




## Article

# Effect of Pre-Harvest Organic Cytokinin Application on the Post-Harvest Physiology of Pepper (*Capsicum annuum* L.)

Seyda Cavusoglu <sup>1</sup>, Suat Sensoy <sup>1</sup>, Arzu Karatas <sup>2</sup>, Onur Tekin <sup>1</sup>, Firat Islek <sup>3</sup>, Nurettin Yilmaz <sup>4</sup>, Selma Kipcak <sup>5</sup>, Sezai Ercisli <sup>6</sup> , Sona Skrovankova <sup>7</sup> , Anna Adamkova <sup>7,\*</sup>  and Jiri Mlcek <sup>7</sup>

<sup>1</sup> Department of Horticulture, Faculty of Agriculture, Yuzuncu Yil University, Van 65080, Turkey; scavusoglu77@gmail.com (S.C.); suatsensoy@yyu.edu.tr (S.S.); onurtekin@yyu.edu.tr (O.T.)

<sup>2</sup> Department of Horticulture, Faculty of Horticulture, Recep Tayyip Erdogan University, Pazar, Rize 53800, Turkey; arzu.karatas@erdogan.edu.tr

<sup>3</sup> Department of Plant and Production Technologies, Faculty of Applied Sciences, Mus Alparslan University, Mus 49250, Turkey; f.islek@alparslan.edu.tr

<sup>4</sup> Institute of Natural and Applied Sciences, Yuzuncu Yil University, Van 65080, Turkey; nrnylmz47@gmail.com

<sup>5</sup> Department of Organic Agriculture, Baskale Vocational School, Yuzuncu Yil University, Baskale, Van 65140, Turkey; selmakipcak@yyu.edu.tr

<sup>6</sup> Department of Horticulture, Faculty of Agriculture, Ataturk University, Erzurum 25240, Turkey; sercisli@gmail.com

<sup>7</sup> Department of Food Analysis and Chemistry, Faculty of Technology, Tomas Bata University in Zlin, Vavreckova 275, CZ-76001 Zlin, Czech Republic; skrovankova@utb.cz (S.S.); mlcek@utb.cz (J.M.)

\* Correspondence: aadamkova@utb.cz; Tel.: +420-576-033-030



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**Abstract:** Peppers are cultivated in almost all provinces in Turkey, making up approximately 7% of the world's production. In this study, the effects of pre-harvest cytokinin application on the postharvest physiology of pepper (*Capsicum annuum* L.) fruits (cv. Akra and Melek F1) grown in ecological conditions in Turkey (Van, eastern Turkey) were investigated. During the growing period, organic cytokinin (nytrozine) at two concentrations (50 and 100 ppm doses) was applied by spraying. Peppers were harvested in green maturity and then stored at 12 °C for 28 days. Changes in color, respiration rate, exogenous ethylene amount, antioxidative enzyme analysis (SOD, CAT and APX), lipid peroxidation, total phenolic content (TP) and antioxidant activity (AA) were determined just after the harvest and then at the 7th, 14th, 24th and 28th days. L\* values for both varieties and the control sample had fluctuations during storage, and they slightly decreased after 28 days, except for the end value of cv. Melek F1 with a 50 ppm addition. In terms of respiration rate values, the difference between applications was found to be statistically significant, except for the 14th and 24th days of storage, for cv. Akra F1; and the 0th and 28th days for cv. Melek F1, respectively. It was observed that cytokinin can delay aging in peppers of both cultivars stored for 28 days. As a result, as the used cytokinin has an organic origin, is not synthetic and does not have a negative effect on human health, it can be used safely and without a significant loss of quality characteristics for peppers. These applications have positive effects on in-package gas composition, antioxidative enzymes, lipid peroxidation, total phenolics and total antioxidant activity.

**Keywords:** pepper; storage; cytokinin; antioxidative enzyme; respiration; bioactive content

## 1. Introduction

Horticulture is practiced across cool temperate to tropical latitudes and over a wide range of elevations and climatic conditions. Horticultural products include all products, raw or processed, that arise from the horticultural industry. Horticulture plants, including pepper, have recently gained popularity [1–3]. They include a high content of non-nutritive, nutritive and bioactive compounds such as flavonoids, phenolics, anthocyanins and phenolic acids, as well as nutritive compounds such as sugars, essential oils, carotenoids,

vitamins and minerals. They also have a distinct flavor and taste, excellent medicinal value and health care functions [4,5].

Peppers (*Capsicum annuum* L.) belong to the *Capsicum* genus of the Solanaceae family. They have a different fruit color, from yellow to green and red, and are preferred due to their sensory qualities (taste, aroma and color) [1,6]. They are mainly used for fresh consumption, in salads, and also in cooked foods [3,7]. Peppers belong to the crops that have an important place in human nutrition as a good source of essential vitamins and many beneficial antioxidants, such as carotenoids, flavonoids and phenolic acids [8,9]. The Akra and Melek F1 hybrid pepper varieties have been very popular lately and the object of a wide economic growth in Turkey.

In recent years, the demand for pepper production has increased due to its commercial value and great market usage worldwide [10]. Turkey, with a production of about 2.5 million tons of peppers, is the third greatest producer in the world, after China and Mexico [11].

Changes in physiological activities, the loss of nutrients and a rapid physical and physiological decomposition are common post-harvest problems for the pepper market [12], with softening, drying and fungal spoilage as the main difficulties [13].

Methods that are effective in reducing weight loss in peppers, due to water loss, use a stretch film [14], a modified atmosphere packaging (MAP) [15], a controlled atmosphere (CA) storage [16] and a new system, Palistore (the storage of palletized goods under CA) [17]. Post-harvest studies on peppers proved that MAP is an effective way of maintaining the quality and extending the storage life of plant-based products [18,19].

Today, one of the greatest challenges faced by the agriculture and food industries is developing sustainable and environmentally friendly systems to meet the nutritional demand of the continuously growing global population. One of the solutions that would enable producers to increase crop yields and protect produce is the implementation of eco-friendly treatments [20–23].

Cytokinin, an essential plant hormone, has both organic and synthetic forms which are known to have an important effect on preventing post-harvest aging. The external application of cytokinin to the plant causes a delay in aging, the breaking of apical dormancy, an increase of chlorophyll production and an encouragement to chloroplast development, a decrease in chlorophyll degradation, the support of protein and nucleic acid synthesis, the transport of nutrients to the area where the cytokine is applied and the maintenance of photosynthetic activity [24]. Moreover, gibberellins, belonging to a major class of plant hormones, and cytokinin have been reported to be effective in plant development and maturation [25]. It was also reported that post-harvest 6-benzylaminopurine (BAP) application preserves product quality, prevents rotting and extends shelf life [26].

The aim of this study is to determine the effect of pre-harvest cytokinin applications (50 and 100 ppm doses) on two hybrid pepper cultivars (cv. Akra F1 and cv. Melek F1), grown at field conditions in eastern Turkey (Van province) and to reveal the impact on physical and biochemical changes during storage.

## 2. Materials and Methods

### 2.1. Material

Green peppers for analyses were planted from the seedlings of two hybrid pepper cultivars (cv. Akra F1 and cv. Melek F1) in an unheated glass greenhouse of Van Yüzüncü Yıl University (eastern Turkey) at a row distance of 30 × 30 cm. Pepper plants were irrigated with a drip irrigation system. When the peppers reached the first fruit set stage, pre-harvest organic cytokinin (nytrozine) was applied. Control (0 ppm), 50 ppm and 100 ppm doses of cytokinin were sprayed on the peppers. The application has been made at sunset to prevent cytokinin from being affected by sunlight. Cytokinin doses are based on previous studies of vegetables [27].

Green peppers were harvested (2 kg of each cultivar) and kept at room conditions (18 ± 2 °C) for 24 h; the internal temperature of the pepper fruits was reduced, and then

the fruits were placed on a foam plate, covered with stretch foil and stored for 28 days in a cold place at 12 °C and 90–95% relative humidity.

## 2.2. Methods

All analyses were performed in three replications, and at five storage times (freshly harvested (0 day), 7th, 14th, 24th and 28th day).

### 2.2.1. Color

The changes in the color values of the peppers were determined by a Minolta CR-400 brand colorimeter. The results are expressed as  $L^*$ ,  $a^*$ ,  $b^*$  C and Hue angle value. Colors represent the color values,  $a^*$  (+red,−green),  $b^*$  (+yellow,−blue) and  $L^*$  (brightness).

### 2.2.2. Respiratory Rate

For the evaluation of the respiration rates, the pepper fruits were placed in gas-tight containers, and the amount of  $\text{CO}_2$  given to the environment at the end of 2 h was determined with the Headspace Gas Analyzer GS3/L analyzer device (Systech Inst., Gaspac Advance, GS3/L; Johnsburg, IL, USA). Respiratory rate values were calculated using the weight and volume values [27].

### 2.2.3. Exogenous Ethylene Content

In the determination of the exogenous ethylene amount of the pepper fruits, the samples were placed in a gas-tight container and, after a 2-h waiting period, the gas samples were injected into the Gas Chromatography (GC) device (Shimadzu, Kyoto, Japan) equipped with a flame ionization detector (FID) and a stainless Restek Rt-Alumina BOND/MAPD 50m, 0.53mm ID, filled with activated alumina. The temperatures of the oven and FID detectors were 40 °C (isothermal) and 250 °C, respectively. The gas flows for high-purity hydrogen ( $\text{H}_2$ ) and dry air used as carrier gas in FID were 30 and 400  $\text{mL min}^{-1}$ , respectively. The formula used for calculating the respiratory rate was modified [27] and calculated as  $\text{ml C}_2\text{H}_4 \text{ kg}^{-1}$ .

### 2.2.4. In-Package Gas Composition

The  $\text{CO}_2$  and  $\text{O}_2$  gas levels in the packages of peppers were determined with the Headspace Gas Analyzer GS3/L device (Johnsburg, IL, USA) Missing manufacturer's brand, producer, conditions and unit expression).

### 2.2.5. Antioxidative Enzyme Analysis (SOD, CAT and APX)

SOD activity was determined by the inhibition of nitroblue tetrazolium (NBT) at 560 nm wavelength [24] (Thermo Scientific Genesys 10S Model UV-VIS spectrophotometer Waltham, MA, USA). For the determination of CAT activity, 0 and 60 s readings were taken at a 240 nm wavelength in the spectrophotometer. The evaluation was made considering the change in absorbance within 1 min [24] (Evolution™ 201/220 UV-Visible Spectrophotometers, USA). APX activity was measured by the reduction of the  $\text{H}_2\text{O}_2$  bound to ascorbic acid at a 290 nm wavelength. At 0 and 60 s, readings were taken at a 290 nm wavelength in the spectrophotometer (Thermo Scientific Genesys 10S Model, Waltham, MA, USA). This was performed considering the change in absorbance within 1 min [28].

### 2.2.6. Lipid Peroxidation

Lipid peroxidation in plants is expressed as malondialdehyde (MDA) content. The MDA content of the pepper samples was determined by reading the absorbance values at 532 and 600 nm wavelengths [29] (Thermo Scientific Genesys 10S Model, Madison, WI, USA).

### 2.2.7. Total Phenolic Content and Antioxidant Activity

The total phenolic content (TP) of peppers was determined spectrophotometrically (Thermo Scientific Genesys 10S Model, Waltham, MA, USA) using the Folin–Ciocalteu colorimetric method [30]. The absorbances of the solutions were read at a wavelength of 700 nm by the spectrophotometer, with gallic acid as a standard. The amount of TP was expressed as gallic acid equivalents (GAE) at  $\text{mg } 100 \text{ g}^{-1}$  fresh weight (FW).

To determine the antioxidant activity (AA), the FRAP (Ferric Reducing Antioxidant Power) method was used [31]. Absorbances of the solutions were read at a wavelength of 593 nm by the spectrophotometer (Thermo Scientific Genesys 10S Model UV-VIS spectrophotometer, Waltham, MA, USA). AA values were given due to the Trolox standard, as  $\mu\text{mol trolox equivalent (TE) g}^{-1}$ .

### 2.2.8. Statistical Analysis

Descriptive statistics for the traits were expressed as average and standard deviations. A one-way analysis of variance was performed in factorial order to determine whether there was a difference between applications and storage times. Following the variance analysis, a Duncan test was used to determine the difference between the mean of applications and storage times. The statistical significance level was taken as 5% in the calculations, and the “SPSS version 13.0” statistics package program was used for the calculations.

## 3. Results and Discussion

### 3.1. Color

The color of peppers is one of the most important parameters for consumer acceptability that also affects their marketability [17,32]. Color values are expressed as  $L^*$ ,  $a^*$ ,  $b^*$ ,  $C$  and Hue angle value. The color parameter  $L^*$  ranges from 0 up to 100; and when it approaches 100, the brightness increases. Hue is the color quality that distinguishes one color from another: a hue angle of  $0^\circ$  indicates red,  $90^\circ$  yellow,  $180^\circ$  green and  $270^\circ$  blue. The chroma value expresses the vividness and dullness of fruits and vegetables. A low chroma value indicates a dull color, while an increase in this value indicates a vivid color.

The changes in color values of cv. Akra F1 and cv. Melek F1 pepper cultivars after cytokinin application during 28 days storage were examined and are shown in Table 1. It was determined that the  $L^*$  values for both varieties and the control sample had fluctuations during storage, and they slightly decreased after 28 days, except for the end value of cv. Melek F1 with 50 ppm addition. Çavuşoğlu and Gökçenay [17] stated that the  $L^*$  value of mushrooms treated with cytokinin was better than that of the control; however, they attributed this to tissue aging. Color change is closely related to the ripening process, and is one of the indicators of physical and chemical development stages [9,33,34]. In terms of the  $L^*$  value results (Table 1), there were similarities with other studies; as for bananas [25], olives [35] and mushrooms [17], the  $L^*$  color value of cv. Akra F1 was found to be statistically significant in terms of storage times except for the 0th and 28th days of storage, while for cv. Melek F1 they were found to be statistically significant only at the 28th day of storage. The difference between both cultivars in terms of all application and storage times was found to be statistically significant (Table 1).

**Table 1.** Changes in the colors ( $L^*$ ,  $a^*$ ,  $b^*$ ,  $C^\circ$  and  $H^\circ$ ) of pepper fruits cultivars (cv. Akra F1 and cv. Melek F1) treated with two concentrations of cytokinin during storage.

	Storage Period (Day)	cv. Akra F1			cv. Melek F1		
		Control	50 ppm	100 ppm	Control	50 ppm	100 ppm
$L^*$	0	51.90 ± 0.59 A ab #	47.80 ± 0.23 B a #	47.73 ± 0.45 B a #	65.42 ± 0.23 A a	65.11 ± 0.65 A ab	66.10 ± 0.04 A a
	7	47.77 ± 0.89 A b #	46.29 ± 0.71 A a #	47.40 ± 0.84 A a #	62.99 ± 2.59 A a	62.66 ± 0.96 A b	64.18 ± 0.81 A ab
	14	52.30 ± 1.67 A a #	45.58 ± 0.75 B a #	46.15 ± 1.71 B ab #	64.18 ± 0.53 A a	64.39 ± 0.68 A b	63.21 ± 1.06 A b
	24	48.61 ± 1.79 A ab #	46.38 ± 1.12 AB a#	43.64 ± 1.06 B b #	65.23 ± 1.27 A a	62.97 ± 0.78 A b	64.96 ± 0.60 A ab
	28	48.43 ± 1.20 A ab #	47.09 ± 0.75 A a #	47.47 ± 0.99 A a #	65.26 ± 0.29 B	67.31 ± 0.78 A a	65.69 ± 0.224 AB a
Significant effects; P <sub>treatment</sub> : 0.001 P <sub>storage</sub> : 0.034 P <sub>treatment</sub> × P <sub>storage</sub> : 0.086 P <sub>treatment</sub> : 0.855 P <sub>storage</sub> : 0.007 P <sub>treatment</sub> × P <sub>storage</sub> : 0.438 Significant effects; P <sub>c</sub> : 0.001 P <sub>c</sub> × P <sub>treatment</sub> : 0.001 P <sub>c</sub> × P <sub>storage</sub> : 0.101 P <sub>c</sub> × P <sub>treatment</sub> × P <sub>storage</sub> : 0.201							
$a^*$	0	−20.72 ± 0.03 B b #	−19.30 ± 0.31 A b #	−19.06 ± 0.06 A b #	−16.89 ± 0.09 A b	−17.62 ± 0.29 A b	−17.30 ± 0.24 A b
	7	−18.25 ± 0.43 A a #	−19.23 ± 0.22 A b #	−18.89 ± 0.30 A b #	−15.64 ± 0.45 A ab	−16.47 ± 0.93 A b	−16.52 ± 0.20 A bc
	14	−18.82 ± 0.55 A a #	−17.59 ± 0.60 A a	−18.08 ± 0.75 A b #	−16.34 ± 0.34 A ab	−16.09 ± 0.24 A b	−15.63 ± 0.33 A ab
	24	−17.38 ± 0.72 A a	−17.88 ± 0.61 A a	−16.38 ± 0.12 A a	−15.35 ± 0.58 A a	−16.30 ± 0.25 A b	−15.67 ± 0.51 A ab
	28	−17.40 ± 0.44 A a #	−17.88 ± 0.21 A a #	−17.88 ± 0.18 A b #	−15.49 ± 0.50 B ab	−14.10 ± 0.12 A a	−15.03 ± 0.28 AB a
Significant effects; P <sub>treatment</sub> : 0.253 P <sub>storage</sub> : 0.001 P <sub>treatment</sub> × P <sub>storage</sub> : 0.001 P <sub>treatment</sub> : 0.799 P <sub>storage</sub> : 0.001 P <sub>treatment</sub> × P <sub>storage</sub> : 0.126 Significant effects; P <sub>c</sub> : 0.001 P <sub>c</sub> × P <sub>treatment</sub> : 0.361 P <sub>c</sub> × P <sub>storage</sub> : 0.061 P <sub>c</sub> × P <sub>treatment</sub> × P <sub>storage</sub> : 0.075							
$b^*$	0	40.38 ± 0.78 A a	34.70 ± 0.61 B ab #	33.76 ± 0.19 B b #	40.32 ± 0.23 B a	42.36 ± 0.27 A a	40.91 ± 0.59 B a
	7	38.59 ± 0.55 A a	33.73 ± 0.48 B b #	34.60 ± 0.55 B ab #	37.36 ± 0.74 A b	39.38 ± 1.62 A bc	38.57 ± 0.60 A b
	14	39.70 ± 1.52 A a	34.64 ± 0.93 B ab #	34.83 ± 1.01 B ab	38.71 ± 0.84 A ab	39.40 ± 0.99 A bc	37.05 ± 0.99 A b
	24	38.50 ± 1.38 A a	37.30 ± 1.04 A a	33.07 ± 1.03 B b #	38.04 ± 0.93 A ab	40.28 ± 0.34 A ab	38.46 ± 0.54 A b
	28	39.56 ± 1.27 A a	36.11 ± 1.55 A ab	36.32 ± 0.51 A a	38.65 ± 0.76 A ab	36.92 ± 0.18 A c	37.43 ± 0.35 A b
Significant effects; P <sub>treatment</sub> : 0.001 P <sub>storage</sub> : 0.347 P <sub>treatment</sub> × P <sub>storage</sub> : 0.179 P <sub>treatment</sub> : 0.040 P <sub>storage</sub> : 0.001 P <sub>treatment</sub> × P <sub>storage</sub> : 0.173 Significant effects; P <sub>c</sub> : 0.001 P <sub>c</sub> × P <sub>treatment</sub> : 0.001 P <sub>c</sub> × P <sub>storage</sub> : 0.001 P <sub>c</sub> × P <sub>treatment</sub> × P <sub>storage</sub> : 0.169							
$C^*$	0	45.41 ± 0.70 A a	39.87 ± 0.59 B a #	38.78 ± 0.13 B a #	43.72 ± 0.25 B a	45.89 ± 0.31 A a	44.43 ± 0.64 AB a
	7	42.75 ± 0.65 A a	38.83 ± 0.51 B a	39.44 ± 0.62 B a #	40.51 ± 0.86 A b	42.69 ± 1.85 A b	41.97 ± 0.62 A b
	14	43.96 ± 1.49 A a	38.90 ± 1.06 B a	39.28 ± 1.22 B a	41.93 ± 0.87 A ab	42.57 ± 1.00 A b	40.22 ± 1.03 A b
	24	42.29 ± 1.54 A a	41.37 ± 1.19 A a	37.00 ± 0.97 B a #	41.03 ± 1.08 A ab	43.46 ± 0.40 A ab	41.55 ± 0.69 A b
	28	43.34 ± 1.08 A a	40.33 ± 1.40 A a	28.49 ± 1.30 B a	41.65 ± 0.89 A ab	39.53 ± 0.20 B c	40.36 ± 0.43 AB b
Significant effects; P <sub>treatment</sub> : 0.010 P <sub>storage</sub> : 0.643 P <sub>treatment</sub> × P <sub>storage</sub> : 0.631 P <sub>treatment</sub> : 0.080 P <sub>storage</sub> : 0.001 P <sub>treatment</sub> × P <sub>storage</sub> : 0.158 Significant effects; P <sub>c</sub> : 0.021 P <sub>c</sub> × P <sub>treatment</sub> : 0.008 P <sub>c</sub> × P <sub>storage</sub> : 0.878 P <sub>c</sub> × P <sub>treatment</sub> × P <sub>storage</sub> : 0.545							

Table 1. Cont.

		cv. Akra F1			cv. Melek F1		
<b>Hue Angle</b>	0	117.2 ± 0.42 B a #	119.5 ± 0.29 A a #	119.5 ± 0.19 A a #	112.7 ± 0.03 A a	112.5 ± 0.28 A a	112.8 ± 0.02 A b
	7	115.0 ± 0.37 C b #	119.7 ± 0.18 A a #	118.7 ± 0.13 B a #	112.5 ± 0.26 A a	112.6 ± 0.36 A a	113.1 ± 0.19 A a
	14	115.4 ± 0.76 B ab #	116.76 ± 0.39 AB b #	117.5 ± 0.40 A ab #	112.9 ± 0.09 A a	112.1 ± 0.16 A a	112.8 ± 0.18 A b
	24	114.2 ± 0.46 A b #	115.6 ± 0.29 A b #	116.1 ± 1.50 A b	111.9 ± 0.26 A b	112.0 ± 0.164 A a	112.1 ± 0.42 A b
	28	113.7 ± 0.85 B b	116.5 ± 0.97 A b #	116.2 ± 0.30 A b #	111.7 ± 0.24 A b	110.86 ± 0.15 A b	111.8 ± 0.19 A c
Significant effects; P <sub>treatment</sub> : 0.001 P <sub>storage</sub> : 0.001 P <sub>treatment × storage</sub> : 0.309 P <sub>treatment</sub> : 0.004 P <sub>storage</sub> : 0.001 P <sub>treatment × storage</sub> : 0.313							
Significant effects; P <sub>c</sub> : 0.001 P <sub>c × treatment</sub> : 0.001 P <sub>c × storage</sub> : 0.001 P <sub>c × treatment × storage</sub> : 0.438							

A, B, C →: The difference between applications that have different capital letters in the same row (same cultivar and storage time) is significant ( $p < 0.05$ ). a, b, c ↓: The difference between storage times that take different lower-case letters in the same column (same cultivar and application) is significant ( $p < 0.05$ ). #: The difference from cv. Melek F1 in the same application and storage time is significant ( $p < 0.05$ ).

The  $a^*$  values of both pepper cultivars and the control were found to increase, again with fluctuations. It was seen that the  $a^*$  color values in the samples treated with cytokinin were lower compared to the control. The highest  $a^*$  values were especially near the end of storage (24th or 28th day), and higher for cv. Melek F1, while the highest was for the 50 ppm cytokinin application. In several studies about broccoli, it was determined that the samples treated with cytokinin preserved the green color better. It was found that the application of cytokinin was related to the delay of chlorophyll degradation [24,36,37]. As for the  $a^*$  color value in this study, there were significant variances in terms of the difference between applications for the same variety and storage time for the 0th day of storage in cv. Akra F1 and the 28th day of storage in cv. Melek F1. The values of cv. Akra F1 were significantly different from those of cv. Melek F1, except for the 14th and 24th days of storage after a 50 ppm cytokine application, and the 24th day of storage after a 50 ppm cytokine application (Table 1).

As for  $b^*$  color values during storage, we found an increase with fluctuations for cv. Akra F1, except for the control group, but there was a decrease with fluctuations for Melek F1 during 28 days of storage. There was a difference in  $b^*$  values between cv. Akra F1 and cv. Melek F1. Statistically,  $b^*$  values were found to be significantly different for samples with applications in the storage period, except for the 28th day of storage in cv. Akra F1 and the 0th day in cv. Melek F1. The obtained data were similar to Al-Qurashi and Awad's [25] study on bananas.

As the chroma value indicates the vividness and dullness of the fruit or skin color, it has been found that the chroma value will be low in dull colors and high in vivid colors [38]. Chroma values in peppers were found to decrease during storage in both cultivars, except in the end of storage for cv. Akra F1 with 50 ppm addition. The applications of cytokinin thus slowed the pepper maturation. Moreover, a slight fluctuation was observed. Especially for some cultivars, the increase in the chroma value is seen as a typical ripening event [39,40]. All storage times for cv. Akra F1, and the 0th and 28th days of storage in cv. Melek F1 were found to be statistically significant in terms of differences between applications. The difference between the storage of cv. Akra F1 on the 0th day in the application of 50 ppm cytokinin and the 0th, 7th and 24th days in the cytokinin application of 100 ppm from cv. Melek F1 was found to be statistically significant (Table 1).

While the hue value expresses the angle of the line passing through the point where the  $a^*$  and  $b^*$  color values intersect the X axis, it is known that the angle corresponds to red when  $0^\circ$ , yellow when  $90^\circ$ , green when  $180^\circ$  and blue when  $270^\circ$ . Hue color values for peppers were found to decrease during storage for both cultivars. The hue values of the samples treated with cytokinin, compared to the control, were higher for both pepper cultivars. The aging of the control group samples is faster than that of the samples treated with cytokinin. The results obtained in the present study are similar to those of Downs et al. [37] for broccoli and Tsantili et al. [35] for olives. For cv. Akra F1, the difference between applications in storage periods, except for the 24th day of storage, was found to be statistically significant. The difference between the applications was not statistically significant in cv. Melek F1. For cv. Akra F1, there were significant differences from cv. Melek F1, except for the 24th day of storage, at 100 ppm cytokine addition (Table 1).

### 3.2. Respiratory Rate

As for the significance of respiration on the shelf life, Lee et al. [41] pointed out that there exists an inverse relationship between the respiration rate and shelf life. In our study, there were differences in the respiration rate of pepper fruits belonging to both cultivars (cv. Akra F1 and cv. Melek F1) during the storage periods (Table 2).

**Table 2.** Changes in respiration rate and exogenous ethylene amount of pepper fruit cultivars (cv. Akra F1 and cv. Melek F1) treated with two concentrations of cytokinin during storage.

	Storage Period (Day)	Akra F1			Melek F1		
		Control	50 ppm	100 ppm	Control	50 ppm	100 ppm
Respiration Rate (mL CO <sub>2</sub> /kgh)	0	111.2 ± 1.78 A c #	96.6 ± 1.08 B b #	56.8 ± 0.00 C c #	88.3 ± 3.03 B a	118.8 ± 2.85 A a	81.5 ± 2.85 B ab
	7	139.6 ± 6.18 A bc #	127.8 ± 3.66 A b #	90.7 ± 9.83 B b #	48.6 ± 3.85 A b	64.1 ± 5.28 A b	57.2 ± 7.07 A b
	14	198.9 ± 14.00 A a #	188.1 ± 16.98 A a #	166.0 ± 8.45 A a #	73.8 ± 2.33 A a	99.6 ± 18.97 A a	89.7 ± 4.18 A a
	24	164.8 ± 28.26 A ab #	128.8 ± 13.44 A b #	103.5 ± 6.72 A b	53.0 ± 10.07 A b	66.15 ± 10.30 A b	56.75 ± 19.31 A b
	28	158.5 ± 6.55 A abc #	102.9 ± 5.70 B b	100.2 ± 6.64 B b #	79.3 ± 5.28 A a	60.3 ± 3.35 B b	57.7 ± 3.59 B b
Significant effects; P <sub>treatment</sub> : 0.001 P <sub>storage</sub> : 0.001 P <sub>treatment × storage</sub> : 0.045 P <sub>treatment</sub> : 0.033 P <sub>storage</sub> : 0.001 P <sub>treatment × storage</sub> : 0.136 Significant effects; P <sub>c</sub> : 0.001 P <sub>c × treatment</sub> : 0.001 P <sub>c × storage</sub> : 0.001 P <sub>c × treatment × storage</sub> : 0.781							
Exogenous Ethylene Amount (mL C <sub>2</sub> H <sub>4</sub> /kgh)	0	0.121 ± 0.01 A ab	0.046 ± 0.02 B b #	0.012 ± 0.00 C b	0.088 ± 0.02 A ab	0.103 ± 0.01 A a	0.019 ± 0.00 B c
	7	0.092 ± 0.01 A b	0.086 ± 0.03 A b	0.057 ± 0.01 A ab #	0.058 ± 0.03 A ab	0.107 ± 0.04 A a	0.144 ± 0.00 A a
	14	0.254 ± 0.09 A a	0.293 ± 0.08 A a #	0.084 ± 0.04 A ab	0.080 ± 0.02 A ab	0.046 ± 0.00 AB ab	0.007 ± 0.01 B c
	24	0.045 ± 0.03 A b	0.110 ± 0.06 A b	0.147 ± 0.05 A a	0.030 ± 0.01 A b	0.023 ± 0.01 A b	0.039 ± 0.01 A b
	28	0.072 ± 0.02 A b	0.079 ± 0.01 A b	0.131 ± 0.05 A ab	0.108 ± 0.01 A a	0.062 ± 0.02 B ab	0.043 ± 0.0q B b
Significant effects; P <sub>treatment</sub> : 0.392 P <sub>storage</sub> : 0.002 P <sub>treatment × storage</sub> : 0.050 P <sub>treatment</sub> : 0.130 P <sub>storage</sub> : 0.001 P <sub>treatment × storage</sub> : 0.001 Significant effects; P <sub>c</sub> : 0.001 P <sub>c × treatment</sub> : 0.835 P <sub>c × storage</sub> : 0.001 P <sub>c × treatment × storage</sub> : 0.052							

A, B, C →: The difference between applications that have different capital letters in the same row (same variety and storage time) is significant ( $p < 0.05$ ). a, b, c ↓: The difference between storage times that take different lower-case letters in the same column (same type and application) is significant ( $p < 0.05$ ). #: The difference from cv. Melek F1 in the same application and storage time is significant ( $p < 0.05$ ).



The general trend for the respiration rate of cv. Akra F1 was an increase up to the 14th day of storage, and then some fluctuations have been seen, with the final value at the 28th day higher than the default value. For the Melek F1 cultivar there were fluctuations in the respiration rate during storage, with a decreased value at the end of storage. In terms of respiration rate values, the difference between applications was found to be statistically significant, except for the 14th and 24th days of storage, for cv. Akra F1; and 0 and for the 28th day for cv. Melek F1, respectively. The difference among the storage days of cv. Akra F1 compared with cv. Melek F1 were found to be statistically significant for all applications, except for the 28th day with the application of 50 ppm cytokinin, and for the 24th day with the application of 100 ppm cytokinin (Table 2). Respiratory rate results are similar with those of the studies performed by Çavuşoğlu [27] for cauliflower and Costa et al. [42] for broccoli, in which different doses of cytokinin were applied before harvest. Koide and Shi [43] reported that the respiration rate of green peppers increased during storage.

There is an aim to reduce weight loss, respiration rate and ethylene production in products, prevent blackening, delay maturation and softening, and prevent pathogens and physiological disorders by providing low O<sub>2</sub> and high CO<sub>2</sub> in the environment with MAP applications to fruits and vegetables [44–46]. In cases where there is no correlation between the respiratory rate of the product in the MAP and the permeability of the packaging, anaerobic respiration and ethyl alcohol accumulation occur in parallel with the increase in the amount of CO<sub>2</sub> in the environment [47]. It is a situation that negatively affects the quality of the products, leading to the loss of taste and to deterioration. The respiration rate in fresh fruits and vegetables is often seen as a good criterion for determining the storage life of the product [17].

### 3.3. Exogenous Amount of Ethylene

Ethylene is one of the few plant growth regulators that affect growth and development processes, including maturation and aging [48]. Therefore, the prevention of ethylene biosynthesis, or its effect, can play an important role in delaying aging.

In our study, fluctuations in the amount of exogenous ethylene, manifesting decreases and increases during storage, occurred for both pepper cultivars (Table 2). For cv. Akra F1 there were higher exogenous ethylene amounts at the end of storage for both cytokinin applications, and the highest value was after 100 ppm addition. Fluctuations of the exogenous ethylene amount were observed during the storage for cv. Melek F1. Statistical significance, in terms of cytokinin applications, was found for fresh peppers of cv. Akra F1, and for the 0th, 14th and 28th days of storage of cv. Melek F1. The difference among the storage days of cv. Akra F1 compared with cv. Melek F1 on the 0th and 14th days with the application of 50 ppm cytokinin, and on the 7th day of storage after the application of 100 ppm cytokinin, were found to be statistically significant (Table 2). In the present study, the application of cytokinin generally increased ethylene production, as seen in the amount of exogenous ethylene. Exogenous ethylene results are similar with those of the studies using different doses of cytokinin before harvest, performed by Çavuşoğlu [27] for cauliflower and Costa et al. [42] for broccoli.

### 3.4. In-Package Gas Composition

In pepper cultivars, there has been a decrease in the oxygen (O<sub>2</sub>) value and an increase of carbon dioxide (CO<sub>2</sub>) for the in-package gas components during storage. At the end of storage, we found the lowest oxygen level and the highest carbon dioxide value after the application of 50 ppm cytokinin for cv. Akra F1, and after the application of 100 ppm cytokinin for cv. Melek F1, respectively (Table 3). Therefore, a decrease in oxygen and an increase in carbon dioxide during storage after cytokinin applications in both pepper cultivars in MAP had positive effects on the products in MAP by reducing the amount of O<sub>2</sub> in the package and increasing the CO<sub>2</sub> amount. When the changes in the oxygen and carbon dioxide values were statistically examined, the difference between the applications was found to be significant.

**Table 3.** Changes in the in-package oxygen (O<sub>2</sub>) and carbon dioxide (CO<sub>2</sub>) values of pepper fruits cultivars (cv. Akra F1 and cv. Melek F1) treated with two concentrations of cytokinin during storage.

	Storage Period (Day)	Akra F1			Melek F1		
		Control	50 ppm	100 ppm	Control	50 ppm	100 ppm
% O <sub>2</sub>	0	20.90 ± 0.00 A a	20.90 ± 0.00 A a	20.90 ± 0.00 A a	20.90 ± 0.00 A a	20.90 ± 0.00 A a	20.90 ± 0.00 A a
	7	19.60 ± 0.06 A bc #	17.80 ± 0.32 B bc	17.80 ± 0.77 B bc	18.13 ± 0.23 AB bc	18.70 ± 0.20 A b	17.80 ± 0.10 B bc
	14	19.00 ± 0.15 A c	17.26 ± 0.41 B c	16.46 ± 0.42 B c #	17.60 ± 0.60 A c	18.23 ± 0.18 A b	18.13 ± 0.18 A b
	24	19.66 ± 0.35 A bc #	18.46 ± 0.29 A b	16.53 ± 0.49 B c	18.16 ± 0.18 A bc	19.20 ± 0.86 A b	17.60 ± 0.15 A c
	28	20.16 ± 0.37 A ab #	18.33 ± 0.29 A b	18.90 ± 1.00 A ab	18.80 ± 0.11 A b	18.60 ± 0.06 AB b	18.10 ± 0.23 B bc
Significant effects; P <sub>treatment</sub> : 0.001 P <sub>storage</sub> : 0.001 P <sub>treatment</sub> × P <sub>storage</sub> : 0.022 P <sub>treatment</sub> : 0.011 P <sub>storage</sub> : 0.001 P <sub>treatment</sub> × P <sub>storage</sub> : 0.155 Significant effects; P <sub>c</sub> : 0.649 P <sub>c</sub> × P <sub>treatment</sub> : 0.001 P <sub>c</sub> × P <sub>storage</sub> : 0.184 P <sub>c</sub> × P <sub>treatment</sub> × P <sub>storage</sub> : 0.066							
% CO <sub>2</sub>	0	0.30 ± 0.00 A d	0.30 ± 0.00 A d	0.30 ± 0.00 A c	0.30 ± 0.00 A b	0.30 ± 0.00 A c	0.30 ± 0.00 A c
	7	0.80 ± 0.00 B b #	1.20 ± 0.06 A b #	1.26 ± 0.08 A ab	1.10 ± 0.06 AB a	0.96 ± 0.03 B a	1.16 ± 0.07 A a
	14	0.93 ± 0.07 B a	1.40 ± 0.06 A a #	1.50 ± 0.00 A a #	1.23 ± 0.14 A a	1.10 ± 0.06 A a	1.23 ± 0.08 A a
	24	0.60 ± 0.00 B c #	0.86 ± 0.08 B c	1.40 ± 0.20 A a	0.93 ± 0.08 A a	0.63 ± 0.12 B b	0.96 ± 0.03 A b
	28	0.73 ± 0.03 A b	1.03 ± 0.03 A bc #	0.93 ± 0.22 A b	0.93 ± 0.08 A a	0.90 ± 0.00 A a	1.10 ± 0.06 A ab
Significant effects; P <sub>treatment</sub> : 0.001 P <sub>storage</sub> : 0.001 P <sub>treatment</sub> × P <sub>storage</sub> : 0.003 P <sub>treatment</sub> : 0.002 P <sub>storage</sub> : 0.001 P <sub>treatment</sub> × P <sub>storage</sub> : 0.393 Significant effects; P <sub>c</sub> : 0.369 P <sub>c</sub> × P <sub>treatment</sub> : 0.001 P <sub>c</sub> × P <sub>storage</sub> : 0.275 P <sub>c</sub> × P <sub>treatment</sub> × P <sub>storage</sub> : 0.015							

A, B, C →: The difference between applications that have different capital letters in the same row (same variety and storage time) is significant ( $p < 0.05$ ). a, b, c ↓: The difference between storage times that take different lower-case letters in the same column (same type and application) is significant ( $p < 0.05$ ). #: The difference from cv. Melek F1 in the same application and storage time is significant ( $p < 0.05$ ).

### 3.5. Antioxidative Enzyme (SOD, CAT and APX) Assays

Antioxidants and phenolics in plants help effectively protect them from the oxidative damage caused by free radicals and reactive oxygen species by interacting with them and stabilizing free radicals [49,50]. The accumulation of reactive oxygen species results from the change in the balance between the production of reactive oxygen species and their elimination process, and it decreases the storage quality and marketability of vegetables [51,52]. The main scavengers for reactive oxygen species include antioxidant enzymes such as CAT, SOD, APX, GR and POX [53], as well as antioxidant compounds such as carotenoids, ascorbate, glutathione, etc. [54]. While SOD catalyzes the separation of the superoxide anion into  $H_2O_2$  and  $O_2$ , CAT and APX are reported to play a role in the elimination of  $H_2O_2$  from plant cells [55].

An increase in antioxidative enzymes (SOD, CAT and APX) was observed, comparing the beginning and the end of storage, for both pepper cultivars, with some fluctuations occurring during storage.

It was seen that the activity of the SOD enzyme increased in nearly all storage times. Higher SOD values were found for the Melek F1 pepper cultivar and for peppers after a 100 ppm cytokinin application. As for the SOD activity, in terms of the difference between applications, the 28th day of storage was found to be statistically significant in cv. Akra F1, while the difference between applications was not statistically significant in cv. Melek F1. The difference among the storage days of cv. Akra F1 compared with cv. Melek F1 on the 28th day with the application of 50 ppm cytokinin was found to be statistically significant (Table 4).

Some fluctuations in the activity of the CAT enzyme were observed. There were increases and decreases according to the applications and the day of storage. The growth was observed up to the 24th day, and then the values slightly decreased. At the end of storage, the highest CAT values were observed after a 50 ppm cytokinin application for both cultivars. While there was no statistically significant difference in the activity of the CAT enzyme among the applications in cv. Akra F1, the results for the 7th and 14th days of storage were statistically significant in cv. Melek F1. The difference among the storage days of cv. Akra F1 compared with cv. Melek F1 on the 14th, 24th and 28th days after the application of 100 ppm cytokinin was found to be significantly different (Table 4).

It was determined that the activity of the APX enzyme increased up to the 14th day for cv. Akra F1 and decreased afterwards, with higher values in the end for peppers with cytokinin application compared to fresh peppers after the harvest. Regular increases were observed for cv. Melek F1 during the storage periods. At the end of storage, it was determined that the highest APX values were found for cv. Melek F1, and for both cultivars after a 100 ppm cytokinin application. In terms of APX values, there were significant differences among the applications of both cultivars after all storage days, except for the fresh products (Table 4).

Considering the data examined in the present study, it was found that the SOD, CAT and APX values of both pepper cultivars were higher than those of the control cytokinin application. The results of SOD, CAT and APX are analogous to the results of Zhang et al. [56], who applied 6-benzylaminopurine (BAP) to litchi fruits.

**Table 4.** Changes in SOD, CAT, APX and MDA values of pepper fruits cultivars (cv. Akra F1 and cv. Melek F1) treated with two concentrations of cytokinin during storage.

	Storage Period (Day)	Akra F1			Melek F1		
		Control	50 ppm	100 ppm	Control	50 ppm	100 ppm
<b>SOD</b> Unit g <sup>-1</sup> FW	0	346 ± 48 A b	346 ± 48 A a	346 ± 48 A b	353 ± 11 A a	353 ± 11 A b	353 ± 11 A b
	7	445 ± 57 A ab	529 ± 108 A a	431 ± 127 A ab	322 ± 81 A a	479 ± 42 A b	550 ± 82 A ab
	14	841 ± 211 A ab	965 ± 274 A a	1197 ± 196 A ab	1109 ± 90 A a	1131 ± 231 A a	1185 ± 191 A ab
	24	962 ± 242 A a	1025 ± 523 A a	1304 ± 533 A a	1215 ± 671 A a	1272 ± 131 A a	1430 ± 302 A a
	28	690 ± 103 B ab	848 ± 68 AB a #	1064 ± 50 A ab	1144 ± 769 A	1331 ± 143 A a	1246 ± 538 A ab
Significant effects; P <sub>treatment</sub> : 0.372 P <sub>storage</sub> : 0.001 P <sub>treatment × storage</sub> : 0.986 P <sub>treatment</sub> : 0.827 P <sub>storage</sub> : 0.001 P <sub>treatment × storage</sub> : 1.000 Significant effects; P <sub>cv</sub> : 0.174 P <sub>cv × treatment</sub> : 0.925 P <sub>cv × storage</sub> : 0.754 P <sub>cv × treatment × storage</sub> : 0.999							
<b>CAT</b> mmol g <sup>-1</sup> FW	0	0.022 ± 0.017	0.022 ± 0.017	0.022 ± 0.017	0.025 ± 0.011 ab	0.025 ± 0.01	0.025 ± 0.02 b
	7	0.023 ± 0.001	0.024 ± 0.000	0.024 ± 0.021	0.040 ± 0.002 A ab	0.033 ± 0.04 AB	0.028 ± 0.01 B b
	14	0.056 ± 0.026	0.047 ± 0.018	0.032 ± 0.002 #	0.045 ± 0.006 B a	0.061 ± 0.000 A	0.049 ± 0.03AB a
	24	0.034 ± 0.002	0.063 ± 0.031	0.062 ± 0.002 #	0.027 ± 0.008 ab	0.04 ± 0.023	0.033 ± 0.003 b
	28	0.024 ± 0.003	0.049 ± 0.037	0.047 ± 0.006 #	0.019 ± 0.011 b	0.038 ± 0.007	0.026 ± 0.0002 b
Significant effects; P <sub>treatment</sub> : 0.729 P <sub>storage</sub> : 0.187 P <sub>treatment × storage</sub> : 0.928 P <sub>treatment</sub> : 0.209 P <sub>storage</sub> : 0.003 P <sub>treatment × storage</sub> : 0.786 Significant effects; P <sub>cv</sub> : 0.617 P <sub>cv × treatment</sub> : 0.944 P <sub>cv × storage</sub> : 0.314 P <sub>cv × treatment × storage</sub> : 0.976							
<b>APx</b> mmol g <sup>-1</sup> FW	0	1.92 ± 0.31 A c	1.92 ± 0.31 A c	1.912 ± 0.31 A c#	4.56 ± 0.92 A a	4.59 ± 0.92 A ab	4.59 ± 0.92 A b
	7	2.62 ± 0.30 C b	3.92 ± 0.16 B b #	5.39 ± 0.28 A b	2.02 ± 0.66 B b	2.24 ± 0.47 B c	4.96 ± 0.85 A b
	14	6.40 ± 0.12 B a#	7.73 ± 0.28 A a #	7.37 ± 0.57 A a	4.34 ± 0.52 B a	3.52 ± 0.25 B bc	6.77 ± 0.92 A ab
	24	1.26 ± 0.06 B d#	4.39 ± 0.84 A b	4.78 ± 0.81 A b #	5.26 ± 0.59 B a	5.86 ± 0.36 B a	7.26 ± 0.14 A a
	28	0.82 ± 0.10 B c#	3.28 ± 0.78 A bc#	4.09 ± 0.18 A b #	5.65 ± 0.34 B a	6.16 ± 0.68 B a	7.10 ± 1.02 A a
Significant effects; P <sub>treatment</sub> : 0.001 P <sub>storage</sub> : 0.001 P <sub>treatment × storage</sub> : 0.004 P <sub>treatment</sub> : 0.001 P <sub>storage</sub> : 0.001 P <sub>treatment × storage</sub> : 0.356 Significant effects; P <sub>cv</sub> : 0.001 P <sub>cv × treatment</sub> : 0.011 P <sub>cv × storage</sub> : 0.001 P <sub>cv × treatment × storage</sub> : 0.306							
<b>MDA</b> nmol g <sup>-1</sup> FW	0	3.42 ± 0.74 A b	3.42 ± 0.74 A b	3.42 ± 0.74 A b	2.32 ± 1.91 A a	2.32 ± 1.91 A a	2.32 ± 1.91 A a
	7	6.31 ± 0.44 A a	5.96 ± 0.28 A a #	5.56 ± 0.67 A ab	4.02 ± 0.92 A a	3.82 ± 0.32 A a	3.91 ± 0.13 A a
	14	7.39 ± 0.85 A a	6.78 ± 0.57 A a #	6.57 ± 0.59 A a #	4.71 ± 0.77 A a	4.12 ± 0.54 A a	4.42 ± 0.33 A a
	24	7.84 ± 0.62 Aa#	7.25 ± 0.53 A a #	7.15 ± 0.35 A a #	4.92 ± 0.14 A a	4.35 ± 0.56 A a	4.53 ± 0.57 A a
	28	8.97 ± 1.18 A a	7.94 ± 1.20 A a	7.71 ± 1.19 A a	5.75 ± 1.22 A	4.74 ± 0.11 A a	4.77 ± 1.19 A a
Significant effects; P <sub>treatment</sub> : 0.338 P <sub>storage</sub> : 0.001 P <sub>treatment × storage</sub> : 0.999 P <sub>treatment</sub> : 0.761 P <sub>storage</sub> : 0.031 P <sub>treatment × storage</sub> : 1.000 Significant effects; P <sub>cv</sub> : 0.001 P <sub>cv × treatment</sub> : 0.899 P <sub>cv × storage</sub> : 0.367 P <sub>cv × treatment × storage</sub> : 1.000							

A, B, C →: The difference between applications that have different capital letters in the same row (same variety and storage time) is significant ( $p < 0.05$ ). a, b, c ↓: The difference between storage times that take different lower-case letters in the same column (same type and application) is significant ( $p < 0.05$ ). #: The difference from cv. Melek F1 in the same application and storage time is significant ( $p < 0.05$ ).

### 3.6. Lipid Peroxidation

Conditions of aging and stress in plants, in different parts of the cells, increase the formation of reactive oxygen species (ROS), including superoxide radicals ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ) and hydroxyl radicals ( $OH^-$ ) [57,58]. In other words, it can be said that cytokinin contributes to the delay of the accumulation of reactive oxygen species in pepper fruits. The loss of membrane integrity causes the decomposition of enzymes and substrates, which initiates enzymatic blackening [59]. Membrane damage in plants is associated with lipid peroxidation caused by reactive oxygen species [60], while malondialdehyde (MDA) is a product of lipid peroxidation.

Regular increases of MDA values were observed for both pepper cultivars during storage. According to the results of the analyses, the highest MDA values were at the end of storage, with similar values for the products after application, and with higher values for the Akra F1 cultivar. The difference among cytokinin applications in cv. Akra F1 and cv. Melek F1 was not statistically significant. The differences among the storage days of cv. Akra F1 compared with cv. Melek F1 were on the 7th, 14th and 24th days after storage with a pre-harvest 50 ppm cytokinin application; and on the 14th and 24th days after a 100 ppm application (Table 4). It was observed that the samples treated with cytokinin in cv. Akra F1 and cv. Melek F1 showed a lower lipid peroxidation compared to the control. The results of MDA are in line with those of Zhang et al. [56], who applied 6-benzylaminopurine to litchi fruits.

### 3.7. Total Phenolic Content and Total Antioxidant Activity

Phenolic compounds are present in many types of fruits and vegetables, often in a large amount, and represent a wide range of chemical compounds. They are secondary metabolites that are effective in important functions in the plant's life cycle, act as antioxidants with free radical scavenging properties and have the ability to catalyze lipid peroxidation [61]. The content of phenolic compounds varies depending on the species, cultivar and environmental conditions [62–64].

The total phenolic content (TP) of both pepper cultivars had fluctuations throughout the storage, with a final increase at the end of storage. The higher phenolic content after the storage was determined for cv. Akra F1, and for a 50 ppm cytokinin application in both cultivars. These increases are thought to be due to the inhibition of catalase activity, which enables the synthesis of phenolic compounds and the stimulation of the phenylalanine lyase gene [65,66]. Plant antioxidants, including anthocyanins and phenolic substances, occur naturally in plants. Free radicals provide beneficial effects in fruits and vegetables [67].

Moreover, the total antioxidant activity (AA) increased at the end of storage in comparison to that of fresh products, with fluctuations during storage for both pepper cultivars. At the end of storage, higher AA values were found for cv. Akra F1, and for a 50 ppm cytokinin application in both pepper cultivars.

As for the total antioxidant activity, the storage periods, except for the 0th day, were significant for cv. Akra F1, while the results for storage periods in the 7th and 28th days were only significant for cv. Melek F1. The difference among the storage days of cv. Akra F1 compared with cv. Melek F1 on the 14th and 28th days with the application of 50 ppm cytokine were found to be statistically significant (Table 5). The difference between the applications in the 7th, 24th and 28th storage days of cv. Akra F1 and the 7th, 14th and 28th days in cv. Melek F1 was statistically significant.

**Table 5.** Changes in the total phenolic content (TP) and antioxidant activity (AA) of pepper fruits cultivars (cv. Akra F1 and cv. Melek F1) treated with two concentrations of cytokinin during storage.

	Storage Period (Day)	Akra F1			Melek F1		
		Control	50 ppm	100 ppm	Control	50 ppm	100 ppm
TP mg GAE/100 g	0	14.351 ± 4.86 A b	14.351 ± 4.86 A c	14.351 ± 4.86 A b	19.25 ± 3.72 A c	19.259 ± 3.72 A b	19.259 ± 3.72 A b
	7	5.890 ± 1.72 A b #	29.593 ± 3.14 A b	6.63 ± 3.14 B b	35.07 ± 7.70 AB ab	46.607 ± 7.88 A a	13.919 ± 6.93 B b
	14	13.111 ± 4.08 A b	7.82 ± 2.02 B c #	13.05 ± 3.14 A b	26.573 ± 4.48 A c	33.057 ± 2.88 A ab	21.012 ± 3.66 A b
	24	61.730 ± 1.27 A a #	51.634 ± 1.27 B a	49.627 ± 3.10 B a	48.476 ± 1.19 A a	42.509 ± 4.28 A a	46.034 ± 1.49 A a
	28	60.649 ± 2.07 A a #	57.868 ± 0.78Aa#	46.952 ± 2.15 B a	39.454 ± 1.73 AB ab	47.064 ± 2.42 A a	38.300 ± 2.64 B a
	Significant effects; P <sub>treatment</sub> : 0.014 P <sub>storage</sub> : 0.001 P <sub>treatment</sub> × P <sub>storage</sub> : 0.001 P <sub>treatment</sub> : 0.004 P <sub>storage</sub> : 0.001 P <sub>treatment</sub> × P <sub>storage</sub> : 0.020 Significant effects; P <sub>c</sub> : 0.027 P <sub>c</sub> × P <sub>treatment</sub> : 0.520 P <sub>c</sub> × P <sub>storage</sub> : 0.001 P <sub>c</sub> × P <sub>treatment</sub> × P <sub>storage</sub> : 0.057						
AA μmol TE/g	0	5.724 ± 0.39 A c	5.724 ± 0.39 A b	5.724 ± 0.39 A b	5.192 ± 0.76 A b	5.192 ± 0.76 A b	5.192 ± 0.76 A b
	7	2.535 ± 0.63 B d #	8.294 ± 1.09 A b	2.558 ± 0.46 B c	11.72 ± 3.19 AB a	15.468 ± 3.26 A a	4.868 ± 1.35 B b
	14	3.757 ± 0.74 A d	2.781 ± 0.85 Ac #	3.369 ± 1.19 A c	7.751 ± 1.32 AB ab	8.713 ± 0.78 A b	4.917 ± 0.99 B b
	24	18.919 ± 0.28 A a #	12.908 ± 1.08 B a	11.841 ± 0.75 B a	11.407 ± 0.53 A a	9.708 ± 1.38 A b	11.474 ± 0.27 A a
	28	15.045 ± 0.52 A b#	14.163 ± 0.39Aa#	10.332 ± 0.40 Ba #	7.796 ± 0.30 B ab	9.647 ± 0.66 A b	6.903 ± 0.45 B b
	Significant effects; P <sub>treatment</sub> : 0.001 P <sub>storage</sub> : 0.001 P <sub>treatment</sub> × P <sub>storage</sub> : 0.001 P <sub>treatment</sub> : 0.006 P <sub>storage</sub> : 0.001 P <sub>treatment</sub> × P <sub>storage</sub> : 0.018 Significant effects; P <sub>c</sub> : 0.713 P <sub>c</sub> × P <sub>treatment</sub> : 0.359 P <sub>c</sub> × P <sub>storage</sub> : 0.001 P <sub>c</sub> × P <sub>treatment</sub> × P <sub>storage</sub> : 0.006						

A. B. C →: The difference between applications that have different capital letters in the same row (same variety and storage time) is significant ( $p < 0.05$ ). a. b. c ↓: The difference between storage times that take different lower-case letters in the same column (same type and application) is significant ( $p < 0.05$ ). #: The difference from cv. Melek F1 in the same application and storage time is significant ( $p < 0.05$ ).

In the present study, it was determined that pre-harvest cytokinin application increased antioxidant substances during storage. In terms of changes in total phenolic content and total antioxidant activity, our results (Table 5) were similar to those of Perez-Ambrocio et al. [68,69], who studied different irradiations in pepper.

### 3.8. Correlations between Traits

In the study, a correlation analysis was performed to determine the relationships between measured parameters in cv. Akra F1 and Melek F1 during storage (Tables 6 and 7). In cv. Akra 1, the highest positive correlation values were obtained from TP and AA ( $r = 0.961^{**}$ ), while the lowest relationships were observed between  $O_2$  and  $CO_2$  ( $r = -0.952$ ). For cultivar Melek F1, the highest positive correlation values were obtained from b and C ( $r = 0.996^{**}$ ), while the lowest relationships were observed between a and C ( $r = -0.926$ ).

**Table 6.** Pearson's correlation coefficients between measured parameters of cv. Akra F1 during storage.

	$L^*$	$a^*$	$b^*$	$C^*$	hue	TP	AA	O <sub>2</sub>	CO <sub>2</sub>	RR	EA	SOD	CAT	APX	MDA
$L^*$	1														
$a^*$	−0.637 **	1													
$b^*$	0.807 **	−0.292	1												
$C^*$	0.455 **	−0.244	0.467 **	1											
hue	−0.192	−0.525 **	−0.640 **	−0.217	1										
TP	−0.154	0.493 **	0.134	−0.075	−0.484 **	1									
AA	−0.097	0.432 **	0.152	−0.015	−0.453 **	0.961 **	1								
O <sub>2</sub>	0.496 **	−0.386 **	0.386 **	0.264	−0.016	−0.039	0.082	1							
CO <sub>2</sub>	−0.449 **	0.416 **	−0.342 *	−0.240	−0.053	−0.008	−0.140	−0.952 **	1						
RR	0.172	0.128	0.396 **	0.259	−0.446 **	0.009	−0.005	−0.294 *	0.357 *	1					
EA	0.051	0.101	0.053	0.085	−0.172	−0.161	−0.227	−0.223	0.296 *	0.548 **	1				
SOD	−0.209	0.473 **	−0.078	−0.179	−0.280	0.366 *	0.246	−0.485 **	0.441 **	0.239	0.191	1			
CAT	−0.152	0.272	−0.023	−0.108	−0.213	0.241	0.142	−0.189	0.180	0.139	0.291	0.382 **	1		
APX	−0.201	0.112	−0.254	−0.125	0.104	−0.368 *	−0.489 **	−0.700 **	0.708 **	0.399 **	0.493 **	0.348 *	0.336 *	1	
MDA	−0.115	0.559 **	0.246	−0.085	−0.632 **	0.586 **	0.448 **	−0.319 *	0.351 *	0.451 **	0.206	0.486 **	0.279	0.147	1

\*: Correlation is significant at  $p < 0.05$ . \*\*: Correlation is significant at  $p < 0.01$ . WL: Weight loss, EA: Ethylene amount, RR: Respiration rate, TP: Total phenolic content, AA: Antioxidant activity.



**Table 7.** Pearson's correlation coefficients between measured parameters of cv. Melek F1 during storage.

	<i>L</i> <sup>*</sup>	<i>a</i> <sup>*</sup>	<i>b</i> <sup>*</sup>	<i>C</i> <sup>*</sup>	<i>hue</i>	TP	AA	O <sub>2</sub>	CO <sub>2</sub>	RR	EA	SOD	CAT	APX	MDA
<i>L</i> <sup>*</sup>	1														
<i>a</i> <sup>*</sup>	0.148	1													
<i>b</i> <sup>*</sup>	0.048	−0.891 **	1												
<i>C</i> <sup>*</sup>	0.011	−0.926 **	0.996 **	1											
<i>hue</i>	−0.352 *	−0.736 **	0.351 *	0.427 **	1										
TP	0.040	0.428 **	−0.221	−0.264	−0.555 **	1									
AA	−0.355 *	0.230	−0.158	−0.173	−0.269	0.791 **	1								
O <sub>2</sub>	0.125	−0.529 **	0.603 **	0.601 **	0.180	−0.344 *	−0.309 *	1							
CO <sub>2</sub>	−0.176	0.531 **	−0.669 **	−0.656 **	−0.091	0.202	0.208	−0.936 **	1						
RR	0.011	−0.515 **	0.548 **	0.551 **	0.271	−0.423 **	−0.393 **	0.437 **	−0.385 **	1					
EA	−0.005	−0.227	0.206	0.214	0.150	−0.061	0.061	0.002	0.025	0.060	1				
SOD	0.000	0.359 *	−0.226	−0.255	−0.388 **	0.337 *	0.113	−0.375 *	0.273	−0.078	−0.254	1			
CAT	−0.140	0.097	−0.129	−0.127	0.008	0.101	0.156	−0.424 **	0.406 **	0.177	−0.247	0.168	1		
APX	0.203	0.215	−0.094	−0.118	−0.281	0.063	−0.233	−0.121	0.039	−0.067	−0.317 *	0.351 *	0.021	1	
MDA	−0.083	0.379 *	−0.374 *	−0.383 **	−0.233	0.211	0.111	−0.469 **	0.450 **	−0.343 *	−0.139	0.257	0.246	0.296 *	1

\*: Correlation is significant at  $p < 0.05$ . \*\*: Correlation is significant at  $p < 0.01$ . WL: Weight loss, EA: Ethylene amount, RR: Respiration rate, TP: Total phenolic content, AA: Antioxidant activity.

#### 4. Conclusions

The effects of two doses of cytokinin (50 and 100 ppm) in a pre-harvest application, by spraying, on the post-harvest physiology of Akra F1 and Melek F1 pepper cultivars (*Capsicum annuum* L.) were investigated. It was observed that cytokinin can delay aging in peppers of both cultivars stored for 28 days. These applications have positive effects on the in-package gas composition, antioxidative enzymes (SOD, CAT and APX), lipid peroxidation (MDA), total phenolics and total antioxidant activity of peppers. The application of up to 100 ppm cytokinin might be suitable for use in pepper cultivars cv. Akra F1 and Melek F1, in terms of their in-package gas composition (O<sub>2</sub> and CO<sub>2</sub>), antioxidative enzymes, lipid peroxidation, total phenolic content and antioxidant activity. A high positive correlation was found between the total phenolic content and antioxidant activity, SOD and APX and ethylene amount and respiration rate.

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