffer (SBB) to prevent potential oxidation of biothiols by atmospheric oxygen. The SBB buffer contained 100 mmol·l^{-1} Tris-HCl, 10 mmol·l^{-1} borate, 5 mmol·l^{-1} serine and 1 mmol·l^{-1} DETAPAC, with the final pH adjusted to 7.0 by a concentrated NaOH solution. The samples were homogenized with tissue tearor D-130 (Wiggen Hauser, Berlin, Germany) on ice during 2 min with homogenization intervals of 5 s, and then centrifuged at 13 000 ×*g* for 15 min at 4 °C. Fifty microlitres of supernatant, 200 μl of distilled water and 750 μl of NPM (1 mmol·l^{-1} in acetonitrile) were added to tubes. NPM reacts with free sulfhydryl groups to form fluorescent derivatives. The resulting solution was mixed and incubated at room temperature for 5 min. After that, 10 μl of 2 mol·l^{-1} HCl were added to stop the reaction and the derivatized samples were filtered through nylon filters (pore size 0.45 μm). Samples were analysed by HPLC using the same column and equipment as for ascorbic acid, but a fluorescence detector was used, operating at an excitation wavelength of 330 nm and an emission wavelength of 376 nm. The mobile phase consisted of 70 % acetonitrile and 30 % water, and it was adjusted to pH 2.5 by addition of 1 ml of acetic acid and 1 ml of orthophosphoric acid per litre. The calibration

curves were constructed by preparing mixed standard solutions, each containing a known concentration of GSH and CYS in the range of 0 nmol·l^{-1} to 2500 nmol·l^{-1}. The r^2 values of the curves for GSH and CYS were 0.994 and 0.999, respectively. To calculate *LOD* and *LOQ*, the procedure used in ascorbic acid analysis was performed. *LOD* and *LOQ* were determined to be 0.05 μmol·l^{-1} and 0.16 μmol·l^{-1} for GSH, 0.03 μmol·l^{-1} and 0.11 μmol·l^{-1} for CYS, respectively.

Determination of antioxidant capacity

The same extract as used for *TPC* analysis was used for determination of DPPH radical-scavenging capacity and FRAP assay. The DPPH radical-scavenging capacity was carried out according to Brand-Williams et al. [21] with some modifications. The extracts were diluted to 37.5 g·l^{-1}, and 200 μl of the diluted extract were transferred into a tube. A volume of 3 ml of freshly prepared DPPH solution (0.05 mmol·l^{-1}) was added and the mixture was incubated in the dark at room temperature for 30 min. Absorbance was determined at 517 nm (Shimadzu UV-1240). The DPPH radical-scavenging activity was calculated in percent inhibition.

The FRAP assay was carried out according to Benzie and Strain [22] with some modifications. Acetate buffer (300 mmol·l^{-1}, pH 3.6), 10 mmol·l^{-1} TPTZ, and 20 mmol·l^{-1} $FeCl_3 \cdot 6H_2O$ were mixed in a ratio of 10 : 1 : 1 to prepare the FRAP reagent. One hundred microlitres of the extract and 1.2 ml of distilled water were added to 1.8 ml of FRAP reagent. After incubation at 37 °C for 15 min, absorbance was measured at 593 nm. The calibration curve was constructed by using standard $FeSO_4$ solutions (r^2 = 0.995). The results were expressed as gram of $FeSO_4$ equivalents per kilogram of dry weight.

Sample extraction for CUPRAC analysis was performed according to the method described by Capanoglu et al. [23]. Three millilitres of 75% methanol were added to 1 g of sample and sonicated for 15 min. After centrifugation at 13000 ×*g* for 10 min at 4 °C, the supernatant was separated. The pellet was mixed with 3 ml of methanol (75%) and the procedure was repeated. The collected supernatants were added 75% methanol to a final volume of 10 ml. A total volume of 1.1 ml of the extract and distilled water were transferred into a tube. One millilitre of 10 mmol·l^{-1} copper (II) chloride (in distilled water), 1 ml of 75 mmol·l^{-1} neocuproine (in 96 % ethanol) and 1 ml of ammonium acetate buffer solution (in distilled water) were added, and the tube was stoppered. Mixture was incubated at room temperature for 1 h and its absorbance was determined spectrophotometrically at 450 nm. The results of analysis were expressed as grams of Trolox per kilogram of dry weight (r^2 = 0.998).

Statistical analysis

The tests were performed in triplicate and the results were expressed as mean ± standard deviation. Storage periods were 0–21 days and active MAP without ethylene absorber (A), active MAP with ethylene absorber (AE), passive MAP without ethylene absorber (P), passive MAP with ethylene absorber (PE), and control (C) were the treatments. Comparison of the means was performed using Duncan's test at 5% level of significance.

RESULTS AND DISCUSSION

Headspace gas

The dynamics of in-package headspace gas composition (O_2 and CO_2) during the storage of red peppers are shown in Fig. 1. Both O_2 and CO_2 concentration of active packages were 5 % at the beginning of shelf life. The final O_2 levels were 8.6 % and 7.9 % for AE and A, respectively. It was seen that O_2 level of active packages first increased and then decreased. The reason could be that O_2 permeability of the packaging film was high enough to allow the oxygen from the outside to pass inside. However, O_2 level decreased towards the end of storage. The CO_2 concentration was 4.1 % and 5.0 % for AE and A, respectively. This was not significantly different from the initial level.

In a study by Manolopoulou et al. [24], O_2 decreased from the initial 5 % to the final 0.5 % and 0.2 % for 0 °C and 5 °C storage, respectively, apparently because of using an impermeable high density polyethylene film. The O_2 concentration decreased to 6.8 % in passive packages with ethylene absorber. On the other hand, it was 2.2 % in passive packages without an ethylene absorber. The CO_2 values were 4.7 % for PE and 5.8 % for P, which were close to the initial level. In headspace gas analysis of active packaging of broccoli, O_2 concentration dropped below 1 % in packages without ethylene absorber but it reached equilibrium and remained 2 % in packages with an ethylene absorber until the end of the storage [25]. When the O_2 concentration is under the 2%, which is the minimum acceptable level, harmful physiological reactions can occur.

In this study, O_2 level was not under that critical value and it was higher in MAP packages with

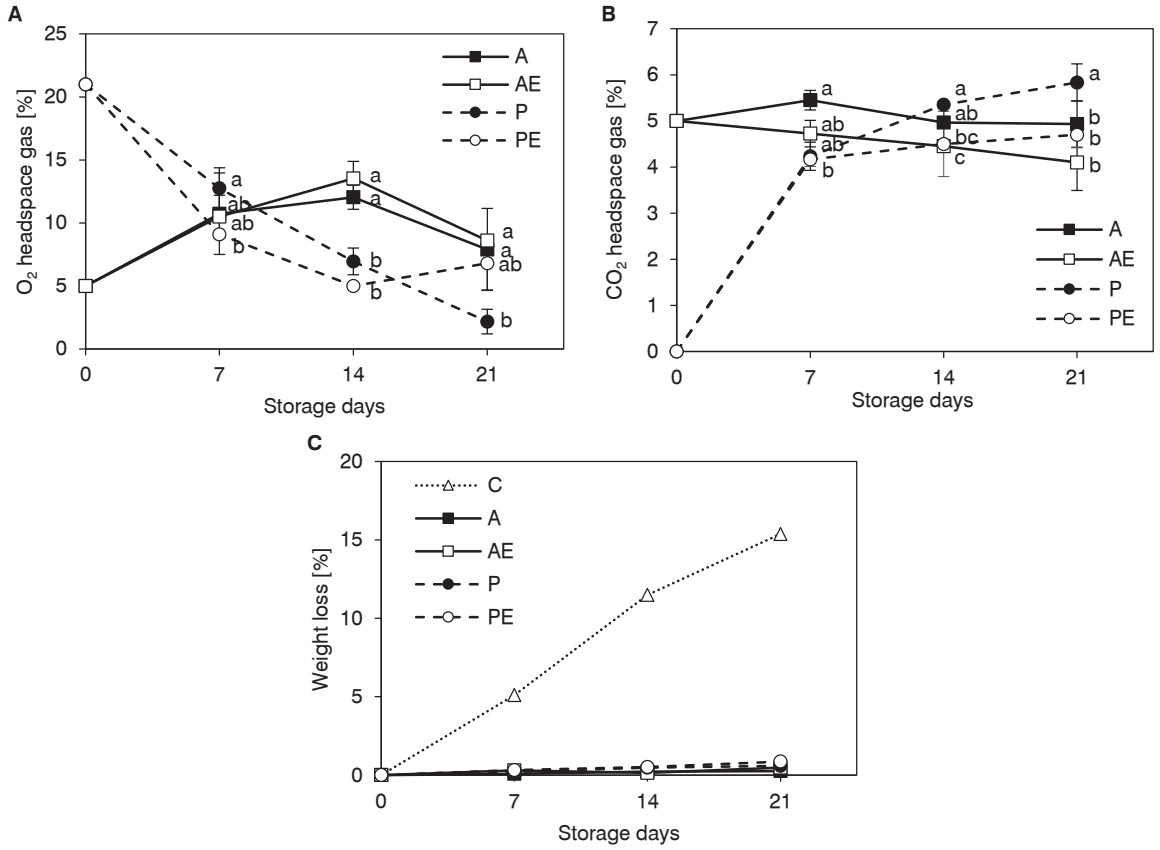

Fig. 1. Headspace gas and weight loss of pepper.

A – O_2 headspace gas, B – CO_2 headspace gas, C – weight loss.
Data are presented as mean ± standard deviation of three samples (n = 3). Different letters denote significant differences ($p < 0.05$) at each storage period. The difference between control and all modified atmosphere packaging groups is important according to weight loss results.
C – control, A – active packages without ethylene absorber, AE – active packages with ethylene absorber, P – passive packages without ethylene absorber, PE – passive packages with ethylene absorber.

ethylene absorbers. Ethylene accelerates respiration rate and this leads to maturity. If the produced ethylene is not removed, it speeds up respiration and causes O_2 depletion [14, 26]. In this study, ethylene was retained by $KMnO_4$ pockets whose colour turned from pink-purple to brown-black with the absorption until the end of the storage (data not shown). This showed that red bell peppers used in this study produced ethylene gas during 21 days of storage.

Weight loss

Weight loss of AE, A, PE and P were determined as 0.5 %, 0.3 %, 0.9 % and 0.6 % at the end of storage, respectively (Fig. 1). The loss was low in both active and passive packages, 15.1 % being observed in control samples at the end of the storage. Similar trend was also observed by SINGH et al. [17] and CHITRAVATHI et al. [16]. The weight loss in MAP packages was limited to < 1 % at the end of shelf life, while it should be lower than 5 % to avoid shriveling appearance [27].

Antioxidants

Red peppers contain antioxidant compounds, such as phenolic substances, carotenoids, ascorbic acid or thiols. These are active as scavengers of free radicals. Percentage loss of *TPC* in active packages, passive packages and control groups is shown in Tab. 1.

After 21 days, the loss of *TPC* in the control group, A and AE were determined as 37.5 %, 5.1 % and 4.9 %, respectively. In passive packages, the loss of *TPC* in P (32.9 %) was higher than in PE (12.3 %). Overall, both active and passive packaging with ethylene absorber protected *TPC* effectively. It was reported that ethylene had stimulative effect on the polyphenol oxidase (PPO) activity. PPO oxidases the phenolic compounds in the presence of O_2 [28]. Therefore, in this

Tab. 1. Percentage loss of antioxidant contents of peppers.

Samples	Total phenolic content [%]			Ascorbic acid [%]			Glutathione [%]			Cysteine [%]		
	Day 7	Day 14	Day 21	Day 7	Day 14	Day 21	Day 7	Day 14	Day 21	Day 7	Day 14	Day 21
C	6.8 ± 0.3^{a}	21.3 ± 0.9^{ab}	37.5 ± 0.3^{a}	60.9 ± 0.2^{a}	59.8 ± 0.0^{a}	76.6 ± 1.1^{a}	75.4 ± 2.0^{ab}	79.8 ± 2.0^{a}	82.1 ± 2.0^{a}	68.8 ± 1.5^{ab}	78.7 ± 1.5^{a}	82.2 ± 1.0^{a}
A	0.2 ± 0.1^{a}	6.8 ± 0.4^{bc}	4.9 ± 0.3^{c}	49.4 ± 2.2^{ab}	44.7 ± 3.0^{b}	44.8 ± 1.4^{bc}	55.6 ± 3.1^{c}	57.6 ± 0.8^{c}	76.6 ± 0.8^{ab}	71.8 ± 0.6^{ab}	69.9 ± 0.1^{c}	69.8 ± 1.0^{c}
AE	2.9 ± 0.3^{a}	4.8 ± 0.2^{c}	5.1 ± 0.1^{c}	42.8 ± 2.2^{ab}	44.7 ± 0.3^{b}	43.2 ± 0.9^{bc}	79.6 ± 0.5^{a}	78.7 ± 3.6^{a}	72.5 ± 1.8^{abc}	61.9 ± 0.1^{b}	63.5 ± 18.5^{d}	66.4 ± 0.7^{d}
P	2.4 ± 0.1^{a}	23.1 ± 0.9^{a}	32.9 ± 3.4^{a}	39.2 ± 1.2^{ab}	42.2 ± 3.4^{b}	49.8 ± 2.1^{b}	71.2 ± 2.2^{ab}	70.0 ± 18.0^{b}	67.3 ± 3.6^{bc}	77.9 ± 3.8^{a}	75.6 ± 1.0^{ab}	75.0 ± 0.9^{b}
PE	1.1 ± 0.1^{a}	8.4 ± 1.4^{abc}	12.3 ± 0.8^{b}	31.4 ± 0.5^{b}	32.7 ± 1.5^{c}	41.1 ± 0.3^{c}	68.9 ± 0.4^{b}	71.1 ± 3.8^{b}	62.9 ± 6.9^{c}	66.2 ± 6.1^{ab}	74.0 ± 2.7^{bc}	71.1 ± 0.7^{c}

Data presented are mean ± standard deviation of three samples (n = 3). Different letters denote significant differences ($p < 0.05$) at each storage period.
C – control, A – active packages without ethylene absorber, AE – active packages with ethylene absorber, P – passive packages without ethylene absorber, PE – passive packages with ethylene absorber.

study, it was thought that PPO activity increased and amount of phenolic compound decreased in the packages without ethylene absorber. Similar results were also observed by Lagnika et al. [29]. However, different results were reported by Rodoni et al. [15], where *TPC* was higher in control samples than for controlled atmosphere peppers after 12 days. As a reason, tissue damage and senescence were hypothesized, causing accumulation of phenolic compounds. However, dry matter of samples could significantly change during storage. Therefore, results in this study were calculated in dry matter.

Data on percentage loss in ascorbic acid content of peppers are shown in Tab. 1. It was determined that the ascorbic acid content decreased during storage. The highest loss was in the control group (76.6 %) at the end of storage. The loss of ascorbic acid was 43.2 %, 44.8 %, 41.1 % and 49.8 % in AE, A, PE, P, respectively. There was no significant difference between active and passive packages, but the effect of using ethylene absorber was important in passive package. Singh et al. [17] investigated the effect of MAP using moisture absorbent on the green bell pepper. When expressed in terms of dry matter, the loss of ascorbic acid in control samples was almost 70 % of the initial value, which was similar to this study. MAP prevents ascorbic acid degradation by eliminating O_2. The ascorbic acid level is known to decrease in the presence of light, oxygen, heat, peroxides and enzymes like ascorbate oxidase or peroxidase [30]. Therefore, in this study, ascorbic acid retention was higher in MAP treated samples than in control peppers.

GSH and CYS contents were determined and it was revealed that these compounds were present in pepper, but there no other biological thiols like CAP, NAC or HCYS were present. Data on the percentage loss of GSH and CYS is shown in Tab. 1. While 82.1 % of GSH was lost in control samples, it was 72.5 % for AE, 76.6 % for A, 62.9 % for PE and 67.3 % for P at the end of storage. The other thiol compound found in pepper was CYS, which is one of the non-essential amino acids. The loss of CYS in control, AE, A, PE and P were 82.2 %, 66.4 %, 69.8 %, 71.1 % and 75.0 %, respectively. It can be seen that control samples had the highest loss of both GSH and CYS. Actually, the loss of GSH and CYS in all treatments was very high. GSH and CYS are easily oxidized during storage, preparation or processing such as drying [31]. In both active and passive packages, the thiol loss was lower in groups that involved ethylene absorbers. Oxidative stress, antioxidant capacity and ethylene pro-

duction during aging of cut carnation petals were investigated previously and it was reported that when the 6 mg·l^{-1} ethylene-enriched atmosphere was supplemented to petals on 6th day after cutting, it decreased GSH content by 17 % and 66 % after 8 and 11 days of detachment, respectively [32]. Therefore, it was thought that since ethylene was absorbed by $KMnO_4$ pockets, retention of GSH and CYS were higher in the packages with ethylene absorbers in this study.

Antioxidant capacity

Bell peppers have become popular also because they contain various kinds of antioxidants, which protect the food, and probably also human body, from oxidative damage induced by free radicals [3]. Antioxidant capacity (DPPH scavenging activity, FRAP and CUPRAC values) of control and MAP groups are shown in Fig. 2. The DPPH scavenging activity of fresh pepper was determined as 64.4 % and showed a decreasing trend during storage. However, DPPH scavenging activity on 21st day was higher than on 14th day in all groups. The reason could be that the dry matter of samples increased at the end of the storage, which caused an apparent increase in antioxidant activity. The results also demonstrated that PE group had the highest DPPH scavenging activity in each storage period.

Regarding FRAP, the fresh pepper had a value of 67.85 g·kg^{-1} (expressed as grams of $FeSO_4$) (Fig. 2). The changes in FRAP values of pepper were lower by MAP application with respect to control sample in each storage period. Moreover, similar to DPPH scavenging activity, FRAP values of peppers in PE were significantly higher than those of other groups in each storage period.

In the CUPRAC method, the cupric neocuprine complex reacts with antioxidants and then it generates cuprous form of copper. The CUPRAC reactive can oxidize thiol-type antioxidants such as GSH and CYS [33]. The CUPRAC value of the fresh sample in this study was 80.34 g·kg^{-1} (expressed as grams of Trolox), while it was 29.73 g·kg^{-1} for control, 56.74 g·kg^{-1} for AE, 54.39 g·kg^{-1} for A, 73.89 g·kg^{-1} for PE and 75.42 g·kg^{-1} for P at the end of storage (Fig. 2). Unlike DPPH scavenging activity and FRAP values, use of ethylene absorber had no effect on CUPRAC values except for passive packaging on 14th day. However, MAP was effective in protecting the antioxidants from the point of view of CUPRAC values. In addition, when active and passive packages were compared, passive packages had significantly higher CUPRAC values.

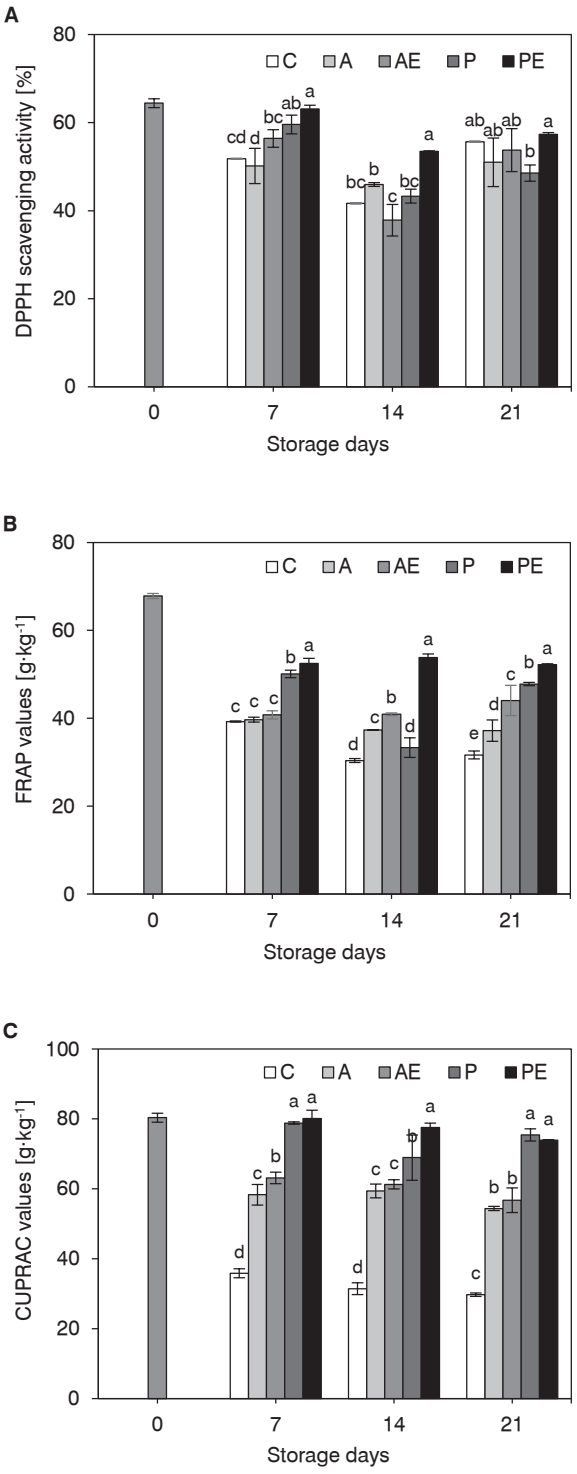

Fig. 2. Antioxidant activities of pepper.

A – DPPH scavenging activity, B – ferric ion reducing antioxidant power (FRAP), C – cupric ion reducing antioxidant capacity (CUPRAC).
Data are presented as mean ± standard deviation of three samples (n = 3). Different letters denote significant differences (p < 0.05) at each storage period.
C – control, A – active packages without ethylene absorber, AE – active packages with ethylene absorber, P – passive packages without ethylene absorber, PE – passive packages with ethylene absorber.

CONCLUSIONS

Effects of active and passive packaging with or without ethylene absorbers on quality of red bell pepper were investigated in this study. As far as we know, this is the first report on MAP application by using ethylene absorber in pepper. The LDPE film for packaging and $KMnO_4$ pockets as ethylene absorbers were used. According to headspace gas analysis, initial 5 % of O_2 increased to 8.6 % and 7.9 % in active packages and decreased from 21 % to 6.8 % and 2.2 % in passive packages with and without ethylene absorber, respectively. The O_2 concentration was not below the critical value (2 %) in any group. In addition, CO_2 concentration was at a desired level in all packages. While the weight loss was 15.1 % in control sample, it was very low level in MAP samples (< 1 %), as expected. Antioxidant contents decreased during storage with ripening, but they were retained more effective by MAP application compared to control samples. Although the *TPC* and CYS contents were higher in active packages, loss of ascorbic acid and GSH was lower in passive packages with ethylene absorber. The antioxidant activity of pepper was also highest in passive packages and using ethylene absorber was effective. The DPPH scavenging activity, FRAP and CUPRAC values after MAP application were 1.0, 1.7 and 2.5 times higher than those of control samples, respectively. As a result, MAP preserved bioactive compounds of red peppers better than unpackaged samples. When passive and active MAP compared, passive MAP was more effective regarding content of antioxidants (except for total phenolic and CYS contents) and regarding antioxidant activity. Moreover, it is more reasonable to use passive MAP when the cost of instruments and gases used in active packaging is taken into account. Further investigations can be done with different packaging films for red bell peppers and their effectiveness may be examined regarding storage stability and retention of bioactive compounds.

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