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Ethylene treatment of 'Hayward' kiwifruits (*Actinidia deliciosa*) during ripening and its influence on ethylene biosynthesis and antioxidant activity

Y.S. Park [a], S.T. Jung [b], S. Gorinstein [c,*]

[a] *Department of Horticultural Science, Mokpo National University, Muan Jeonnam 534-729, Republic of Korea*
[b] *Department of Food Engineering, Mokpo National University, Muan Jeonnam 534-729, Republic of Korea*
[c] *Department of Medicinal Chemistry and Natural Products, School of Pharmacy, The Hebrew University, Hadassah Medical School, P.O. Box 12065, Jerusalem 91120, Israel*

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Abstract

The aim of this investigation was to assess the influence of ethylene treatment on ethylene biosynthesis and on antioxidant activity in kiwifruits during ripening. Kiwifruits were treated with ethylene of 100 μg ml^{-1} at 20 °C for 24 h and then the ripening process at the same temperature was observed for 10 additional days. It was found that in treated fruits: (a) the flesh firmness in the early stage of ripening was significantly decreased in treated samples, (b) the contents of free sugars, soluble solids, ethylene, respiration and sensory value were increased and were significantly higher than in untreated fruits, (c) the ethylene biosynthesis was increased simultaneously with increase in 1-aminocyclopropane-1-carboxylic acid (ACC) content, ACC synthase (ACS) and ACC oxidase (ACO) activities, (d) the polyphenols content and the related antioxidant activity were increased significantly higher than in the untreated fruits and (e) the acidity and pH were not influenced by ethylene treatment.

In conclusion, the ethylene treatment of kiwifruits significantly increases its ethylene biosynthesis, the contents of total polyphenols and the antioxidant activity in comparison with untreated samples. ACS and ACO are the key enzymes, which control the rate of ethylene biosynthesis in kiwifruits.

Keywords: Ethylene treatment and biosynthesis; ACS and ACO enzymes; Polyphenols; Antioxidant activity

1. Introduction

Citrus and other fruits play a special role in diseases prevention (Guo et al., 2003; Gorinstein et al., 2004a,b). The positive effect of these fruits is attributed to their bioactive compounds and first of all phenolics (Gorinstein et al., 2001, 2004c).

Kiwifruits are one of the main crops in south-east region of Korea. In recent years, it became an important commercial fruit and its growing area has spread in United States, Japan and other countries. However, even after the maturation stage kiwifruits have hard firmness and high acidity and therefore could be eaten only after ripening.

It is well known that ripening of kiwifruits requires a long time and its quality reduces during this process at ambient temperature (Park, 1996; Park and Kim, 2002).

It was shown that ethylene treatment shortens ripening duration and enhances edible quality of kiwifruits by increasing free sugars and flavor (Mashmichi, 1995). Also Shinji et al. (1983) and Shinji (1996) have reported that ethylene treatment of kiwifruits during ripening improves the fruit's quality by increasing fructose and sucrose contents. Mashmichi and Hasegawa (1993) found that the content of soluble solids content (SSC) during kiwifruit ripening was much higher in fruits treated with ethylene than in untreated samples.

Kiwifruit is a climacteric fruit, which possesses negligible ethylene content at harvest. This fruit is very sensitive to post harvest ethylene treatment, showing a typical pattern of increase in ethylene production accompanied by an increase in respiration (Hyudo and Fukasawa, 1985; Park and Kim, 1995; Park, 1996, 2002).

* Corresponding author. Tel.: +972 2 6758690; fax: +972 2 6757076.
E-mail address: gorin@cc.huji.ac.il (S. Gorinstein).

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The ripening of kiwifruits can be induced by a very low concentration of exogenous ethylene (Beever and Hopkirk, 1990). Ethylene is synthesized in higher plants via the following pathway: L-methionine → *S*-adenosyl-L-methionine (SAM) → 1-aminocyclopropane-1-carboxylate (ACC) → C_2H_2 (Adams and Yang, 1979). Two enzymes are involved in this pathway: 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS) and ACC oxidase (ACO), which catalyze the conversion of SAM to ACC and then ACC to ethylene. Some researchers tried to enhance ethylene production of fruits by increasing ACS and ACO enzyme activities to induce ripening in kiwifruit (Mashmichi and Hasegawa, 1993; Ikoma, 1996). So, Mashmichi and Hasegawa (1993), reported that ethylene treatment increased endogenous ethylene levels by enhancing ACC content and ACO activity in kiwifruits. Also Ikoma (1996), had shown that ethylene production and ACO activity in kiwifruits increases after 100 μg ml^{-1} ethylene treatment.

Investigations of the changes in contents of bioactive compounds and in the antioxidant activity during ripening of kiwifruits are important for understanding of the effectiveness of the ethylene treatment. There have previously been almost no investigations on the relationship between the ripening of kiwifruits, ethylene treatment and the antioxidant activity (Leong and Shui, 2002).

Therefore, it was decided to study the possible changes in free sugar content, polyphenols, the pattern of ethylene biosynthesis, the ACS and ACO enzymes, and antioxidant activity during ripening at 20 °C following exogenous ethylene treatment.

Antioxidant assays give different antioxidant activity trends (Ou et al., 2002). Therefore, it was decided to apply two complementary assays: 2,2′-azino-bis-(3-ethyl-benzothiazoline-6-sulfonate) radical cation ($ABTS^{\bullet+}$) and the Folin–Ciocalteu method (Miller et al., 1996).

As far as we know there are not such comprehensive investigations.

2. Materials and methods

2.1. Chemicals

Trolox (6-hydroxy-2,5,7,8,-tetramethyl-chroman-2-carboxylic acid), Folin–Ciocalteu reagent and 2,2′-azino-bis-(3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and other routine chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All reagents were of analytical grade.

2.2. Sample preparation

'Hayward' kiwifruits were picked at a commercial orchard near Muan county, Jeonnam. The harvest date was October 26, 2002. At this stage the initial SSC and firmness were 6.8% and 33.4 N, respectively. Fruits with defects were discarded and 40 good fruits of average weight of 80–100 g were placed in glass jar. These fruits were divided into two groups (treated and control) and were ripened immediately after harvest: one (treated) was treated with 100 μg ml^{-1} ethylene for 24 h at 20 °C in a growth chamber (Percival, USA) and the other one (control) was not treated. Kiwifruits were put into an 18 l glass jar and ventilated with humidified flow of air (control) or air mixed with ethylene (treated) at 300 ml min^{-1}. Then the ethylene treated and untreated kiwifruits were ripened separately under the same conditions at 20 °C in a growth chamber (Percival, USA) for 10 days.

2.3. Determination of the studied variables

Ten fruits from each replicate were analyzed for firmness by measuring penetration force in kilogram using a fruit firmness tester (Model KM, Fruit Test Tech, Japan). A sample of 10 fruit halves was ground with mixer and the clear juice was analyzed for SSC, pH and titratable acidity (TA). SSC was measured using a refractometer, pH—with a pH meter. For TA measurement, a sample of 4 ml juice was diluted with 20 ml of distilled water and titrated with 0.1 N NaOH. Free sugars were extracted from juice and analyzed using HPLC (Waters, USA) with carbohydrate analysis column (Park, 1996). The ethylene production was measured at 2 days intervals during ripening at 20 °C in a growth chamber. Ten fruits were sealed in a 1.8 l jar for 24 h and headspace gas was sampled with a 1 ml syringe. A gas chromatography (Hewlett Packard, USA) with Pora Plot Q aluminum column and FID, TCD detector were used to analyze the respiration and ethylene production, respectively (Park and Kim, 2002). For ACC analysis 10 g of flesh tissue was taken and extracted with acetone up to a final volume of 20 ml, filtered and kept at −70 °C until use. Five millilitres of aliquot was concentrated in vacuum and assayed for ACC as described by Lizada and Yang (1979). ACS and ACO activities were assessed as described by Gorney and Kader (1996). Portions of 10 g of peeled kiwifruit were homogenized with 125 ml of 95% ethanol for 1 min and then gently boiled. After this procedure, the fruit samples were cooled and filtered under vacuum using Whatman no. 1. The filtrates were evaporated under vacuum at 60 °C until 10 ml and then made up to 100 ml by distilled water. Total polyphenols were determined by Folin–Ciocalteu method and measured at 765 nm. The results were given in mg/100 g fresh weight (FW) of gallic acid equivalent (Singleton et al., 1999).

The antioxidant activities were determined using $ABTS^{\bullet+}$ with $K_2S_2O_8$ and with MnO_2. $ABTS^{\bullet+}$ radical cation was generated by the interaction of ABTS (250 μM) and $K_2S_2O_8$ (40 μM). After addition of 990 μl of ABTS solution to 10 μl of fruit extracts (0.2 mg ml^{-1}) or Trolox standards (final concentration 0–20 μM) in ethanol or phosphate buffered saline (PBS), the absorbance was monitored exactly 1 and 6 min after the initial mixing (Miller et al., 1996). ABTS was prepared as well by passing a 5 mM aqueous stock solution of ABTS through manganese dioxide on a Whatman no. 5 filter paper. Excess manganese dioxide was removed from the filtrate by passing it through a 0.2 μM Whatman PVDF syringe filter. This solution was then diluted in a 5 mM phosphate buffered saline, pH 7.4 to an absorbance of 0.70.

The percentage decrease of the absorbance at 734 nm was calculated and plotted as a function of the concentration of the extracts and of Trolox for the standard reference data (Miller et al., 1996). Two assays are compared by their percentage of inhibition.

2.4. Statistical analyses

To verify the statistical significance of the studied parameters, means and standard deviation (mean ± S.D.) of five measurements were determined. Where it was appropriate, differences between groups were tested by two-way ANOVA. The *P*-values of <0.05 were considered significant.

3. Results

The changes in firmness and sensory value of the treated and untreated kiwifruits are shown in Fig. 1. As can be seen, the firmness was decreased significantly and reached minimum levels after 2 days of ethylene treatment. The fruits firmness of untreated samples decreased gradually and remained at low level after 10 days of the ripening process. The loss of the firmness in ethylene treated fruits was significantly higher than in untreated samples ($P < 0.05$).

Sensory value of both ethylene treated and untreated kiwifruits was increased, but the increase was significant only in treated samples ($P < 0.05$). The increase in the sensory value was registered together with the decrease in the firmness.

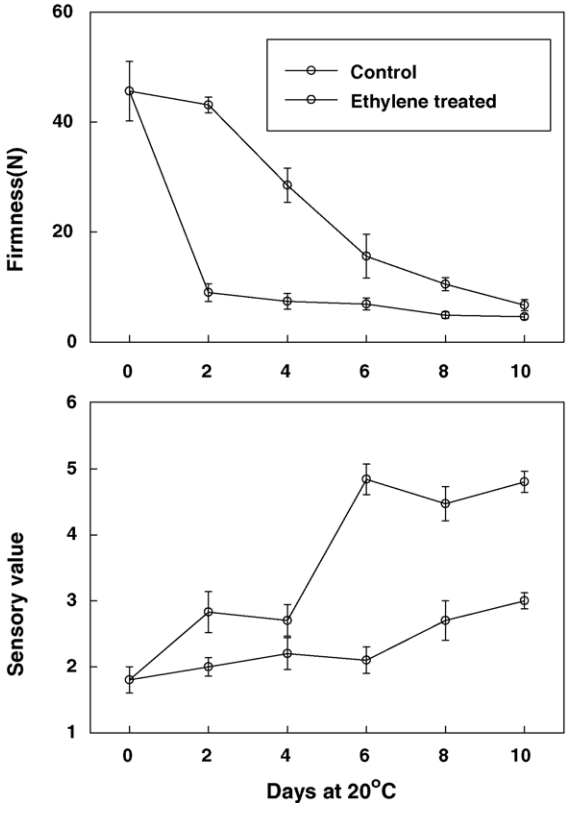

Fig. 1. Changes in firmness and sensory value of kiwifruits as influenced by ethylene treatment.

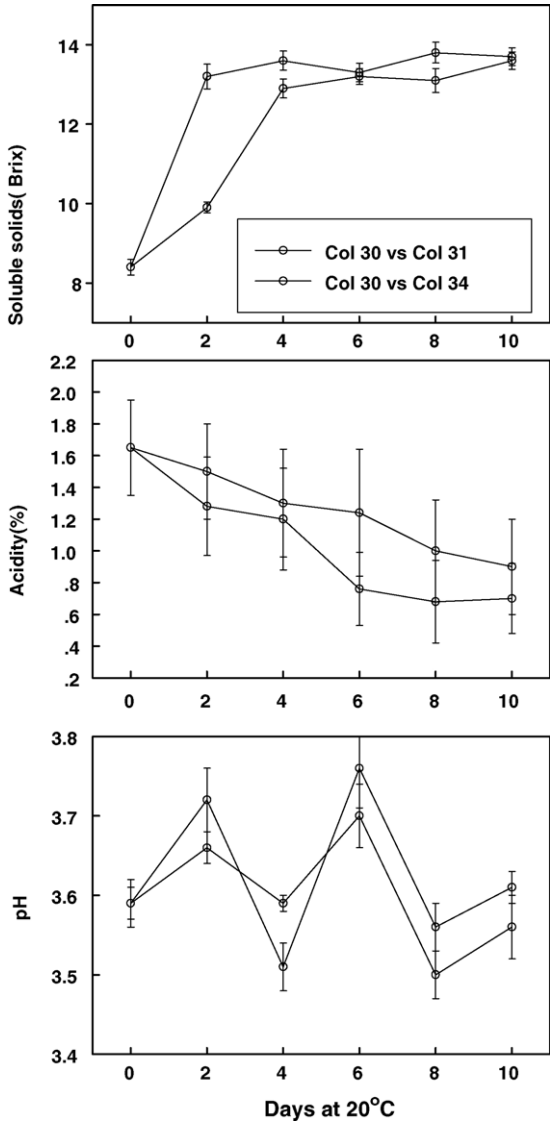

Fig. 2. Changes in soluble solids, acidity and pH of kiwifruits as influenced by ethylene treatment.

The changes in SSC, TA content and pH in the treated and untreated kiwifruits are summarized in Fig. 2. As can be seen, the SSC was significantly increased, while TA content was gradually decreased during ripening duration regardless of treatments. The pH value fluctuated with ripening duration.

Sensory value increased rapidly with ethylene treatment and resulted in high SSC and low acidity. The rise in the ethylene production was accompanied by an increase in SSC and a decrease in flesh firmness. Amount of TA tended to a decrease during the ripening process.

It was also observed that the time required for ripening became shorter and uniform in fruits treated with ethylene (data was not shown).

Fructose, glucose and sucrose contents in kiwifruits treated with ethylene were increased significantly ($P < 0.05$) within first 4 days of ripening and then slightly decreased (Fig. 3). The contents of the studied variables were significantly higher in the treated than in the untreated fruits ($P < 0.05$).

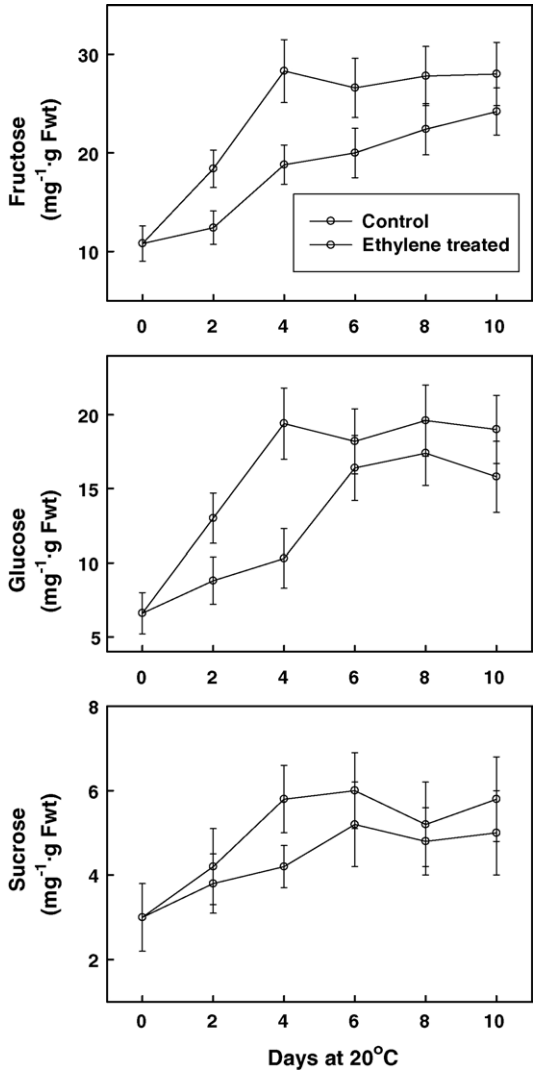

Fig. 3. Changes in fructose, glucose and sucrose contents in kiwifruits as influenced by ethylene treatment.

The changes in contents of ethylene and respiration in treated and untreated kiwifruits are shown in Fig. 4. As can be seen, the content of ethylene and respiration in the ethylene treated fruits was increased only during the first 2 days of ripening and then was decreased. In the untreated fruits, their contents have increased in the first 4 days of ripening time and then decreased. The increase in ethylene production was accompanied by rise in respiration, regardless of treatments.

The changes in ACC content, ACS and ACO activities are summarized in Fig. 5. As can be seen, the ACC concentration in ethylene treated kiwifruits was increased during the first 2 days of treatment and decreased significantly thereafter. ACC content, ACS and ACO activities had the highest increase between 0 and 2 days and were significantly higher in treated than in untreated fruits ($P < 0.05$). Untreated fruits produced a small amount of ACC, and relatively low ACS and ACO activities during first 4 days of ripening and then these variables were decreased with duration of the ripening process.

The changes in the content of total polyphenols and of the antioxidant activity are shown in the Figs. 6 and 7, respectively.

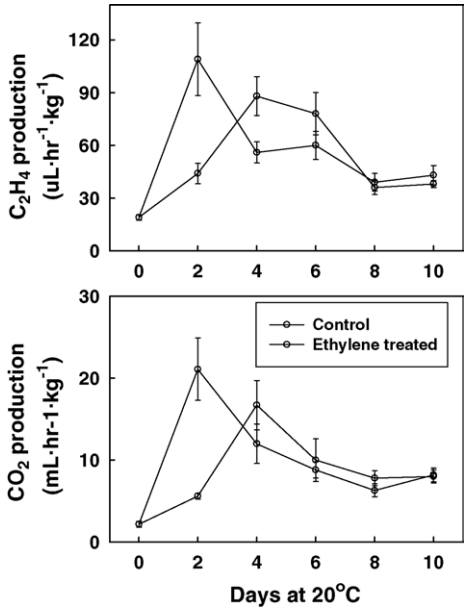

Fig. 4. Changes in ethylene and respiration contents of kiwifruits as influenced by ethylene treatment.

As can be seen, the content of total polyphenols and the antioxidant activity was significantly increased only in ethylene treated kiwifruits at the period of 4–6 days after at the beginning of the ripening process ($P < 0.05$).

The results of the changes in the antioxidant activity (AA) in kiwifruits (KW) are also shown in Fig. 8. As can be seen, the results of the determination of AA with manganese dioxide (M) at 0 (KWM0), 2 (KWM2), 4 (KWM4) and 6 days (KWM6) were compared with the results of another variation of ABTS decolorization assay (KWP0, KWP2, KWP4 and KWP6) where ABTS radical cation was produced by reacting ABTS with potassium persulfate (P).

ABTS with $K_2S_2O_8$ showed that the AA (mmol TE g^{-1}) for KWPO, KWP2, KWP4 and KWP6 were 10.25 ± 2.5 a, 14.48 ± 0.9 b, 15.51 ± 4.5 b and 20.73 ± 3.7 c, respectively, and with MnO_2 were 13.32 ± 1.4 a, 19.83 ± 4.1 b, 20.63 ± 2.1 b and 27.15 ± 3.7 c for KWMO, KWM2, KWM4 and KWM6, respectively.

The AA of these samples had comparative results against ABTS at the end point of 6 min as determined by spectrophotometric measurement (Fig. 8). According to these results, KWP6 had the highest % of inhibition, as well as the highest AA (20.73 mmol TE g^{-1}). The other extracts especially KWP0 showed the lowest AA (10.25 mmol TE g^{-1}). The untreated samples were examined at the same concentration of 0.25 mg ml^{-1} and were comparable with caffeic acid. BHA was lower than the treated samples. The manganese dioxide method gave slightly higher results than with potassium persulfate (about 10% less).

4. Discussion

Kiwifruits are one of the subtropical fruits, which is very popular among consumers (Mashmichi, 1995; Ikoma, 1996). However, even after the maturation stage kiwifruits have hard

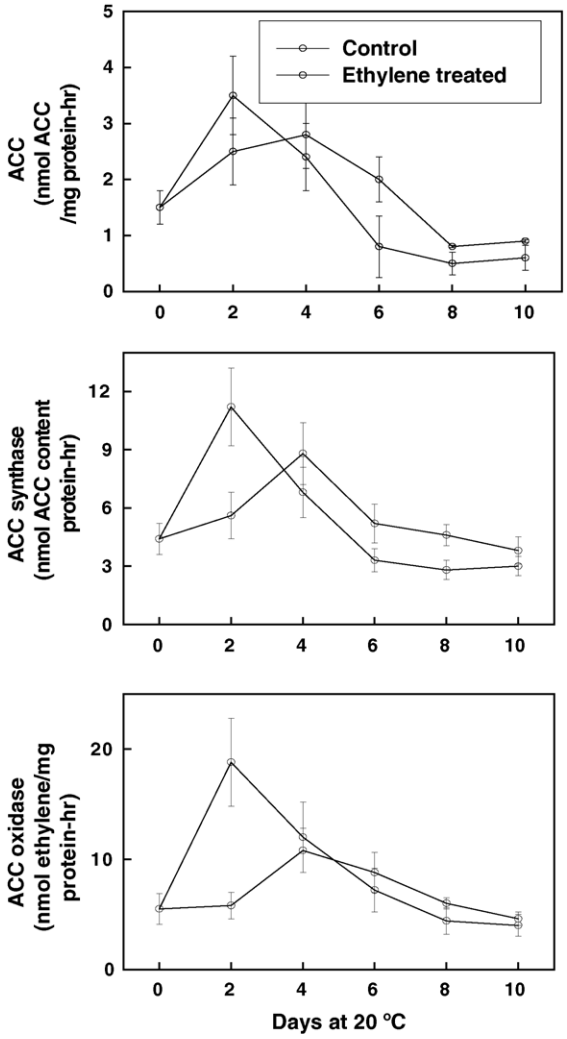

Fig. 5. Changes in ACC content, ACC synthase and ACC oxidase activities of kiwifruits as influenced by ethylene treatment. In all results shown on Figs. 1–5, fruits were treated with 100 μg ml^{-1} of ethylene at 20 °C for 24 h. ACC, 1-aminocyclopropane-1-carboxylic acid; ACS, synthase; ACO, oxidase.

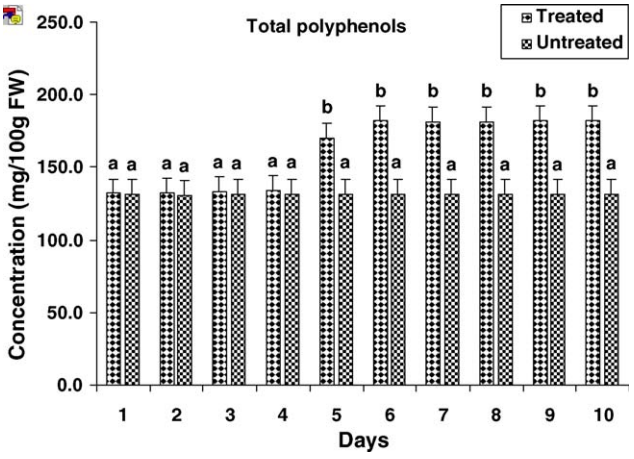

Fig. 6. Total polyphenols in treated and untreated kiwifruits during 10 days of ripening. Mean ± S.D. (vertical lines). Bars with different letters differ significantly ($P < 0.05$).

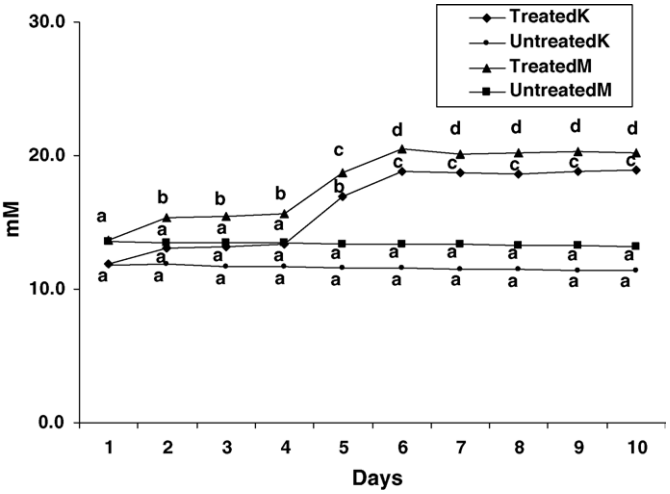

Fig. 7. Antioxidant activity in treated and untreated kiwifruits during 10 days of ripening. Means in lines with different letters differ significantly ($P < 0.05$).

firmness and high acidity and therefore could be eaten only after ripening.

The ripening of kiwifruit requires a long time during which the quality deteriorated (Park, 1996, 2002; Park and Kim, 2002). Therefore, it is important to avoid a long ripening.

Some authors claim that ethylene treatment is the best solution (Park and Kim, 2002). It was shown that ethylene treatment enhanced edible quality of kiwifruits, by decrease in its flesh firmness and acidity, and increase in the contents of free sugars, fructose and sucrose contents, soluble solids content (SSC), ethylene, respiration and sensory value (Park and Kim, 1995; Ikoma, 1996; Park and Kim, 2002). Therefore, it was decided to investigate the possible changes in these and other

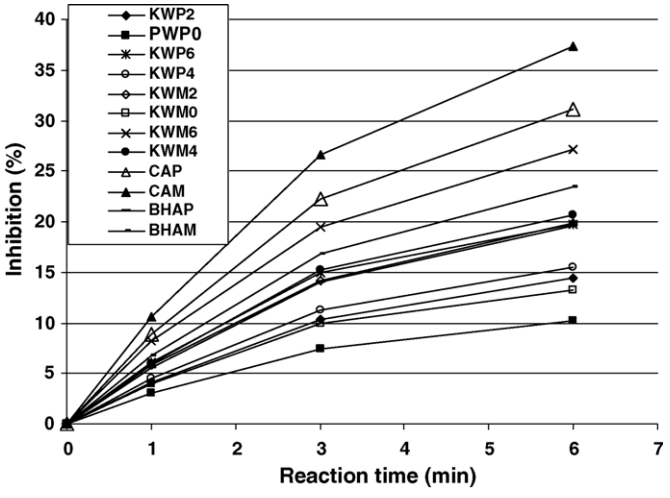

Fig. 8. Kinetics of ABTS scavenging effect of kiwifruit extracts. The concentration of the samples was 0.25 mg ml^{-1}. ABTS radical cation was produced by reacting with manganese dioxide (M): kiwifruit at 0 (KWM0), 2 (KWM2), 4 (KWM4) and 6 days (KWM6). ABTS was produced by reacting with potassium persulfate (P): KWP0, KWP2, KWP4 and KWP6. CAM, CAP, BHAM and BHAP, caffeic acid, butylated hydroxyanisole (BHA), respectively with manganese dioxide and potassium persulfate.

variables in ethylene treated and untreated kiwifruit samples and to compare with the data of others.

The changes in total polyphenol content and in antioxidant activity in ethylene treated kiwifruits were less investigated (Leong and Shui, 2002). Therefore, in this investigation also the possible changes in total polyphenol content and in antioxidant activity were studied.

It was found that the firmness in the fruits treated with ethylene was decreased significantly and reached minimum levels after 2 days, while in untreated samples the firmness decreased gradually and remained at low level after 10 days of ripening. The loss of firmness in fruits treated with ethylene was significantly higher than in the untreated samples. These data correspond with the data of others (Shinji et al., 1983; Ikoma, 1996).

It was observed that the sensory value of the ethylene treated and untreated kiwifruits was increased, but the increase was significant only in treated samples. This increase was observed together with the decrease of the fruit firmness during ripening. Also these data are in accordance with the results of others, who reported that the loss of firmness was significantly decreased at an early stage and was closely related to ethylene production in kiwifruits (Shinji et al., 1983; Mashmichi and Hasegawa, 1993; Park and Kim, 1995; Park, 1996). The same phenomenon was also observed in some vegetables: in avocado (Cheverry et al., 1988) and tomato (Mathook et al., 1993).

It was found that SSC was significantly increased, while TA content was gradually decreased during ripening duration regardless of the treatments. This phenomenon was observed also by Ikoma (1996).

As in our previous investigations (Park and Kim, 1995), we found that the SSC increase was correlated with the decrease in acidity during ripening progress of kiwifruits. Others have also reported that there is also a significant correlation between SSC and the rate of ethylene production [$r = 0.755$] (Hyudo and Fukasawa, 1985).

Fructose, glucose and sucrose contents in kiwifruits treated with ethylene were increased and were significantly higher in than in the untreated samples. We have already reported that free sugar contents increases with ethylene production and reached maximum levels before full softening (Park, 1996). Also Shinji et al. (1983) and Shinji (1996) have found that free sugar content was significantly higher in kiwifruits treated with ethylene than in untreated samples. We suppose that ethylene treatment enhances free sugar content by improving sugar metabolism in kiwifruits.

The increase in ethylene production through the autocatalytic effect of endogenous ethylene is a well-known feature in climacteric fruits. Ikoma (1996) showed that endogenous ethylene content is not enough to induce ripening of kiwifruits, and therefore it is necessary to use exogenous ethylene treatment. The results of this experiment show that the contents of ethylene and respiration in the ethylene treated fruits were increased only in the first 2 days of ripening and then were decreased. In the untreated fruits, the increase in the content of ethylene and respiration was in the first 4 days of ripening and then was decreased. The increase in ethylene production was accompanied by the rise in respiration, regardless of treatments. Also others found the same tendencies (Hyudo and Fukasawa, 1985; Ikoma, 1996).

It was observed that the ACC concentration in ethylene treated kiwifruits was increased in the first 2 days and was decreased significantly thereafter. ACS and ACO activities were increased significantly in the initial time of the process and were progressing only during the ethylene treatment. Contrary, untreated fruits produced small amount of ACC, and relatively low ACS and ACO activities during first 4 days of ripening and then these variables were decreased with duration of this process. These observations are in accordance with the data of others (Mashmichi and Hasegawa, 1993; Ikoma, 1996). So, Mashmichi and Hasegawa (1993) reported that ethylene treatment increased endogenous ethylene levels by enhancing ACC content and ACO activity in kiwifruits. Also Ikoma (1996) and Zhong et al. (1998) have shown that ethylene production in kiwifruits increases with the increase of the ACO activity as a result of 100 $\mu g\ ml^{-1}$ exogenous ethylene treatment. These data suggest that ethylene treatment is effective in accelerating endogenous ethylene production in the treated fruits. ACC content in treated fruits rose progressively with increasing rate of ethylene production, which may indicate that ACS is a limiting step in ethylene biosynthesis. ACC content in the kiwifruits was considerably higher as long as the rate of ethylene production was remaining high. Its content increases proportionally to the rate of ethylene production, which may indicate that ethylene production is regulated by availability of ACS.

The results of the determination of the total polyphenols content and AA in treated and untreated kiwifruits samples show that the ethylene treatment significantly increases both variables in treated fruits. We cannot compare our data with the data of others: we did not find such data. However, our results can be compared with the results of authors, who observed similar trends in traditional fruits (Guo et al., 2003; Leong and Shui, 2002). They found that hawthorn pulp had the highest antioxidant value among all fruit pulps, followed by date, guava, purple mulberry, strawberries and others.

In conclusion, the ethylene treatment of kiwifruits is effective: it leads to a significant decrease in the flesh firmness in the early stage of ripening and to a significant increase in the contents of free sugars, soluble solids, total polyphenols, ethylene biosynthesis, respiration, sensory value and antioxidant activity determined as ability to scavenge ABTS free radicals.

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