International Journal of Food Sciences and Nutrition,
February/March 2006; 57(1/2): 107–122

informa
healthcare

In vitro studies of polyphenols, antioxidants and other dietary indices in kiwifruit (*Actinidia deliciosa*)

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Abstract
The main aim of the present study was the evaluation of proteins and antioxidant potential in ethylene-treated kiwifruit during the first 10 days of ripening. Kiwifruit samples were randomly divided into two groups: treated and untreated. Flesh firmness, sensory value, visual score, free sugars, soluble solids, ethylene biosynthesis, proteins, dietary fibers, total polyphenols and antioxidant potential were determined in both groups. Ethylene (100 ppm) at 20°C for 24 h was used in the treated group. The flesh firmness and acidity in treated samples decreased significantly in the early stage of ripening simultaneously with significant increase in the contents of free sugars, soluble solids, endogenous ethylene production, sensory value, 1-aminocyclopropane-1-carboxylic acid (ACC) content, ACC synthase and ACC oxidase activities, total polyphenols and related antioxidant potential, and was significantly higher than in untreated samples ($P < 0.05$). Proteins were extracted from kiwifruit and separated by modified sodium dodecyl sulphate polyacrylamide gel electrophoresis. The separation was resolved into 14 protein bands. Some minor quality changes were found only in the 32 kDa band, which was more pronounced in the treated samples. In conclusion, ethylene treatment of kiwifruits leads to positive changes in most of the studied kiwifruit compounds and to an increase in the fruit antioxidant potential. It shortens the ripening time and improves fruit quality by decreasing its flesh firmness and acidity. Some minor changes in the protein profile did not affect the fruit taste and quality.

Keywords: *Treated and untreated kiwifruits, bioactive compounds, antioxidant potential, proteins*

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ISSN 0963-7486 print/ISSN 1465-3478 online © 2006 Informa UK Ltd
DOI: 10.1080/09637480600658385

Introduction

Consumption of fruits and vegetables plays a special role in prevention and even treatment of various diseases (Duttaroy and Jorgensen 2004; Gorinstein et al. 2004). Among these fruits is kiwifruit, one of the main crops in the south-east region of Korea. In recent years it became very popular in USA and Europe. Kiwifruit is effective even in the prevention of the dangerous coronary atherosclerosis (Duttaroy and Jorgensen 2004). These authors reported that consumption of two or three kiwifruit per day lowers the blood triglyceride level by 15% and reduces platelet aggregation response by 18% compared with controls ($P < 0.05$). However, even after maturation kiwifruit has hard flesh firmness and high acidity, and therefore it can be eaten only after ripening (Park 1996).

The natural ripening of kiwifruit is a long process and leads to a decrease in fruit quality (Park 1996, 2002). Some authors therefore propose to shorten this process through 1-methylcyclopropene treatment (Kim et al. 2001; Boquete et al. 2004), acetylsalicylic acid treatment (Zhang et al. 2003), 1-aminocyclopropane-1-carboxylic acid (ACC) treatment (Haji et al. 2003) and ethylene treatment (Park 2002). Ethylene treatment effectively shortens the ripening duration and enhances edible quality of kiwifruit by increasing fructose, sucrose and soluble solids contents. This makes such a treatment more preferable than the others (Shinji 1996). Kiwifruit, as other climacteric fruits, possesses a negligible content of ethylene at harvest and its ripening can be induced by a very low concentration of exogenous ethylene (Beever and Hopkirk 1990; Park 1996). Two enzymes are involved in the endogenous ethylene production—ACC synthase and ACC oxidase—which catalyze the conversion of S-adenosyl-L-methionine to ACC and then ACC to ethylene. Some researchers tried to enhance ethylene production in fruits by increasing enzyme activities (Ikoma 1996).

To evaluate the effectiveness of the ethylene treatment, it is important to record the possible changes in bioactive compounds and the antioxidant potential of kiwifruit. However, until now nearly no investigations have been carried out on the relationship between antioxidant potential and ethylene treatment of kiwifruit (Leong and Shui 2002). Therefore, the main bioactive compounds with known antioxidant properties (total polyphenols and dietary fibers) were studied.

Antioxidant assays give different antioxidant activity trends (Ou et al. 2002). In order to receive reliable results of the antioxidant potential, three other complemented assays were used in this investigation.

Food proteins supply the required building blocks for protein biosynthesis in humans. Now some authors suggest that proteins have much wider biological functions (Larson et al. 1996). Inter alia, dietary plant proteins play a positive role in plasma cholesterol control (Anderson et al. 1995). The main aim of the present report was therefore the evaluation of possible changes in proteins by electrophoresis, some other bioactive compounds and in the antioxidant potential of ethylene-treated kiwifruit during the first 10 days of ripening.

Materials and methods

Chemicals

Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid), Folin–Ciocalteu reagent, 2,2′-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt

(ABTS), β-carotene, butylated hydroxyanisole, sodium dodecyl sulphate (SDS), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and other routine chemicals were purchased from Sigma Chemical Co. (St Louis, MO, USA). All reagents were of analytical grade.

Sample preparation

'Hayward' kiwifruits harvested in October 2003 were from Muan County and were purchased from the same farmer. Fruits with defects were discarded and good fruits of average weight 100 g were cleaned with tap water and placed in a glass jar. They were randomly divided into two groups, treated and untreated, and were ripened immediately after harvest. Kiwifruit samples of the treated group were treated with 100 ppm ethylene for 24 h at 20°C in a growth chamber (Percival Scientific Inc., Perry, Iowa, USA). The samples were put into an 18 l glass jar and ventilated with humidified flow of air (untreated) or air mixed with ethylene (treated) at 300 ml/min. Then the ethylene-treated and untreated kiwifruits were ripened separately at the same conditions at 20°C in a growth chamber (Percival) for 10 days.

Ripening assessment

Firmness of the fruit samples was analyzed by measuring the penetration force (kg) using a fruit firmness tester and a destructive plunger (Model KM, diameter 5 mm; Fruit Test Tech Com, Tokyo, Japan) from the equator of peeled flesh.

The soluble solids content (SSC) was measured using a hand refractometer (NI, ATAGO Company, Tokyo, Japan), and pH was measured with a pH meter. For titratable acid measurement, a sample of 4 ml juice was diluted with 20 ml distilled water and titrated with 0.1 N NaOH.

Free sugars were extracted from juice freshly prepared for this purpose and determined using high-performance liquid chromatography (Waters, Milford, MA, USA) with a carbohydrate analysis column ($3.9 \times 25 cm^2$, 10μm), a detector (Waters 410 differential refractometer) and an isocratic solvent (83% acetonitrile + 17% water, 1 ml/min) (Park 1996).

Carbon dioxide and ethylene production were measured at 2-day intervals. Fruits were sealed in a 1.8 l jar for 24 h and headspace gas was sampled with a 1 ml syringe. A gas chromatograph (Hewlett Packard, Waterford, FL, USA) with a Pora Plot Q aluminum column and TC and FI detectors were used to analyze carbon dioxide and ethylene production, respectively (Park 2002).

In ethylene analysis, the flow gas carrier was 13, 33, and 420 ml nitrogen, oxygen and air, respectively. For CO_2 analysis, all the conditions were the same as with ethylene, except for the carrier gas (300 ml/min helium) with a TCD detector. The identified peak was compared with the standard curve and retention time.

Dietary fiber in the selected samples was analyzed by the modified Association of Official Analytical Chemists method (Prosky et al. 1992). Samples were treated with heat-stable α-amylase, protease, and amyloglucosidase, followed by centrifugation (15 min, 3000 × g) to separate the soluble and insoluble fractions and dialysis against water.

Protein determination

The protein content of the samples was determined by the Bradford (1976) method using an Uvikon 930 spectrophotometer (Kontron instruments, Watford, UK).

SDS-polyacrylamide gel electrophoresis

Approximately 30 μl freshly prepared kiwifruit juice was filtered through filter paper. Then 20 μl filtrate was centrifuged at 15,000 × g for 20 min. The sediment was resuspended in 550 μl of 0.5 M Tris–HCl (pH 8.3) buffer and mixed with an equal volume of sample buffer. The solution was boiled for 3 min. After centrifugation at 15,000 × g for 15 min the supernatant was used for SDS-PAGE, which was carried out according to Laemmli (1970) using a Hoeffer SE-600 apparatus. The Laemmli method was modified for juice proteins: the resolving gel was 13.7% T and 1.7% C, and the stacking gel was 3.8% T and 1.8% C. The gel size was 140 × 160 × 1.5 mm^3. Juice supernatant (5 μl) was loaded onto each of the lanes of the gel. The run was carried out at a constant current of 25 mA per gel. Gels were stained with 0.25% Coomassie Brilliant Blue R-250 in methanol/water/glacial acetic acid solution (5:5:1 v/v) and distained in methanol/water/glacial acetic acid solution. The following Sigma molecular weight markers (kDa) were used: 205-kDa myosin, 116-kDa β-galactosidase, 97-kDa phosphorylase b, 66-kDa albumin, 45-kDa ovalbumin, 29-kDa carbonic anhydrase, 20-kDa trypsin inhibitor, 14-kDa α-lactalbumin.

With the use of software (BIO-GENE version 98; Vilbert Lourmat, Marne-la-Valle, Cedex, France), function 'Calculation of molecular weights', the molecular weights of protein bands were determined.

ACC content and enzyme activity

For ACC determination, 10 g flesh tissue was taken and extracted with acetone up to a final volume of 20 ml, was filtered and kept at −70°C until use. A 5 ml aliquot was concentrated in vacuum and assayed for ACC as described by Lizada and Yang (1979).

ACC synthase and ACC oxidase activities were assessed as described by Gorney and Kader (1996). Portions of 10 g peeled kiwifruit were homogenized with 125 ml of 95% ethanol and then gently boiled for 30 min. The fruit samples were cooled and filtered under vacuum using Whatman No 1 filter paper. The filtrates were evaporated under vacuum at 60°C until 10 ml and then made up to 100 ml with distilled water.

Bioactive compounds

Total polyphenols were determined by the Folin–Ciocalteu method and measured at 765 nm. The results are given in milligrams of gallic acid equivalent (Singleton et al. 1999) per 100 g fresh weight.

Despite the high antioxidant potential of individual compounds, the antioxidant effect of whole fruit could be low (Lotito and Frei 2004). Therefore, in addition to the determination of individual bioactive compounds, three other complemented total antioxidant assays were used. In these assays different scavenging radicals were used in order to determine the antioxidant activities of the bioactive compounds that mostly

were represented by polyphenols. The described methods show the relative scavenging activity of the extracted polyphenols from kiwifruit.

1. Radical scavenging activity using DPPH was expressed as an inhibition percentage: % radical scavenging activity = (control OD – sample OD/control OD) × 100, where OD is optical density. Changes in the absorbance of the samples were measured at 517 nm (Singh et al. 2002).
2. Antioxidant activity using the β-Carotene Linoleate Model System was evaluated in terms of bleaching of the β-carotene, measuring the absorbance at 470 nm: $AA = 100\ [1 - (A_0 - A_t)/(A_0^\circ - A_t^\circ)]$, where A_0 and A_0° are the absorbance values measured at zero time of the incubation for test sample and control, respectively, and A_t and A_t° are the absorbance measured in the test sample and control, respectively, after incubation for 180 min. The results were expressed as the percentage of inhibition. Butylated hydroxyanisole was used for comparison in both methods (Singh et al. 2002).
3. The antioxidant activities were determined using $ABTS^{\bullet+}$ with $K_2S_2O_8$. The $ABTS^{\bullet+}$ radical cation was generated by the interaction of ABTS (250 μM) and $K_2S_2O_8$ (40 μM). After addition of 990 μl ABTS solution to 10 μl fruit extracts (0.2 mg/ml) or Trolox standards (final concentration 0–20 μM) in ethanol or phosphate-buffered saline, the absorbance was monitored exactly 1 and 6 min after the initial mixing. 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. The results are expressed in nanomoles of Trolox equivalent/g (Miller et al. 1996).

Statistical analyses

To verify the statistical significance of the studied parameters, the mean and standard deviation of five measurements was determined. Where appropriate, the data were tested by two-way analysis of variance using GraphPad Prism, version 2.0 (GraphPad Software, San Diego, CA, USA) followed by Duncan's new multiple-range test to assess differences between groups means. $P < 0.05$ was considered significant.

Results (*in vitro* studies)

SSC and firmness

At harvest date, the SSC and firmness were 6.8% and about 40 N, respectively. A significant decrease in fruit firmness with the peak after 2 days of ethylene treatment was registered. The firmness of untreated samples decreased gradually and remained at a 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.

Fructose, glucose and sucrose

The contents of fructose, glucose and sucrose in the treated samples increased significantly within the first 4 days of ripening and then slightly decreased (Figure 1). The contents of the aforementioned compounds were significantly higher in the treated than in the untreated fruits.

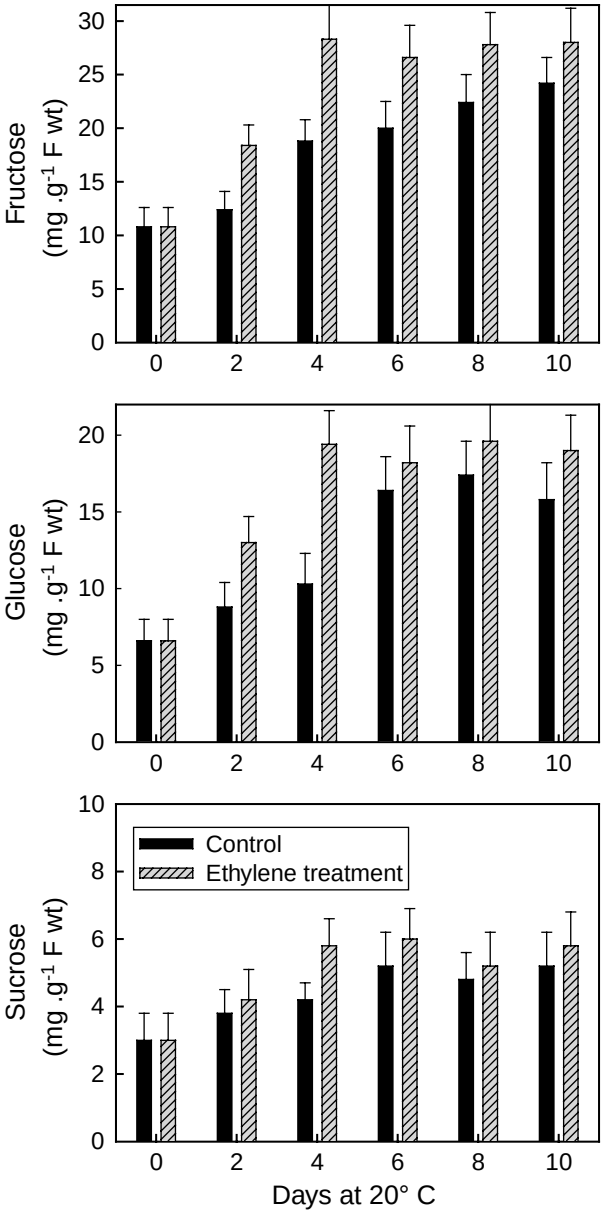

Figure 1. Changes in fructose, glucose and sucrose contents in kiwifruits as influenced by ethylene treatment. F wt., fresh weight.

Soluble solids, CO_2 and C_2H_4 production, ACC, ACC synthase and ACC oxidase

The changes in the soluble solids, CO_2 and C_2H_4 productions, ACC, ACC synthase and ACC oxidase are summarized in Table I. As can be seen, the soluble solids content increased significantly during the ripening process regardless of treatment. Only at the second day of ripening was the soluble solid content in treated fruits significantly higher than in the untreated fruits.

Table I. Changes in some variables in treated and untreated kiwifruits.

Compound	Sample	Days of ripening					
		0	2	4	6	8	10
Soluble solids (°Brix)	Untreated	8.4±0.8[a]	9.9±0.7[a]	12.9±1.24[a]	13.2±1.20[a]	13.1±1.3[a]	13.6±1.3[a]
	Treated	8.5±0.8[a]	13.2±1.3[b]	13.6±1.41[a]	13.3±1.23[a]	13.8±1.3[a]	13.1±1.2[a]
C_2H_4 production (µl/h/kg)	Untreated	19.0±1.5[a]	44.0±5.8[a]	88.0±11.1[a]	78.8±12.6[a]	36.0±4.4[a]	38.0±4.0[a]
	Treated	19.1±1.5[a]	85.0±20.7[b]	62.0±6.6[a]	64.0±8.8[a]	48.0±5.4[a]	43.0±5.5[a]
CO_2 production (ml/h/kg)	Untreated	2.17±0.3[a]	5.6±0.5[a]	16.7±1.5[a]	10.0±0.9[a]	7.8±0.9[a]	8.0±0.8[a]
	Treated	2.16±0.3[a]	21.1±2.8[b]	12.0±1.24[a]	8.8±1.0[a]	6.3±0.8[a]	8.2±0.83[a]
ACC (nmol ACC/mg protein/h)	Untreated	1.51±0.2[a]	2.5±0.3[a]	2.8±0.3[a]	2.0±0.16[a]	0.8±0.06[a]	0.9±0.08[a]
	Treated	1.52±0.2[a]	3.5±0.4[b]	2.4±0.2[a]	0.8±0.06[a]	0.5±0.05[a]	0.6±0.06[a]
ACC synthase (nmol ACC content/mg protein/h)	Untreated	2.22±0.3[a]	3.2±0.2[a]	8.8±0.8[a]	7.2±2.2[a]	4.6±0.4[a]	3.8±0.70[a]
	Treated	2.21±0.3[a]	11.2±1.6[b]	6.8±0.6[a]	3.3±0.3[b]	2.8±0.3[a]	3.0±0.3[a]
ACC oxidase (nmol ethylene/mg protein/h)	Untreated	3.51±0.4[a]	5.8±0.2[a]	10.0±1.1[a]	8.8±1.8[a]	6.0±0.5[a]	4.6±0.4[a]
	Treated	3.52±0.4[a]	18.8±4.0[b]	12.0±1.2[a]	7.2±1.7[a]	4.4±0.4[a]	4.0±0.4[a]

Means in rows without common letters differ significantly ($P < 0.05$).

Endogenous ethylene

The increase of the content of endogenous ethylene in the treated samples appeared in the first 2 days, followed by a sharp decrease. On the contrary, in the untreated fruit samples this increase was recorded only after the first 6 days of ripening.

CO_2 production

The CO_2 production in the treated samples increased significantly during the first 2 days of ethylene treatment and decreased significantly thereafter. On the contrary, the CO_2 production in the untreated samples increased significantly during the first 4 days of the ripening and only then began the decrease. At the end of the ripening process the CO_2 production in both kiwifruit samples was without significant differences.

Similar changes were observed in ACC content, ACC synthase and ACC oxidase activities.

C_2H_4 production and firmness

Figure 2 shows the correlation between C_2H_4 production and firmness in treated and untreated kiwifruit, respectively. The correlation between firmness and C_2H_4 production in the treated was higher than in the untreated samples.

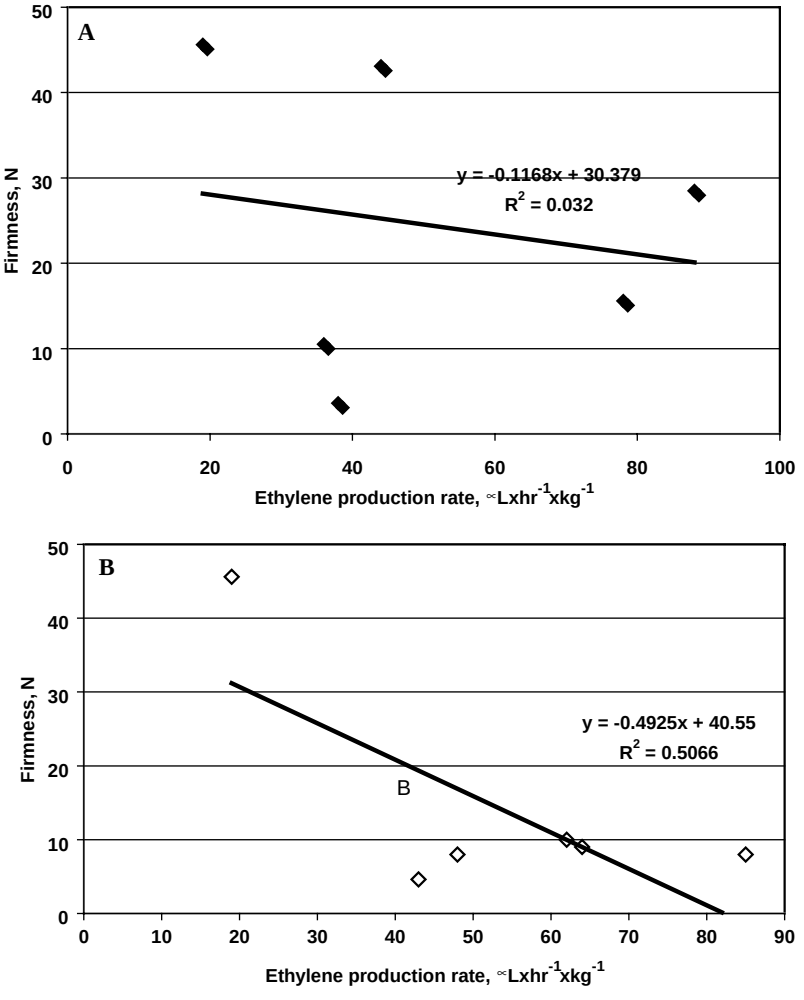

Figure 2. Correlation between the ethylene production and firmness of kiwifruit during 10 days ripening: (A) untreated and (B) treated samples.

Acidity and pH

The acidity and pH in the treated and untreated kiwifruit were gradually decreased (Table II). The acidity decrease began from the sixth day of ripening and was significantly higher than in the untreated samples.

The pH value fluctuated during ripening.

Dietary fiber

At the beginning of the ripening process, the total dietary fiber content was 24.7%, the soluble dietary fiber was 6.4%, while the insoluble dietary fiber was about 18.3% on a dry weight basis.

There were no differences in the contents of total insoluble and soluble dietary fibers in the treated and untreated kiwifruit samples after 10 days of ripening (data not shown).

Table II. Changes in acidity and pH in treated and untreated kiwifruits.

Parameter	Sample	Days of ripening					
		0	2	4	6	8	10
Acidity (%)	Untreated	1.65 ± 0.3^{a}	1.5 ± 0.3^{a}	1.3 ± 0.3^{a}	1.2 ± 0.3^{a}	1.0 ± 0.3^{a}	0.9 ± 0.3^{a}
	Treated	1.64 ± 0.3^{a}	1.3 ± 0.3^{a}	1.2 ± 0.3^{a}	0.8 ± 0.2^{b}	0.7 ± 0.2^{b}	0.6 ± 0.2^{b}
pH	Untreated	3.59 ± 0.4^{a}	3.67 ± 0.4^{a}	3.59 ± 0.4^{a}	3.70 ± 0.4^{a}	3.50 ± 0.4^{a}	3.56 ± 0.4^{a}
	Treated	3.57 ± 0.4^{a}	3.72 ± 0.4^{a}	3.51 ± 0.4^{a}	3.76 ± 0.4^{a}	3.55 ± 0.4^{a}	3.61 ± 0.4^{a}

Means in rows without common letters differ significantly ($P < 0.05$).

Protein content

The protein content of the extract was 29 mg/ml and the application to the gel was about 3.88 mg/ml. After separation with SDS-PAGE electrophoresis 14 bands were detected, with sharpness in the range of 36 kDa and diffusion in the range of 20 kDa and lower (Figure 3). Nevertheless, we obtained more detected bands in a zone of 32 kDa, lower than those of Watsuji et al. (1992) and Möller et al. (1997).

The use of a longer gel (14 cm instead of 7 cm) gave us more distinct and sharp bands, especially in the zone higher than 36 kDa. At the end of the ripening period, the protein qualities of both groups of samples were without significant differences. The protein profile of the sampling dates of the experiment are not shown, because the comparison between the electrophoretic bands of non-treated fruits and the treated ones during the treatment period did not show drastic changes.

Total polyphenols and of the antioxidant activity

The changes in the content of total polyphenols and of the antioxidant activity tests (DPPH, β-carotene and ABTS with $K_2S_2O_8$) are presented in Table III. As it can be seen, the content of total polyphenols and the antioxidant potential in treated kiwifruit significantly increased on the sixth day after the beginning of the ripening process.

Figure 4 shows that there is a linear correlation between the total polyphenol content and the antioxidant activity as determined by ABTS test with $K_2S_2O_8$ as with other antioxidant methods.

Discussion

Kiwifruit is one of the main crops of Korea, which in recent years has become popular in the USA and Europe. However, this fruit is not edible even at the maturation stage: it has hard firmness and high acidity (Park 1996, 2002). There are different proposals to eliminate the hard firmness and high acidity of the kiwifruit (Park 2002; Antunes and Sfakiotakis 2002a) and the ethylene treatment seems preferable. Ethylene treatment decreases the firmness and acidity, and increases contents of fructose, glucose, sucrose and soluble solids and enhances the edible quality of kiwifruits; the best results could be achieved when the ripening process takes place at a temperature of 20°C (Park 2002; Antunes and Sfakiotakis 2002a). It was therefore decided to investigate the possible changes in the aforementioned and other variables using ethylene treatment of kiwifruit samples at a temperature of 20°C.

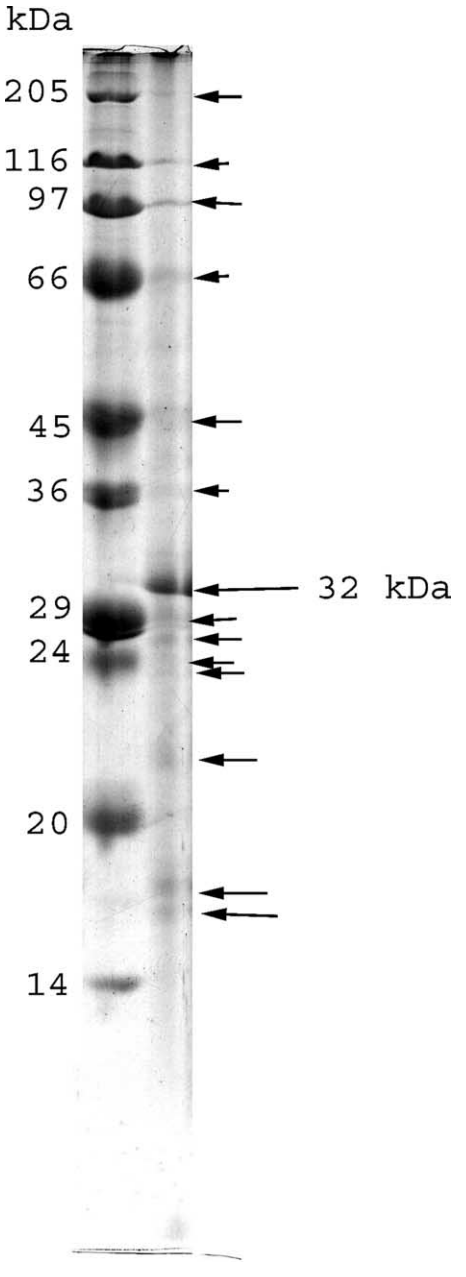

Figure 3. Band intensity of proteins extracted from kiwifruits and separated by SDS-PAGE: lane 1, molecular markers 205-kDa myosin, 116-kDa β-galactosidase, 97-kDa phosphorylase b, 66-kDa albumin, 45-kDa ovalbumin, 29-kDa carbonic anhydrase, 20-kDa trypsin inhibitor, 14-kDa α-lactalbumin; lane 2, last day of ripening phase.

The sensory value ranged from 1 to 5 (bad, normal, good, very good, excellent) and it consisted of sweetness, texture and flavor. Visual score also ranged from 1 to 5 (bad, normal, good, very good, excellent) and related to the flesh (flesh color, browning).

Table III. Changes in total polyphenols and in the antioxidant potential as determined by different scavenging assays in treated and untreated kiwifruits.

Compound	Sample	Days of ripening					
		0	2	4	6	8	10
Total polyphenols (mg/100 g fresh weight)	Untreated	131.8 ± 10.1^{a}	131.7 ± 10.1^{a}	131.8 ± 10.2^{a}	131.6 ± 10.3^{a}	131.5 ± 10.2^{a}	131.4 ± 10.1^{a}
	Treated	131.9 ± 10.2^{a}	132.1 ± 10.2^{a}	134.4 ± 10.3^{a}	180.8 ± 10.5^{b}	180.6 ± 10.4^{b}	180.9 ± 10.4^{b}
DPPH (% of inhibition)	Untreated	57.1 ± 4.9^{a}	57.2 ± 4.9^{a}	57.3 ± 4.9^{a}	57.2 ± 4.9^{a}	57.1 ± 4.9^{a}	57.2 ± 4.9^{a}
	Treated	56.9 ± 4.8^{a}	57.9 ± 5.1^{a}	61.1 ± 5.2^{a}	81.3 ± 6.3^{b}	81.1 ± 6.3^{b}	81.2 ± 6.3^{b}
β-carotene (% of inhibition)	Untreated	48.2 ± 4.1^{a}	48.1 ± 4.1^{a}	48.2 ± 4.1^{a}	48.2 ± 4.1^{a}	48.2 ± 4.1^{a}	48.2 ± 4.1^{a}
	Treated	48.3 ± 4.2^{a}	48.2 ± 4.2^{a}	49.9 ± 4.2^{a}	63.3 ± 5.3^{b}	63.2 ± 5.3^{b}	63.4 ± 5.3^{b}
ABTS (nM Trolox equivalent/g)	Untreated	12.1 ± 1.1^{a}	12.2 ± 1.1^{a}	12.0 ± 1.1^{a}	12.1 ± 1.1^{a}	12.2 ± 1.1^{a}	12.0 ± 1.1^{a}
	Treated	12.2 ± 1.1^{a}	12.3 ± 1.1^{a}	12.9 ± 1.1^{a}	22.8 ± 1.8^{b}	22.9 ± 1.9^{b}	22.7 ± 1.7^{b}

Means in rows with different letters differ significantly ($P < 0.05$).

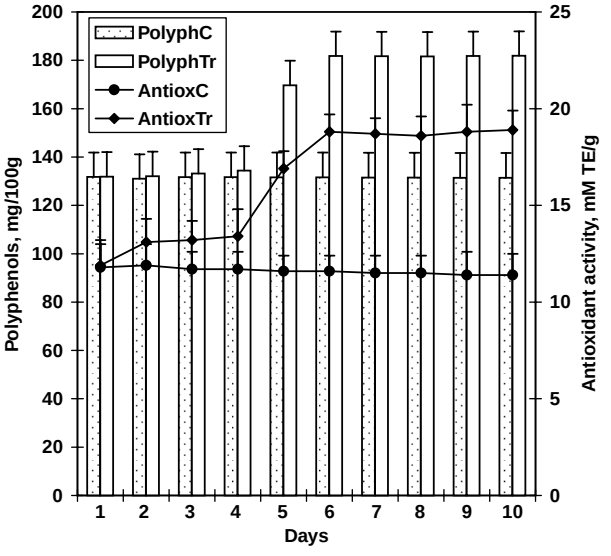

Figure 4. The content of total polyphenols and antioxidant activity determined by the ABTS method during the time of treatment. PolyphC, PolyphTr, total polyphenols in untreated and treated samples; AntioxC, AntioxTr, total antioxidant activities in untreated and treated samples.

The visual score did not change at the initial stage, and even after 10 days of the ripening process was minimal in both groups. The sensory value was increased in both kiwifruit groups, but the increase was significant only in treated samples. The increase in the sensory value was registered together with the decrease in the firmness.

The changes in proteins, total polyphenol content and in antioxidant potential in kiwifruit were less investigated (Yamanaka et al. 2004). Therefore, in this investigation study on the possible changes in kiwifruit proteins, total polyphenol content and antioxidant potential was the main aim.

The results of our investigation have shown that the flesh firmness and acidity in the treated samples were decreased significantly and reached minimum levels after 2 days of treatment. The decrease in these variables in the untreated samples was gradual and reached a minimum only at the end of the ripening. The loss of firmness and acidity in fruits treated with ethylene was significantly higher than in the untreated samples, and the results were similar to those of Mashmichi and Hasegawa (1993).

The decrease of the fruit firmness was accompanied by an increase in the sensory value in both ethylene-treated and untreated kiwifruit samples. However, the increase was significant only in treated samples.

We found that the SSC increase was correlated with the decrease in acidity during ripening of kiwifruits. Also others have reported that there is a significant correlation between these two variables (Hyudo and Fukasawa 1985; Leong and Shui 2002).

The contents of fructose, glucose and sucrose in kiwifruit samples treated with ethylene were increased and were significantly higher than in the untreated samples. These results were expected: we have already reported that free sugar content increased with ethylene production and reached maximum levels before full softening

(Park 2002), and these results corresponded with Beever and Hopkirk (1990), who found that the free sugar content was significantly higher in kiwifruit treated with ethylene than in untreated samples. We suppose that ethylene treatment enhances the free sugar content by improving sugar metabolism in kiwifruit.

We found that the increase in ethylene biosynthesis, CO_2 production, ACC, ACC synthase and ACC oxidase in the ethylene-treated fruits began after 2 days of ripening and was followed by a decrease. The same futures were observed also in the untreated samples but the increase began after 4 days of the ripening. These observations are in accordance with the data of others as well as with Ikoma (1996), who showed that endogenous ethylene content is not enough to induce ripening of kiwifruits, and therefore it is necessary to use exogenous ethylene treatment. A different explanation is given by Antunes and Sfakiotakis (2002a), who showed that exposing kiwifruit to chilling temperatures (0–10°C) for 12 days advanced ethylene biosynthesis and ripening when compared with fruit held continuously at 20°C. According to Antunes and Sfakiotakis (2002a, 2002b), the autocatalytic ethylene production correlated with increased ACC content, and increased the activities of ACC synthase and ACC oxidase. Fruit held continuously at 20°C started autocatalytic ethylene production after 20 days, with concomitant increases in ACC content, ACC synthase and ACC oxidase activities and ripening. It is concluded that kiwifruit stored at 20°C behaves as a typical climacteric fruit, while at 10°C it behaves like a non-climacteric fruit. Considerable variation in ethylene production rates between individual fruit was observed on fruits of similar firmness. The fruit itself does not produce ethylene until it softens to a flesh firmness less than 10 N (Feng et al. 2003), and these results correspond to the present report.

As can be seen, the study of the aforementioned variables did not produce new findings: the observed changes were similar to data described by other workers. However, the investigation of kiwifruit protein reveals some changes that are shown for the first time.

The purity of the isolated peptides was tested by SDS-PAGE and the characteristic of peptides were identified after the extraction with TRIS and sample buffers. The displayed numbers of SDS patterns from the kiwifruit (180, 116, 97, 66, 45, 36, 32, 29, 26, 25, 24, 22, 18, 16 kDa) showed the molecular masses of the specific peptides.

The data about the electrophoretic profiles of treated and untreated samples are very limited; therefore it was difficult to compare our results with other investigations.

Our data confirm the results shown by Watsuji et al. (1992) that the electrophoretic patterns of soluble proteins of three cultivars showed a band around 60 kDa. It was also shown that some bands around 30 kDa were apparently observed during the development of fruit. The highest protease activity in a gradient polyacrylamide gel was found around the 13 kDa protein. The ripening of kiwifruit was accelerated by ethylene treatment, and the protease activity in the fruit slightly declined. Möller et al. (1997) also used the SDS-electrophoretic separation of proteins extracted with acetone/diethylether. In this report proteins were not precipitated previously, but directly applied on the gel using the procedure of Sass-Kiss and Sass (2002).

There was only a slight difference in the protein profile of these two samples; the 32 kDa protein band was more distinguished (protein content about 20% higher than in untreated samples). The results are not shown for the untreated sample because the electrophoretic lane was similar. The results of isolated protein

bands are in agreement with other studies showing the isolated proteins were concentrated in the range of 67, 43, 27 and 22 kDa (Watsuji et al. 1992; Tello-Solis et al. 1995; Möller et al. 1997).

In the present report the proteins were preliminary, not precipitated, but the method was modified using different gel concentrations. We suppose that bigger amount of detected bands may be a favorable factor in searching species-specific peptides in kiwifruit.

We did not find a clear relation between the changes in kiwifruit proteins and other studied variables. In our opinion, these changes could be related to the amount of used ethylene. However, it has to be proved in future experiments.

Ethylene treatment had significantly increased the total polyphenol content and the related antioxidant potential in kiwifruit as determined by three different assays (DPPH, β-carotene and ABTS with $K_2S_2O_8$). Our data are in accordance with other workers who have shown that high total polyphenol content increases antioxidant activity and that there is a linear correlation between these two variables (Velioglu et al. 1998).

The comparison between the protein profiles of the treated samples with non-treated ones showed that the only difference was in the 32 kDa band, in its amount of the protein. The 13 kDa band was also more prominent on the last day of ethylene treatment.

The changes in the protein profile can be connected with the increase in the activity of enzymes and probably with the increase of the quality of the fruit. Electrophoresis may therefore be used as a tool for control of protein changes after ethylene treatment.

Conclusions

The ethylene treatment of kiwifruits leads to positive changes in most of the studied kiwifruit compounds and increases fruit antioxidant potential.

Ethylene treatment shortens the ripening time and improves fruit quality by increasing the antioxidant activity.

Some minor changes in the protein profile did not affect the fruit taste and quality.

Acknowledgements

This paper was partly supported by International Cooperation Program from Mokpo National University, Korea, 2004. The authors are thankful to Mrs Elena Katrich (Hebrew University of Jerusalem, School of Pharmacy) for technical assistance.

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