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Efeects of pretreated ozone and modified atmosphere packaging on the quality of fresh-cut green asparagus

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A b s t r a c t. The changes in lignifying, antioxidant enzyme activities and cell wall composition of fresh-cut green asparagus (*Asparagus officinalis* L.), pretreated in 1 mg l^{-1} aqueous ozone and subsequently packaged in modified atmosphere (MAP), were investigated during storage at 3°C for 25 days. The enzyme activities in fresh-cut asparagus including phenylalanine ammonia lyase (PAL), superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR) were inhibited by aqueous ozone treatment and subsequent modified atmosphere packaging. Changes in lignin, cellulose and hemicellulose contents were also monitored during storage. Similarly, the increase of the cell wall composition elements under the aqueous ozone treatment and/or MAP were significantly reduced ($P<0.05$). The possible mechanisms in which changes in enzymatic activities and cell wall composition occurred were discussed. Compared to the control group, enzyme activities in MAP and/or group remained high, which means that asparagus in the two latter groups remained a high-ability scavenger of free radicals. It was found that as a result of treatment in O_3 and/or MAP, PAL activity presented a peak at day 10 of storage, but its activities were significantly lower than in the control group. The increase in lignin levels during storage is attributed to the toughening which occurs in asparagus a few days after harvest.

K e y w o r d s: asparagus (*Asparagus officinalis* L.), aqueous ozone, modified atmosphere packaging, lignifying, pretreatment

INTRODUCTION

Asparagus (*Asparagus officinalis* L.) is a highly perishable vegetable which is difficult to keep fresh due to its high mechanical and physiological fragility. Fresh-cut asparagus is more susceptible than whole asparagus to spoilage through microbial and pathogen contaminations due to tissue damage.

Toughness of asparagus is a major factor in determining spear quality. Toughening of asparagus is mainly related to the degree of lignification of the spears in both the fiber and the vascular bundles. The lignification is controlled by enzymes, such as phenylalanine ammonia lyase (PAL), peroxidase and isoperoxidases (Chang, 1987). In plants, lignifying peroxidases were involved in polymerization of hydroxy cinnamyl alcohols to lignin. PAL is responsible for the conversion of L-phenylalanine to *trans*-cinnamic acid, a key intermediate in the pathway of lignin production.

Conventional fresh-cut vegetable production uses rinse water, usually chlorinated at 100 mg l^{-1}. However, the relatively low inactivation rate of chlorine at concentrations limited by regulations, as well as the adverse effect of chlorine byproducts, has raised concerns in the food industry. Scientists have been searching for alternative methods of protecting fresh-cut produce from decaying, of prolonging shelf life, and of securing product safety (Hua Wang *et al*., 2004).

Ozone has been declared by an expert panel as Generally Recognized as Safe (GRAS) for use in food processing (Graham, 1997). A petition submitted in August 2000 to the US FDA for approval of ozone as a direct food additive for the treatment, storage and processing of foods in the gas and aqueous phases has been accepted in 2001 (Khadre *et al*., 2001). Both decisions have triggered interest

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in ozone applications among academic researchers and food processors (Manousaridis *et al*., 2005).

Ozone has been used in several studies to sterilize bacon, beef, bananas, eggs, mushrooms, cheese and fruit (Gammon and Karelak, 1973), to decontaminate freshly caught fish (Goche and Cox, 1999), poultry products (Dave, 1999), meat and milk products (Gorman *et al*., 1997), and to preserve lettuce (Kim *et al*., 1999a).

Treating fruits and vegetables with ozone has been used to increase shelf-life (Norton *et al*., 1968; Rice *et al*., 1982). Treatment of apples with ozone resulted in lower weight loss and spoilage. An increase in the shelf-life of apples and oranges caused by ozone has been attributed to the oxidation of ethylene. Fungal deterioration of blackberries and grapes was decreased by ozonation of the fruits (Beuchat, 1992). Ozone has been used experimentally as a substitute for ethylene oxide for the decontamination of whole black peppercorns and ground black pepper (Zhao and Cranston, 1995). Ozone treatment of ground black pepper resulted in slight oxidation of volatile oil constituents, but ozone had no significant effect on the volatile oils of whole peppercorns. Because ozonation successfully reduced microbial loads and did not cause significant oxidation of the volatile oils in whole black peppercorns, this method was recommended for industrial treatment of the spice (Zhao and Cranston, 1995). Shredded lettuce in water bubbled with ozone gas had decreased bacterial content (Kim *et al*., 1999b). Onions have been treated with ozone during storage. Mold and bacterial counts were greatly decreased without any change in chemical composition and sensory quality (Song *et al*., 2000).

Given the extremely limited data available in the literature on the application of ozone to fresh-cut asparagus, the objective of the present work was to study the effect of aqueous ozone and/or modified atmosphere packaging (MAP) application on the enzyme activity and the cell composition of fresh-cut green asparagus during storage.

MATERIALS AND METHODS

Aqueous ozone solution was prepared by circulating water through an ozone generator (ZP-98, Zibang Science and Technology Inc., Shanghai, China) and a plexiglass water tank (50 l). The ozone generator was equipped with a vortexer to facilitate dissolving of gaseous ozone in the water, and a degassing system to remove undissolved ozone. The ozone concentration was determined by the official Indigo Colorimetric method (Bader and Hoigne, 1981; Clesceri *et al*., 1989). The ozone solution was used immediately after the ozone concentration reached 1 mg l^{-1} (Hua Wang *et al*., 2004).

Fresh green asparagus (*Asparagus officinalis* L. cv. 'UC800') spears were obtained from commercial farms in Suzhou, Jiangsu province. The spears were cut at ground level between 8 and 9:30 a.m., placed in crushed ice and transported to the laboratory within 3 h on the day of harvest. Straight, undamaged spears, 8-20 mm in diameter and 22 cm in length with closed bracts were used. The spears were cut into approximately 3 cm segments using a stainless steel knife. The treatments were into divided four groups: a) control; b) O_3 pretreatment; c) modified atmosphere packaging; d) O_3 plus modified atmosphere packaging. O_3 pretreatment and/or MAP were as follows: the fresh-cut asparagus were dipped in aqueous ozone. The time was 30 min. Dipped samples were centrifuged at 550 r.p.m. for 1.5 min with a centrifugal dryer (L-508, Shanghai, China) to remove excess water. Dewatered samples of 300 g each were packaged in sealed low-density polyethylene (LDPE) bags (20x30 cm). The permeability of films for CO_2 was 10.2 10^{-12} mol s^{-1} mm^{-2} kPa^{-1}, and for O_2 3.2 10^{-12} mol $s^{-1}mm^{-2}$ kPa^{-1}, respectively, at 23°C and 90% of relative humidity.

Plant material was extracted in 50 mM potassium phosphate buffer (pH 7) containing 5 mM sodium ascorbate and 0.2 mM ethylene diamine tetraacetic acid (EDTA). The homogenate was centrifuged at 13 000 g for 15 min. The resulting supernatant was used for assays of superoxide dismutase (SOD). SOD activities were determined as described by Polle *et al*. (1989).

Ascorbate peroxidase (APX) activity was monitored through the decrease in A_{290} for 4 min in 3 ml of reaction mixture containing 100 mM potassium phosphate buffer (pH 7.5), 0.5 ml ascorbate, 0.4 mM H_2O_2 (Nakano and Asada, 1981).

Glutathione reductase (GR) activity was determined by the oxidation of γ-nicotinamide adenine dinucleotide phosphate (NADPH) at 340 nm as described by Rao (1992) and Rao *et al*. (1996). The reaction mixture contained 100 mM potassium phosphate buffer (pH 7.8), 0.2 mM NADPH, 0.5 mM oxidized glutatione (GSSG), and the tissue extract. Corrections were made for NADPH oxidation in the absence of the addition of GSSG to the reaction mixture.

Phenylalanine ammonia lyase (PAL) was extracted and assayed by the method of Zucker (1968). Frozen tissue was homogenized in acetone, filtered, extracted again in cold ethanol and filtered once more. Acetone powder was dried in a desiccator and added to cold 0.2 M sodium borate buffer at pH 8.8. The beaker was shaken for 30 min at 4°C and the suspension was filtered and centrifuged. During the preparation of the enzyme extract, the temperature was kept at 4°C. The assay medium contained 3 ml of enzyme extract and 2 ml of phenylalanine. The mixture was incubated at 37°C for 1 h, and the reaction was stopped by adding 0.5 ml of 5N HCl. PAL activity was determined by measuring the absorbance at 290 nm. One unit of PAL activity was defined as the change in absorbance per milliliter of enzyme extract. Protein content in the enzyme extracts was estimated using the Bradford's method (1976). The activity of the enzyme was expressed as units per milligram protein.

Lignin is a polymer of phenolic compounds insoluble in all solvents; therefore, it was necessary to break them down

chemically in order to analyse their structural components. Thioacidolysis is a method used for breaking down lignin in which the 8-0-4'aryl-ether structures are broken down (Önnerud *et al*., 2002). Based on the conditions described by Bruce and West (1989), the freeze-dried tissue (0.5 g) was homogenized with 150 ml of 80% ethanol for 4 min. The mixture was vacuum filtered, the residue washed with 20 ml of 80% ethanol and then dried at 50°C for 24 h. Fifteen milliliters of 2 mol l^{-1} HCl and 1ml of thioglycolic acid were added to the dry residue which was then boiled with stirring for 4 h, and centrifuged at 10,000 g for 15 min. The residue (lignin thioglycolate) was washed with 10 ml of water, suspended again in 20 ml of NaOH 0.5 mol l^{-1} with stirring for 18 h at room temperature and centrifuged; 4 ml of HCl concentrate was added to the supernatant liquid. The lignin thioglycolic acid was precipitated at 4°C for 4 h, centrifuged (10,000 g, 15 min) and the residue was dissolved in 10 ml of NaOH 0.5 mol l^{-1}, its absorbance was read at 280 nm. Quantification was carried out using a standard coumaric acid curve (Aquino-Bolaños and Mercado-Silva, 2004).

Cellulose and hemicellulose contents were determined by the procedures outlined by Van Soest and Wine (1967). The samples were analysed in triplicates and the average was recorded.

Data were analysed using the Statistical Analysis System (SAS Institute, Cary, NC, USA) by the analysis of variance (ANOVA). The Fisher's LSD test was used to determine differences at α=0.05.

RESULTS AND DISCUSSION

Changes in lignin contents were evaluated during storage. During storage of asparagus, very rapid increases were observed in the lignin levels (Fig. 1a). However, O_3 and/or MAP treatments slowed the rate of increase in this component levels during storage. The increase in lignin levels during storage is attributed to the toughening which occurs in asparagus a few days after harvest. This suggests that one of the determining factors leading to the toughening is the thickening of the structural polysaccharide components of the cell walls. O_3 and/or MAP could slow down the increase in the content of this component.

The levels of cellulose and hemicellulose play important roles in the textural attributes of asparagus cell walls. Data showed that the levels in both cellulose and hemicellulose contents increased significantly during storage (Figs 1b and 1c). Increases in cellulose, hemicellulose and lignin during storage may lead to the thickening and lignification of the cell walls. However, the rate of increases was higher in asparagus in the control group than those in O_3 pretreated and MAP groups. Cellulose and hemicellulose contents in the former group increased by approximately 359 and 283%, respectively, during the 25 days of storage at 3°C, while increases of approximately 72 and 54% were noted in the cellulose and hemicellulose contents in the later group, respectively. This indicates that the post-harvest changes in cellulose and hemicellulose contents are influenced by O_3 plus MAP. These additional cellulose and hemicellulose contents is thought to be brought about by a rapid polymerization and epimerization of cell wall microfibrils leading to the thickening of the cell walls, causing textural changes. Analysis of variance showed that aqueous ozone treatment and MAP affected significantly ($P<0.05$) the cell wall constituents of asparagus during storage.

Higher activities of scavenger antioxidant enzymes may help in protecting plants from oxidative stress, and can slow the senescence process of vegetables (Asada, 1997). However, enzyme activities in asparagus including SOD, APX and GR were decreased with the extension of storage time, but there was a significant difference ($P<0.05$) in the three enzyme activities between the treatments at the end of 25 days of storage (Fig. 2). Compared to the control group, enzyme activities in MAP and/or group remained high, which means that asparagus in the two latter groups retained its high ability to scavenge free radicals. However, we did know the detailed mechanism, so more research must be conducted to confirm the effect of MAP and/or O_3 on the function of antioxidant enzymes.

As a key intermediate of phenylpropanoid pathway, PAL activity is induced not only by wounding but also by other stimulations (Camm and Towers, 1973). Increased PAL activity has been correlated with a decrease in the shelf life and overall visual quality of minimally processed lettuce (Lopez-Galvez *et al*., 1996). Therefore, understanding PAL behaviour would allow us to predict the effect of storage process in asparagus. Figure 3 shows that PAL activity increased substantially in control groups for the first 10 days, before decreasing during the latter period of storage. Similarly, after treatment in O_3 and/or MAP, PAL activity presented a peak at day 10 of storage, but its activity was significantly lower than in the control group. This indicates that treatment in O_3 and/or MAP inhibited the increase in PAL activity; a similar effect resulted from subjecting cut lettuce to heat shock treatments (Loaiza-Velarde and Saltveit, 2001).

CONCLUSIONS

1. The increase in lignin levels during storage is attributed to the toughening which occurs in the asparagus a few days after harvest.

2. Compared to the control group, enzyme activities in MAP and/or group remained high, which means that asparagus in the two latter groups retained its high ability to scavenge free radicals.

3. It was found that following treatment in O_3 and/or MAP, PAL activity presented a peak at day 10 of storage, but its activity was significantly lower than in the control group.

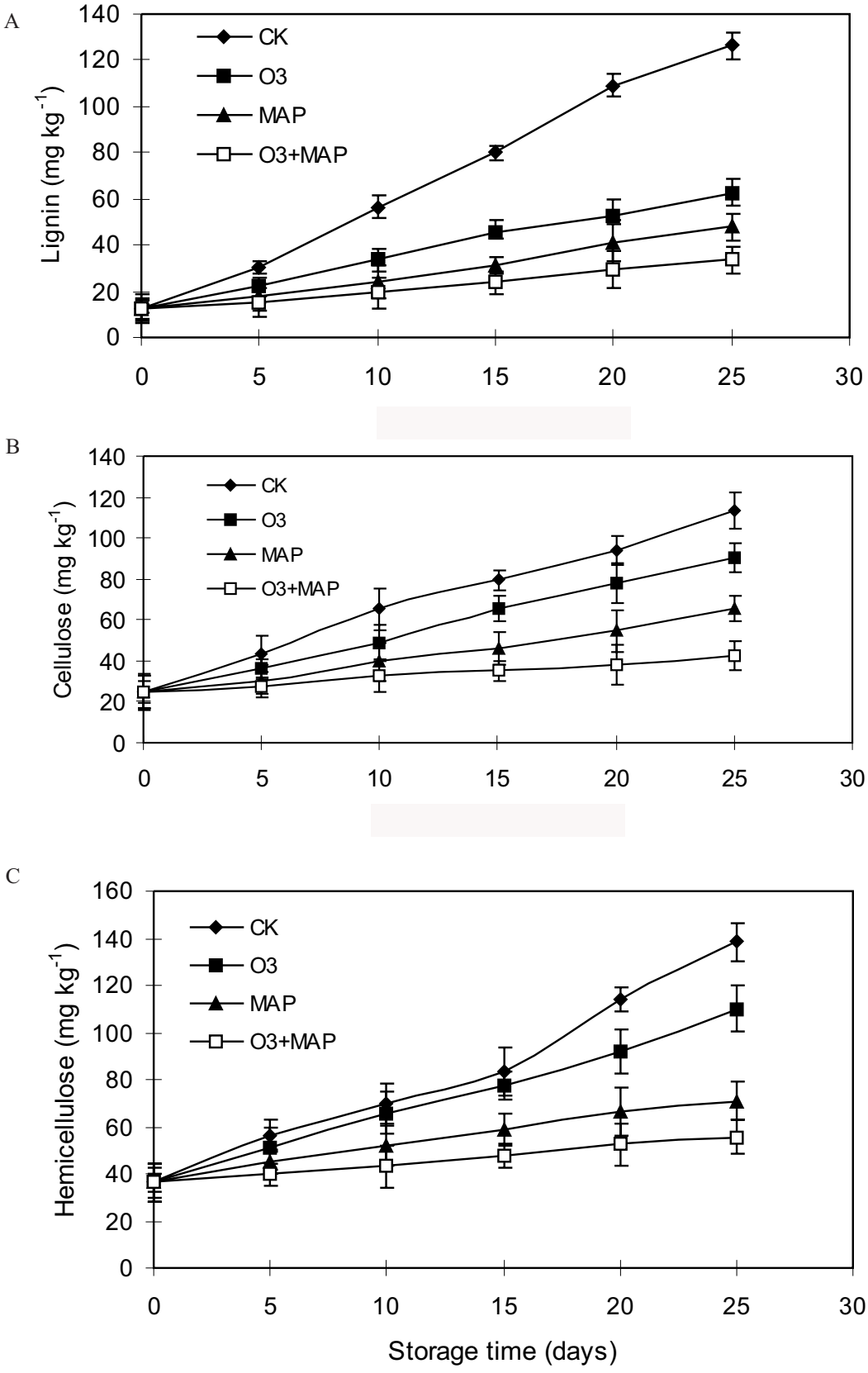

Fig. 1. Changes in the content of lignin (A), cellulose (B) and hemicellulose (C) in asparagus spears at 3°C during 25 days of storage (◆ control; ■ 1 mg l^{-1} O_3 treatments for 30 min; ▲ sealed in 15 μm LDPE bags, □ sealed in 15 μm LDPE bags with 1 mg l^{-1} O_3 treatments for 30 min before packaging). Data are the means of three replicates. The vertical bars represent the standard deviation.

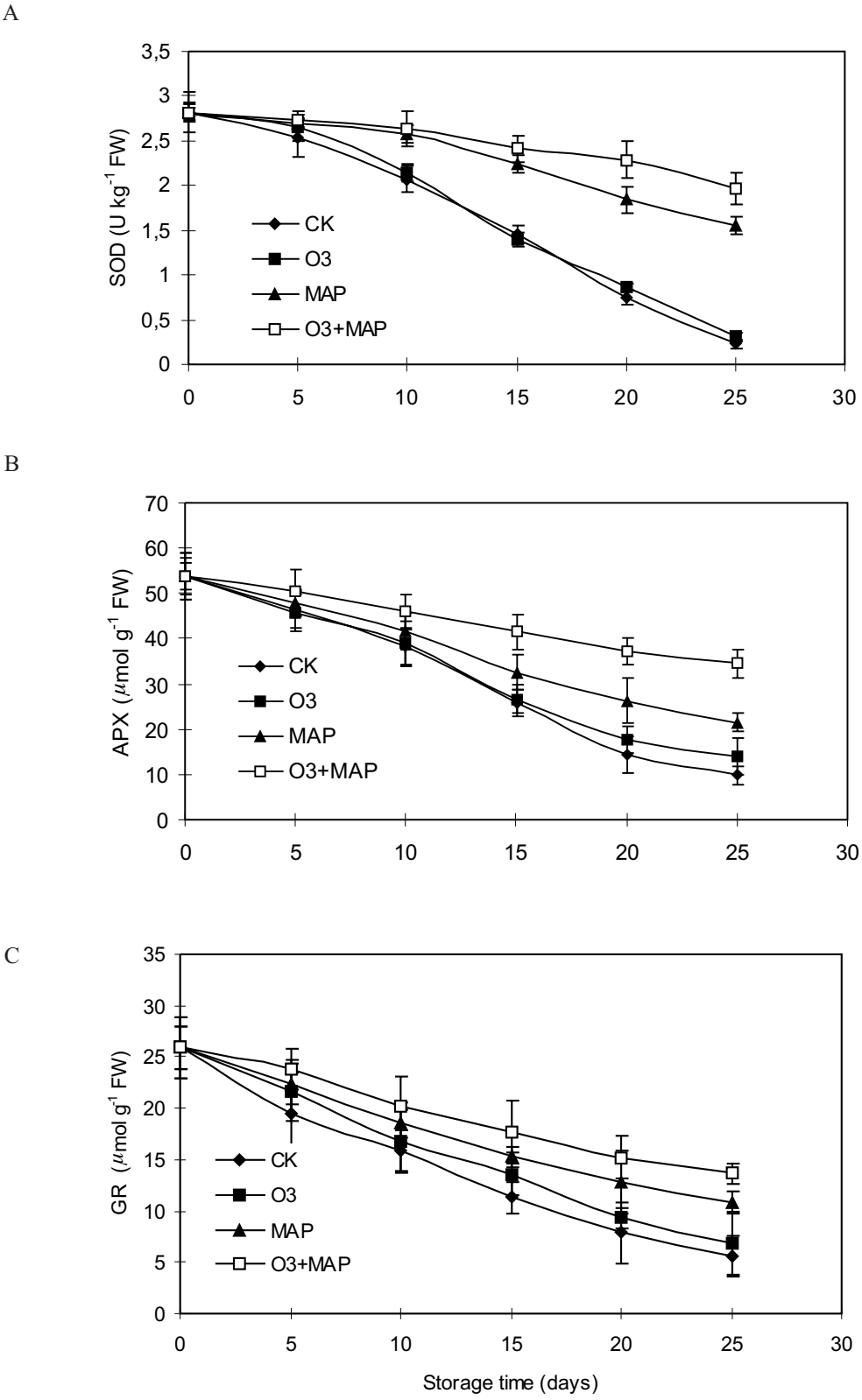

Fig. 2. Changes in: SOD (A), APX (B), and GR (C) enzyme activities in asparagus spears at 3°C during 25 days of storage. Explanations as in Fig. 1.

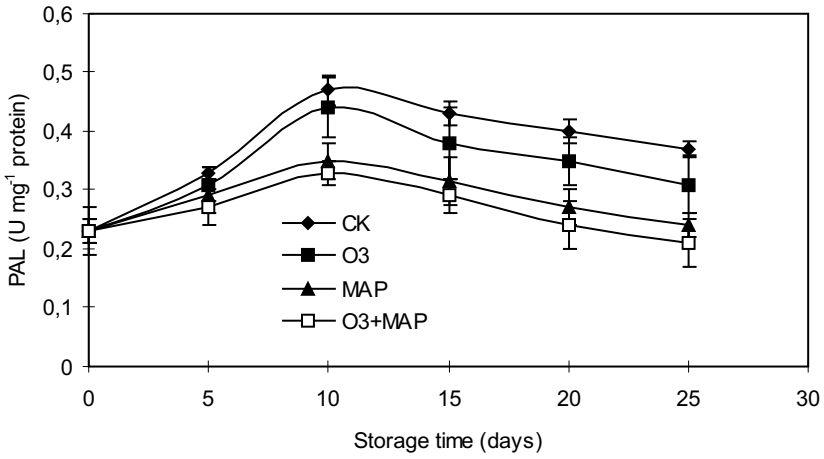

Fig. 3. Changes in PAL enzyme activities in asparagus spears at 3°C during 25 days of storage. Explanations as in Fig. 1.

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