DOI: 10.1111/j.1745-4514.2009.00244.x

RADICAL SCAVENGING CAPACITY OF ETHYLENE-TREATED KIWIFRUIT

YONG-SEO PARK[1], SOON-TECK JUNG[2], SEONG-GOOK KANG[2], BUK GU HEO[3], SANG-HYUN LEE[4], FERNANDO TOLEDO[5], PATRICIA ARANCIBIA-AVILA[5], JERZY DRZEWIECKI[6] and SHELA GORINSTEIN[7]*†

[1]*Department of Horticultural Science*

[2]*Department of Food Engineering*
Mokpo National University
Muan, Jeonnam, Korea

[3]*Naju Foundation of Natural Dyeing Cultural Institute*
Naju, Korea

[4]*Pear Experiment Station, RDA*
Naju, Jeonnam, Korea

[5]*Department of Basic Sciences*
Universidad del Bio-Bio
Chillan, Chile

[6]*Plant Breeding and Acclimatization Institute*
Radzikow, Poland

[7]*Department of Medicinal Chemistry and Natural Products*
School of Pharmacy, The Hebrew University – Hadassah Medical School
Jerusalem, Israel

Accepted for Publication January 14, 2008

ABSTRACT

The aim of this investigation was to compare the content of the polyphenols and ascorbic acid and the radical scavenging capacity of ethylene-treated and nontreated kiwifruit. It was found that the contents of these bioactive compounds determined by UV spectroscopy and fluorometry were higher in ethylene-treated kiwifruit than in the nontreated ones. In order to support the obtained data, two different antioxidant assays were used. Antioxidant

† S.G. is affiliated with the David R. Bloom Center for Pharmacy.
* Corresponding author. TEL: 972-2-6758690; FAX: 972-2-6757076; EMAIL: gorin@cc.huji.ac.il

Journal of Food Biochemistry **33** (2009) 674–692.

capacity in total polyphenol extracts of treated kiwifruit measured by ferric-reducing/antioxidant power (FRAP) and Trolox equivalent antioxidant capacity (TEAC) assays were significantly higher than that of nontreated. Higher correlation coefficients were obtained with polyphenols and antioxidant activity than with ascorbic acid. FRAP produced the most consistent measurements for ethylene-treated kiwifruit. The investigated samples showed similar protein bands during the whole period of treatment. Main protein bands appeared in the range of 20–35 kDa. The radical scavenging capacity of the kiwifruit can be used for the determination of the ripening.

PRACTICAL APPLICATIONS

It is an established fact that supplementation of diet with fruits prevents atherosclerosis and other diseases. A new method was developed and applied for estimation of the duration of kiwifruit ethylene treatment, which is based on the comparison of the radical scavenging capacity and polyphenols in treated and nontreated fruits. Radical scavenging capacity can be determined by different antioxidant tests, which makes such application easy to use practically. The results of this research showed that the bioactivity of kiwifruit increased during the ethylene treatment and its maximum was registered at the sixth day. This is the maximum of the treatment of the fruit, and the bioactive compounds achieved the peak amounts. The main bioactive compounds in kiwifruit with high antioxidant activity prevent the oxidation of low density cholesterol, and can be recommended as a supplementation to the diet and as a part in functional foods.

INTRODUCTION

Kiwifruit is rich in bioactive compounds especially in polyphenols (Park *et al.* 2006a). It is an established fact that supplementation of diet with fruits and vegetables prevents atherosclerosis and other diseases (Motohashi *et al.* 2002; Duttaroy and Jørgensen 2004). Macrophages and arteriosclerosis, the leukocyte activation action and the antioxidant effect of kiwifruit were discussed (Yamazaki *et al.* 2000; Collins *et al.* 2001). It was shown that consumption of kiwifruit lowered blood triglycerides levels by 15% compared with control. All these data indicate that consuming kiwifruit may be beneficial in cardiovascular disease. The authors reported that consuming two or three kiwifruit per day for 28 days reduced platelet aggregation response to collagen (Duttaroy and Jørgensen 2004). It was demonstrated that consumption of certain berries and fruits such as blueberries, mixed grape and kiwifruit, was

associated with increased plasma hydrophilic (H-) or lipophilic (L-) antioxidant capacity (AOC) measured as Oxygen Radical Absorbance Capacity (ORAC). AOC in the postprandial state and consumption of an energy source of macronutrients containing no antioxidants was associated with a decline in plasma AOC (Prior *et al.* 2007).

In our recent studies, different fruits, among them kiwifruit, were tested (Park *et al.* 2006a; Haruenkit *et al.* 2007). There are a number of reports dealing with different varieties and cultivars of kiwifruit. Okamoto and Goto (2005), Nishiyama *et al.* (2004) compared different cultivars with the imported kiwifruit (cv. Hayward). The comparison was based on the amount of bioactive compounds in order to evaluate their compositional characteristics, especially as a possible "healthy fruit."

The mechanism of ethylene biosynthesis, ethylene production during the ripening period of fruits and the effect of ethylene on ripening, the production of ethylene in crisp hard-to-soften peach, the softening of pear and low temperatures treatment and kiwifruit that does not soften on the tree was discussed by Tatsuki (2007).

The parameters analyzed for postharvest quality were: weight loss, external appearance, firmness, color, total titrable acidity, total soluble solids and total soluble sugar content. Even in one of the cited reports, the changes in the bioactive and protein compounds were not mentioned. In our previous investigations (Park *et al.* 2006a,b), the comparison was done on the change in bioactive compounds.

This report shows the composition of the main bioactive compounds during ripening of ethylene-treated kiwifruit.

2, 2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenger and reduction of ferric-tripiridyltriazine [Fe(III)-TPTZ] to a ferrous form Fe(II) assays were applied in order to choose the most suitable radical scavenging method for the process of ripening.

As far as we know there are not such published reports.

MATERIALS AND METHODS

Chemicals

Trolox (6-hydroxy-2,5,7,8,-tetramethyl-chroman-2-carboxylic acid); butylated hydroxyanisole (BHA); 2,2′-azobis-2-methyl-propanimidamide; $FeCl_3x6H_2O$; Folin-Ciocalteau reagent; Cu(II)-neocuproine (Nc), lanthanum (III) chloride heptahydrate, potassium persulfate, sodium dodecyl sulfate (SDS), β-mercaptoethanol (β-ME), acrylamide, polyacrylamide, Coomassie Brilliant Blue R and molecular weight marker (14–66 kDa) were obtained

from Sigma Chemical Co. (St. Louis, MO). 2, 4, 6-tripyridyl-*s*-triazine (TPTZ) was purchased from Fluka Chemie (Buchs, Switzerland). All reagents were of analytical grade. Deionized and distilled water were used throughout.

Samples and Preparation

"Hayward" kiwifruits harvested in October 2006 were from Muan, Korea, and were purchased from the same farmer.

Fruits with an average weight of 100 g were cleaned with tap water and placed in a glass jar. The fruits were randomly divided into two groups: nontreated (NT) and ethylene treated (T), and were ripened immediately after harvest. Kiwifruit samples of the T group were treated with ethylene in concentration of 100 ppm for 24 h at 20C in a growth chamber (Percival Scientific Inc., Perry, IA). The samples were put into an 18 L glass jar and ventilated with humidified flow of air (NT) or air mixed with ethylene (T) at 300 mL/min. Then, the ethylene and air-treated kiwifruits were ripened separately at the same conditions at 20C in a growth chamber (Percival Scientific Inc.) for 10 days.

Extraction and Hydrolysis of Total Polyphenols

A 50 mg aliquot of lyophilized sample was accurately weighed in a screw-capped tube. The total phenols were extracted with 5 mL of 1.2 M HCl in 50% methanol/water (TP). The samples were vortexed for 1 min and heated at 90C for 3 h with vortexing every 30 min. The samples were cooled, diluted to 10 mL with methanol and centrifuged for 5 min at 4,000 × g with a bench-top centrifuge to remove solids. This procedure was described in detail in Park *et al.* (2006b).

Fluorescence

Fluorescence measurements were done using a model FP-6500, Jasco spectrofluorometer, serial N261332 (Tokyo, Japan). Fluorescence emission spectra measurements were performed at a concentration of 0.02 mg/mL and taken at three excitation wavelengths of 270 nm and emission of 290 nm, and recorded over the frequency range from the excitation wavelength to a wavelength of 500 nm. Standards of gallic acid, quercetin and trolox of 0.01 mM in methanol were used (Gorinstein *et al.* 2001).

Total Polyphenols Determination

The Folin-Ciocalteu method was used, and the measurement was performed at 765 nm with gallic acid as the standard (Singleton *et al.* 1999).

Total Condensed Tannins

The analysis of condensed tannins (procyanidins) was carried out according to Heimler *et al.* (2006). A 3 mL amount of a 4% methanol vanillin solution and 1.5 mL of concentrated hydrochloric acid were added to 50 μL of the diluted sample. The absorption was measured at 500 nm against methanol as a blank. The amount of total condensed tannins is expressed as (+) catechin equivalents (CT, mg (+) catechin/g sample).

Total Ascorbic Acid

The water extract was prepared from 100 mg of dry sample and 5 mL of water, mixed and stirred for 24 h, then centrifuged. Then, 1 mL of the extract was mixed with 2 mL of 3.0×10^{-3} M of lanthanum (III) chloride heptahydrate, then extracted with ethylacetate (EtAc) in order to avoid the interference of flavonoids. The aqueous phase was used for determination of ascorbic acid by CUPRAC assay. One mL of Cu(II)-neocuproine (Nc), in ammonium acetate-containing medium at pH 7, was mixed with 1 mL of the obtained extract, where the absorbance of the formed bis(Nc)-copper(I) chelate was measured at 450 nm (Ozyurek *et al.* 2007).

Determination of Total Anthocyanins

The total anthocyanins were measured by a pH differential method. Absorbance was measured in a Beckman spectrophotometer at 510 nm and at 700 nm in buffers at pH 1.0 and 4.5, using $A = [(A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}]$. Results were expressed as mg of cyanidin-3-glucoside equivalent (CGE)/100 g of FW (Cheng and Breen 1991).

Determination of the Antioxidant Capacity

ABTS scavenging assay: the $ABTS^{+}$ was generated by the interaction of ABTS (250 μM) and $K_2S_2O_8$ (40 μM). The absorbance was monitored exactly 1 and 6 min after the addition of 990 μL of $ABTS^{+}$ solution to 10 μL of kiwifruit extracts or Trolox standards (final concentration 0–20 μM) in methanol or phosphate-buffered saline (pH 7.4). For the modified assay, ABTS was dissolved in 20 mM acetate buffer (pH 4.5) and prepared with potassium persulfate as described above. Trolox equivalent antioxidant capacity (TEAC) was estimated.

Ferric-tripiridyltriazine [Fe (III)-TPTZ] is reduced to a ferrous form Fe (II), which absorbs light at 593 nm in FRAP (ferric-reducing/antioxidant power) assay.

FRAP reagent (2.5 mL of a 10 mM ferric-tripiridyltriazine solution in 40 mM HCl plus 2.5 mL of 20 mM $FeCl_3 \cdot H_2O$ and 25 mL of 0.3 M acetate

buffer, pH 3.6) of 900 µL was mixed with 90 µL of distilled water and 30 µL of kiwifruit samples or methanol as the appropriate reagent blank. The absorbance was measured at 595 nm.

The antioxidant activities of standards such as synthetic antioxidants, phenolic acids and flavonoids were determined using these methods.

Two antioxidant assays (ABTS and Fe [III] – TPTZ) were compared at the same time durations (30, 60 and 90 min) and the same concentration of the investigated fruit's methanolic extracts. For each individual antioxidant assay, a trolox aliquot (Ozgen *et al.* 2006) was used to develop a standard curve. All data were then expressed as trolox equivalents (TE).

SDS-polyacrylamide Gel Electrophoresis

Kiwifruit juice was used for SDS-PAGE, which was carried out according to Laemmli (1970) using a Hoeffer SE-600 vertical gel apparatus (Hoefer Scientific, San Francisco, CA) with the resolving gel was 13.7% T and 1.7% C, and the stacking gel was 3.8% T and 1.8% C, as detailed in Park *et al.* (2006a).

Statistical Analyses

The results of this investigation are means ± SD of three measurements. Differences between groups were tested by two-way analysis of variance. In the assessment of the antioxidant potential, Spearman correlation coefficient (R) was used. Linear regressions were also calculated. $P < 0.05$ was considered significant.

RESULTS

Fluorimetric Spectra, Total Polyphenols, Flavonoids, Anthocyanins, Tannins and Ascorbic Acid

The fluorimetric data supported our previous results that polyphenol methanol extracts have the maximum absorptions of their spectra from 198.3 to 205.4 nm; therefore, we present only the fluorimetric spectra, where various contents of phenolic compounds were detected in the extracts, depending on the extraction solvent and the stage of ripening. Total polyphenols (Fig. 1A) at different stage of ripening (1, 4, 6, 10 days) of NT samples showed only one maximum in their spectra of 340–352 nm, changing only the absorbance units from 19.26 to 52.84. The maximum in their spectra slightly changed at the sixth day of ripening to 341 nm with an increase of absorbance units of about 2.5 times, and then on the 10th day moved to the wavelength of about 352 nm and with lower absorbance units than on the sixth day.

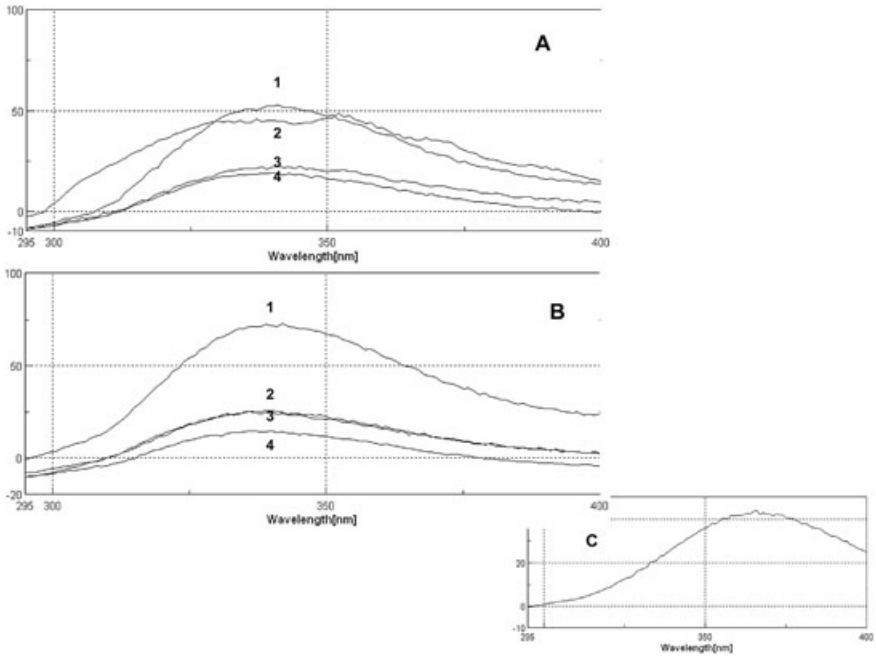

FIG. 1. FLUORIMETRIC SPECTRA OF METHANOL EXTRACTS TAKEN AT EXCITATION WAVELENGTHS OF 270 Nm AND EMISSION OF 290 Nm AND RECORDED OVER THE FREQUENCY RANGE FROM THE EXCITATION WAVELENGTH TO A WAVELENGTH OF 500 NM

(A) total polyphenols (0.02 mg/mL) of NT kiwifruit during storage time of 6 days (1), 10 days (2), 4 days (3) and 1 day (4). (B) total polyphenols (0.02 mg/mL) of T kiwifruit during storage time of 6 days (1), 10 days (2), 4 days (3) and 1 day (4). (C) gallic acid (0.01 mM).

The total polyphenol extracts (Fig. 1B) at different stages of ripening (1, 4, 6, 10 days) of the treated samples showed only one maximum in their spectra of 336–342 nm, changing the absorbance units from 72.73 to 14.73.

The maximum in the spectra was slightly changed on the 6-th day of ripening to 342 nm with an increase of absorbance units of about five times. Then, the maximum shifted on the 10th day to the wavelength of 340 nm without changing the absorbance units in comparison with the fourth day. The standards of gallic acid (Fig. 1C) had the maximum at 366 nm. The contents of phenolic compounds (mg GAE/g DW) in the T kiwifruit extracts (Table 1) during 10 days treatment were in the range from 14.54 ± 2.47 to 26.54 ± 2.56; flavonoids (mgCE/g): from 0.27 ± 0.02 to 0.47 ± 0.04; anthocyanins (mg CGE/g): from 0.063 ± 0.004 to 0.134 ± 0.100; tannins (mg/g): from 2.65 ± 0.9 to 4.20 ± 0.9; and ascorbic acid: from 0.207 ± 0.02 to

TABLE 1.
BIOACTIVE COMPOUNDS IN DIFFERENT KIWIFRUIT SAMPLES

Kiwifruit samples	Polyphenols (mgGAE/g)	Flavonoids (mgCE/g)	Anthocyanins (mg CGE/g)	Tannins (mg/g)	Ascorbic acid (mg/g)
NT 1	12.47 ± 0.90^{a}	0.15 ± 0.01^{a}	0.010 ± 0.001^{a}	2.20 ± 0.9^{a}	0.165 ± 0.01^{a}
NT 4	13.25 ± 0.90^{a}	0.22 ± 0.02^{b}	0.021 ± 0.002^{b}	2.52 ± 0.9^{a}	0.170 ± 0.01^{a}
NT 6	13.92 ± 1.00^{a}	0.24 ± 0.02^{b}	0.023 ± 0.002^{b}	2.67 ± 0.9^{a}	0.194 ± 0.01^{b}
NT 10	14.37 ± 1.00^{b}	0.27 ± 0.02^{b}	0.042 ± 0.003^{c}	2.64 ± 0.9^{a}	0.201 ± 0.02^{b}
T 1	14.54 ± 2.47^{b}	0.27 ± 0.02^{b}	0.063 ± 0.004^{d}	2.65 ± 0.9^{a}	0.207 ± 0.02^{b}
T 4	21.14 ± 1.30^{c}	0.32 ± 0.03^{c}	0.077 ± 0.005^{e}	2.65 ± 0.9^{a}	0.221 ± 0.02^{b}
T 6	26.50 ± 2.53^{d}	0.41 ± 0.04^{d}	0.117 ± 0.009^{f}	3.47 ± 0.9^{b}	0.230 ± 0.02^{b}
T 10	26.54 ± 2.56^{d}	0.47 ± 0.04^{d}	0.134 ± 0.100^{f}	4.20 ± 0.9^{c}	0.233 ± 0.02^{b}

Values are means ± SD of 3 measurements. Means in columns without superscript letters in common differ significantly ($P < 0.05$).
NT, nontreated kiwifruit samples; NT1, NT4, NT6, NT10, nontreated kiwifruit samples after 1, 4, 6 and 10 days storage; T, treated kiwifruit samples; T1, T4, T6, T10, treated kiwifruit samples after 1, 4, 6 and 10 days storage; GAE, gallic acid equivalent; CE, catechin equivalent; CGE, cyanid-3-glucoside equivalent.

0.233 ± 0.02 mg/g. Bioactive compounds changed in T and NT samples mostly at 1, 4, 6 and 10 days; therefore, in Table 1, only these days are shown.

Antioxidant Capacities and Correlation

The content of total polyphenols, and related total antioxidant capacities by Fe(III) –TP and ABTS significantly increased in ethylene-treated (Fig. 2B) than in the NT kiwifruit samples (Fig. 2A) after the middle of the treatment, starting from the 5th day ($P < 0.05$).

The Fe (III)-TP and ABTS values for each extract were compared and correlated with the total phenolic contents. The relationship between the values of total polyphenol concentrations of NT samples versus antioxidant capacities for Fe (III)-TP and ABTS were 0.8845, 0.7229, respectively. For ethylene-treated samples, the calculations showed the following order between the polyphenols and the antioxidant capacities determined by the two methods: 0.9321 and 0.7423, respectively. The results are rather interesting, as there is an excellent linear response, especially for the ethylene-treated samples only for Fe (III)-TP ($R^2 = 0.93$). The overall estimation of the presented data showed that after ethylene treatment, the correlation coefficients were higher than in the NT kiwifruits. The ascorbic acid showed lower correlation of about 0.76 in comparison with 0.93.

The expected correlation between the polyphenols and Fe (III)-TP and ABTS values is known, because all these assays are similar and working by the same mechanism (single electron transfer). The antioxidant activities of fruit

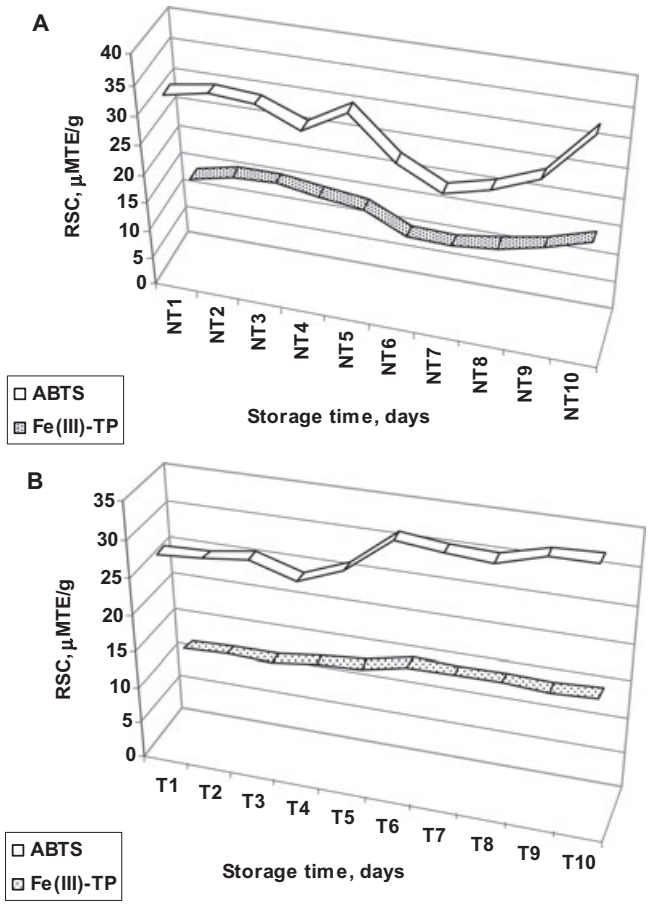

FIG. 2. CHANGES IN TOTAL POLYPHENOL EXTRACTS OF KIWIFRUIT BIOACTIVITY DURING 10 DAYS OF RIPENING
(A) antioxidant capacity (µMTE/g) by ABTS and Fe (III)-TP in NT kiwifruits; (B) antioxidant capacity (µMTE/g) by ABTS and Fe (III)-TP in kiwifruits under ethylene treatment (T). Abbreviations: NT, air-treated samples; T, ethylene treated samples; ABTS, 2, 2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid); Fe (III)-TP, ferric-tripiridyltriazine; RSC, radical scavenging capacity, expressed as µMTE (Trolox equivalent)/g DW.

extracts depend on the time of assays used, therefore the samples were measured at the same concentration and the same periods of time: 30, 60 and 90 min (Fig. 3A,B). As it was explained above, ABTS and Fe (II)-TP were used also as a comparison for a longer time and with different pH for ABTS. The obtained results using these methods were higher for Fe (II)-TP

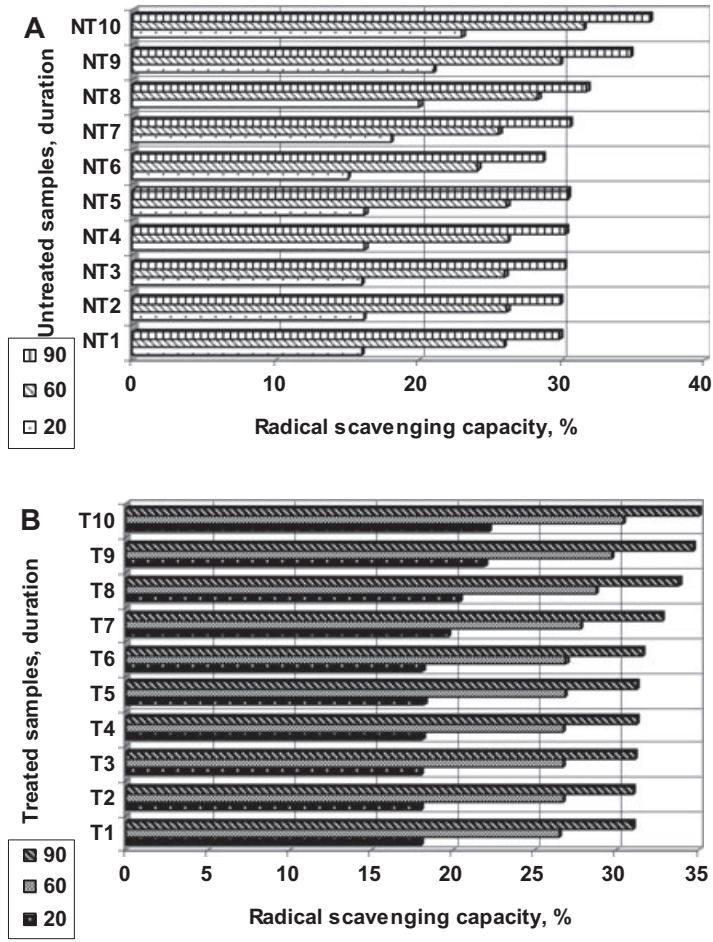

FIG. 3. CHANGES IN RADICAL SCAVENGING CAPACITY OF TOTAL POLYPHENOL EXTRACTS OF KIWIFRUIT DURING 10 DAYS OF RIPENING, USING ABTS ANTIOXIDANT TEST DURING DIFFERENT PERIODS OF TIME
(A) %RSA by ABTS of nontreated kiwifruit samples (NT); (B) %RSA by ABTS of kiwifruit samples under ethylene treatment (T).

(Fig. 4A,B) than in the same method, but only for a short time. The data for ABTS did not change (Fig. 3A,B) as much as for Fe (II)-TP (Fig. 4A,B).

Protein Profiles

The comparison between the electrophoretic bands of NT fruits and the ethylene-treated ones during the 10 days period did not show drastic changes

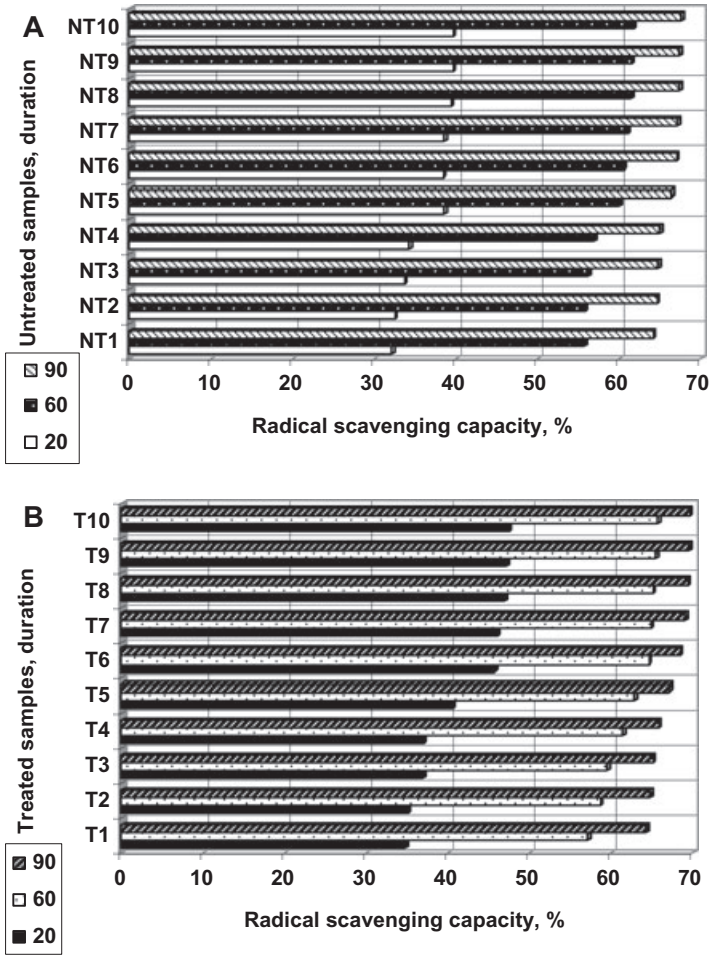

FIG. 4. CHANGES IN RADICAL SCAVENGING CAPACITY OF TOTAL POLYPHENOL EXTRACTS OF KIWIFRUIT DURING 10 DAYS OF RIPENING USING FE (III)-TP, FERRIC-TRIPIRIDYLTRIAZINE. ANTIOXIDANT TEST FOR DIFFERENT PERIODS OF TIME (A) %RSA by Fe(III)-TP, ferric-tripiridyltriazine of nontreated kiwifruit samples (NT); (B) %RSA by Fe(III)-TP, ferric-tripiridyltriazine of kiwifruit samples under ethylene treatment (T).

(Fig. 5). Therefore, only some bands are shown on the SDS-PAGE. After separation with SDS-PAGE electrophoresis, four bands were detected at the molecular weights of 16 (average bands), 20 and 24 (diffused and minor), and the major ones with sharpness are in the range of 32 kDa.

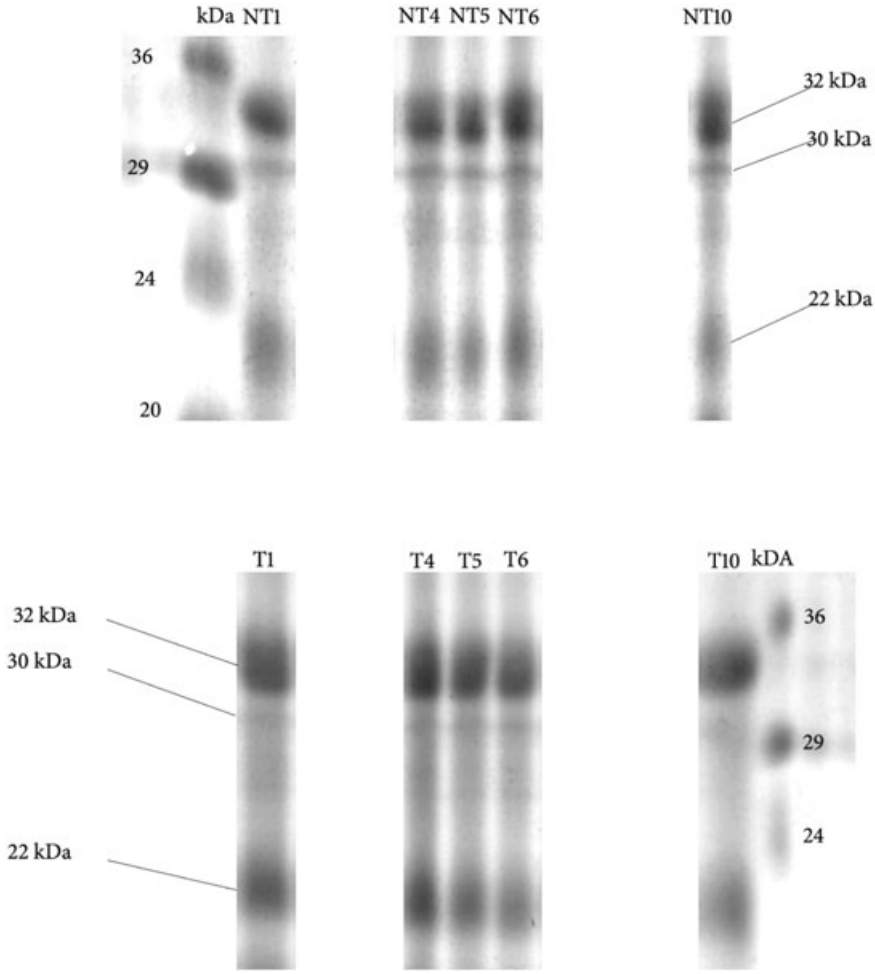

FIG. 5. BAND INTENSITIES OF PROTEINS EXTRACTED FROM KIWIFRUITS AND SEPARATED BY SDS-PAGE: LANES NT1, NT4, NT5, NT6, NT10, NONTREATED; LANES T1, T4, T5, T6, T10, TREATED SAMPLES, RESPECTIVELY FROM 1, 4, 5, 6 AND 10 DAYS. MOLECULAR WEIGHT MARKERS: (KDA): 36-GLYCERALALDEHYDE-3-PHOSPHATE DEHYDROGENASE; 29-CARBONIC ANHYDRASE; 24-TRYPSINOGEN; 20-TRYPSIN INHIBITOR, LOADING 2 μL

DISCUSSION

The present investigation is in agreement with Schröder and Atkinson (2006) and Vilas-Boas and Kader (2007), who showed the best results extend-

ing the postharvest life of fruits with the time-dependent ethylene treatment. In this connection, kiwifruit is an excellent model to study the fruit softening, with the phase associated to the most extensive and rapid loss of firmness being well separated temporally from that associated with the respiratory climacteric and ethylene production.

The results of polyphenols content in the last harvest were higher that in the previous (Park *et al.* 2006b), because then, only free polyphenols were extracted.

Our results correspond with Okamoto and Goto (2005) who compared cv. Hayward with other cultivars and apple. Vitamin C content in kiwifruit was higher than in lemon and apple. Total polyphenol content and DPPH radical scavenging activity in cv. Hayward was significantly higher than in apple. Another comparison was done by Scalzo *et al.* (2005). It was shown that the polyphenol and antioxidant activity of kiwifruit was equal to apples, apricots and peaches. The total antioxidant capacity (6-hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylic acid equivalent antioxidant assay; TEAC) of kiwifruit was similar to the data of the discussed report. The TEAC (considering lipophilic and hydrophilic contributions) was determined for only for 1, 4, 6 and 10 days ripening and was compared with Scalzo *et al.* (2005), and showed that the hydrophilic antioxidants are the main contributors to the overall antioxidant activity.

Our results about the polyphenol content of kiwifruit and its comparison with other fruits were in agreement with Yamazaki *et al.* (2000), comparing favorably with the known antioxidant beverage green tea.

Nishiyama *et al.* (2004) investigated different kiwifruit cultivars and found that the most common available cultivar of *A. deliciosa* cv. Hayward contained 65.5 mg/100 g FW of ascorbic acid. Ascorbic acid was measured in fruit from six genotypes of *Actinidia chinensis* (Rassam and Laing 2005). Levels of whole fruit mean ascorbic acid in the different genotypes ranged from 98 to 163 mg/100 g of fresh weight. Also, these data correspond with our data.

The amount of anthocyanins in this report can be compared with Montefiori *et al.* (2005), where, in the *A. deliciosa* genotypes, analyzed the major anthocyanins identified were cyanidin 3-O-galactoside and cyanidin 3-O-glucoside.

Kiwi gold fruits were extracted with hexane, acetone, methanol and 70% methanol, More hydrophilic fractions of 70% methanol extract displayed higher anti-HIV activity, radical generation and O-2(-) scavenging activity. The antibacterial activity of 70% methanol extracts was generally lower than that of more lipophilic fractions (hexane, acetone, methanol extracts), although each fraction did not show any specific antimicrobial action. These results demonstrate that gold kiwifruit extracts contain valuable, various

bioactive materials (Motohashi *et al.* 2002). Our results showed the highest content of total polyphenols in the NT sample at the first day and decreased significantly at the last day. In the ethylene-treated samples, it was a slight increase of polyphenols after 1 day, and then the increase was at the fifth and sixth days. The patterns of the changes in the content of the tannins, anthocyanins, flavonoids and ascorbic acid were slightly different from those of total polyphenols.

Antioxidant activities varied among the kiwifruit samples as determined by various used assays: the highest was with FRAP, and the lowest with ABTS, regardless of reaction time. The initial values (reaction time about 6–10 min) are comparable to those reported in the presented reviewed articles (Ozgen *et al.* 2006). The modified TEAC assay with pH lower (pH 4.5) than in the previous used assay (pH 7.4) yields antioxidant values that are lower than those obtained by FRAP (Ozgen *et al.* 2006).

Ozgen *et al.* (2006) compared berries and the results have shown that comparison of all three methods gave different numbers, but the relationship for the same fruit was found in all methods. For example, strawberry showed the following antioxidant capacity (μM TE/g) in ABTS (11.5 ± 0.4), and in Fe (II)-TP (24.9 ± 0.7). Our data differed from the reviewed ones, because kiwifruit has lower antioxidant activity than strawberry, but the relationship between the three methods was the same one. For T1–T10 samples by ABTS, the range was from 6.01 ± 0.76 to 6.28 ± 0.87, and for NT1–NT10 samples, from 5.31 ± 0.53 to 6.25 ± 0.75, respectively.

For T1–T10 samples determined by Fe (II)-TP the range was from 5.45 ± 0.76 to 5.95 ± 0.87 and for NT1–NT10 samples: from 5.63 ± 0.56 to 5.85 ± 0.61, respectively.

The obtained results can be compared with the data of Scalzo *et al.* (2005), where, for kiwifruit *Actinidia*, the result of total ABTS was about 2.72 ± 0.01. Scalzo *et al.* (2005) showed that the different portions of kiwifruit (green and white) and the flesh of whole fruit from the same cultivar "Hayward" had different antioxidant capacities: the green had a significantly higher total ABTS value than did the white portion.

Nilsson *et al.* (2005) compared as well some fruits of water soluble extracts using ABTS and Fe (III) TP. Our data on methanolic extracts of kiwifruit antioxidant activity can be compared only with the data of water soluble fractions of banana in Fe (III)-TP and mango using ABTS with the ratio of ABTS/Fe (III)-TP in the range of 1.1–2.0 reported by Nilsson *et al.* (2005). Our results have shown the same ratio between the two methods of 1.1.

The obtained results have shown that major components of antioxidant activity in kiwifruits are dietary natural antioxidant polyphenols. This is in the same line with other reports (Halvorsen *et al.* 2002) showing that in edible plants and fruits polyphenols play the main role and contribution to the

antioxidant overall activity. As it was mentioned above the way of polyphenol extraction is very important; therefore, it is necessary to extract polyphenolic compounds effectively when antioxidant capacities are measured.

The results of the determined antioxidant activities of the studied kiwifruit samples are in a wide range of reported literature data (Leong and Shui 2002), as well as within our recently published data (Park *et al.* 2006a,b). The data depended on the extraction procedure of the kiwifruit: solvent used (acetone, methanol and water), duration and the temperature of extraction. To our knowledge, this is the first study to present findings on the total phenolics, antioxidant activity and protein profile of hardy kiwifruit comparing the ethylene-treated with the NT samples. This is expected because ascorbic acid (vitamin C) plays a large antioxidant role, and research on "Hayward" kiwifruit has shown that there is little effect of maturity at harvest and only a negligible effect of refrigerated storage on ascorbic acid concentrations. The effects of refrigeration depend largely on maturity of the fruit at harvest. Comparison of the antioxidant activity observed in this study with that of other studies would be important; however, differences in method of measurement and in units reported makes direct comparison difficult.

Moyer *et al.* (2002) explored total phenolic content for a variety of berry crops and reported 1.7 to 9.6 mg GAE/g *Vaccinium* blueberries and huckleberries, 1.3 to 10.8 mg GAE/g *Rubus* blackberries, raspberries and black raspberries and 1.9 to 17.9 mg GAE/g *Ribes* gooseberries, currants and jostaberries.

Correlation of the antioxidant activity with ascorbic acid was lower than with the polyphenols. These results were in agreement with Collins *et al.* (2001), who showed that in vitro, a simple extract of kiwifruit, buffered to pH 7, was more effective than a solution of vitamin C (of equivalent concentration) at protecting DNA from damage. It was demonstrated that kiwifruit has significant antioxidant activity ex vivo and in vitro, which is not entirely attributable to the vitamin C content of the fruit.

Our results on the phenolic content and antioxidant activity of *A. arguta* "Ananasnaya" further support the significant health benefits of hardy kiwifruit. The antioxidant activity of standard phenolic acids was estimated. The antioxidant capacity of caffeic, ferulic and *p*-coumaric acids and catechin was determined by the same methods. The two methods gave similar values for caffeic acid. Ferulic acid and catechin gave higher results with ABTS method than with the Fe(II)-TP. Nilsson *et al.* (2005) found that caffeic acid showed 1.13 and 1.18; ferulic −1.40 and 3.51, catechin 1.26 and 3.30 and quercetin, 3.73 and 3.74 by Fe(III)-TP and ABTS, respectively. Nenadis *et al.* (2004) reported the following data for antioxidant capacity (μM) in ethanol (eth) and buffered pH 7.4 (buf) environment: caffeic acid: 1.01 ± 0.05 (eth) and 1.15 ± 0.09 (buf); ferulic acid: 1.32 ± 0.07 (eth) and 1.97 ± 0.02 (buf);

p-coumaric acid: 2.00 ± 0.12 (buf); 2.39 ± 0.09 (buf) and quercetin: 1.85 ± 0.08. The relative order of activity on the basis of radical scavenging activity (%) by DPPH was caffeic acid (76.6), ferulic (30.9), *p*-coumaric (3.6) and quercetin (68.2).

The ripening of kiwifruit was accelerated by ethylene treatment, and the protease activity in the fruit slightly declined. Protein decreased gradually toward maturation and reached the lowest level at the ripening stage. The electrophoretic separation of proteins of kiwifruit samples can be compared only with our previous report (Park *et al.* 2006a) or with other not-really-similar plants such as banana (Ho *et al.* 2007), where a 20-kDa protein with substantial N-terminal sequence homology to thaumatin-like proteins was isolated from ripe fruits of the emperor banana, *Musa basjoo* cv. "emperor banana". In other reports, it was shown that the main protein band was concentrated in 24 and 30 kDa. Some bands around 30 kDa were apparently observed during the development of the fruit, and the highest protease activity in a gradient polyacrylamide gel was found around the 13 kDa protein. In a search for aspartic proteinase inhibitors (APIs) in kiwifruit seeds was found a report by Rassam and Laing (2006). Observed pepsin inhibitory activity (PIA) in an abundant globulin fraction extracted in high salt buffer with a Mr of 148 kDa by gel-filtration. On a SDS-polyacrylamide gel, a major protein band of 54 kDa was observed under nonreducing conditions. This band was largely replaced by two subunits of Mr 33.5 and 20 kDa under reducing conditions. There was only a slight difference in the protein profile of our samples; the 32 kDa protein band was more distinguished in our experiment. The use of a longer gel gave the possibility to obtain more distinct and sharp bands, especially in the zone higher than 32 kDa. At the end of the ripening period, the protein qualities of both groups of samples were without significant differences or with a small decrease (Park *et al.* 2006a).

It was shown in this investigation that ethylene treatment of kiwifruit is preferable:

1. In the 10th day of ethylene treatment, a significant increase of the following bioactive compound of T versus NT fruits was registered

(1) Polyphenols – 26.70 ± 1.5 versus 14.37 ± 1.0 mg GAE/g;
(2) Flavonoids – 0.47 ± 0.04 versus 0.27 ± 0.02 mg GAE/g;
(3) Anthocyanins – 0.134 ± 0.100 versus 0.042 ± 0.003 mg CGE/g;
(4) Tannins – 4.20 ± 0.9 versus 2.64 versus mg/g ($P < 0.05$ in all 4 cases).

2. Together with an increase in the contents of the previously mentioned bioactive compounds, a significant increase in the antioxidant capacity of T versus NT fruits was registered ($P < 0.05$).

Therefore, in order to increase the bioactivity of kiwi fruits, ethylene treatment is recommended.

CONCLUSIONS

The bioactivity of kiwifruit increased during the ethylene treatment, and its maximum was registered at the sixth day of the treatment; total polyphenols are the main contributors to the overall antioxidant activity of kiwifruit; the antioxidant activity of treated kiwifruits increased during ripening and ethylene treatment. The amount of other bioactive substances including the vitamin C influenced as well as the overall antioxidant activity.

ACKNOWLEDGMENTS

This research was partly supported by the Regional Research Development Program from Rural Development Administration (RDA), Korea, 2007. The authors are thankful to Dr. Elena Katrich (Hebrew University of Jerusalem, School of Pharmacy) for her technical assistance in determination of antioxidant activity of kiwifruit.

REFERENCES

CHENG, G.W. and BREEN, P.J. 1991. Activity of phenylalanine ammonia-lyase (PAL) and concentrations of anthocyanins and phenolics in developing strawberry fruit. J. Am. Soc. Hortic. Sci. *116*, 865–869.

COLLINS, B.H., HORSKA, A., HOTTEN, P.M., RIDDOCH, C. and COLLINS, A.R. 2001. Kiwifruit protects against oxidative DNA damage in human cells and in vitro. Nutr. Cancer *39*, 148–153.

DUTTAROY, A.K. and JØRGENSEN, A.U.D. 2004. Effects of kiwi fruit consumption on platelet aggregation and plasma lipids in healthy human volunteers. Platelets *15*, 287–292.

GORINSTEIN, S., DELGADO-LICON, E., PAWELZIK, E., PERMADYM, H.H., WEISZ, M. and TRAKHTENBERG, S. 2001. Characterization of soluble amaranth and soybean protein proteins based on fluorescence, hydrophobicity, electrophoresis, amino acid analysis, circular dichroism and differential scanning calorimetry measurements. J. Agric. Food Chem. *49*, 5595–5601.

HALVORSEN, B.L., HOLTE, K., MYHRSTAD, M.C.W., BARIKMO, I., HVATTUM, E., REMBERG, S.F., WOLD, A.B., HAFFNER, K., BAUGEROD, H., ANDERSEN, L.F. *ET AL.* 2002. A systematic screening of total antioxidants in dietary plants. J. Nutr. *132*, 461–471.

HARUENKIT, R., POOVARODOM, S., LEONTOWICZ, H., LEONTOWICZ, M., SAJEWICZ, M., KOWALSKA, T., DELGADO-LICON, E.,

ROCHA-GUZMÁN, N.E., GALLEGOS-INFANTE, J.-A., TRAKHTENBERG, S. *ET AL*. 2007. Health properties and nutritional value of durian (*Durio zibethinus* cv. Mon Thong): Experiments in vitro and in vivo. J. Agric. Food Chem. *55*, 5842–5849.

HEIMLER, D., VIGNOLINI, P., DINI, M.G., VINCIERI, F.F. and ROMANI, A. 2006. Antiradical activity and polyphenol composition of local Brassicaceae edible varieties. Food Chem. *99*, 464–469.

HO, V.S.M., WONG, J.H. and NG, T.B. 2007. A thaumatin-like antifungal protein from the emperor banana. Peptides *28*, 760–766.

LAEMMLI, U.K. 1970. Cleavage of structural proteins during the assembly of bacteriophage T4. Nature *227*, 680–685.

LEONG, L.P. and SHUI, G. 2002. An investigation of antioxidant capacity of fruits in Singapore markets. Food Chem. *76*, 69–75.

MONTEFIORI, M., MCGHIE, T.K., COSTA, G. and FERGUSON, A.R. 2005. Pigments in the fruit of red-fleshed kiwifruit (*Actinidia chinensis* and *Actinidia deliciosa*). J. Agric. Food Chem. *53*, 9526–9530.

MOTOHASHI, N., SHIRATAKI, Y., KAWASE, M., TANI, S., SAKAGAMI, H., SATOH, K., KURIHARA, T., NAKASHIMA, H., MUCSI, I., VARGA, A. *ET AL*. 2002. Cancer prevention and therapy with kiwifruit in Chinese folklore medicine: A study of kiwifruit extracts. J. Ethnopharmacol. *81*, 357–364.

MOYER, R.A., HUMMER, K.E., FINN, C.E., FREI, B. and WROLSTAD, R.E. 2002. Anthocyanins, phenolics, and antioxidant capacity in diverse small fruits: Vaccinium, Rubus, and Ribes. J. Agric. Food Chem. *50*, 519–525.

NENADIS, N., WANG, L.F., TSIMIDOU, M. and ZHANG, H.Y. 2004. Estimation of scavenging activity of phenolic compounds using the ABTS•+ assay. J. Agric. Food Chem. *52*, 4669–4674.

NILSSON, J., PILLAI, D., OENNING, G., PERSSON, C., NILSSON, A. and AKESSON, B. 2005. Comparison of the 2,2′-azinobis-3-ethylbenzotiazoline-6-sulfonic acid (ABTS) and ferric reducing antioxidant power (FRAP) methods to asses the total antioxidant capacity in extracts of fruit and vegetables. Mol. Nutr. Food Res. *49*, 239–246.

NISHIYAMA, I., YAMASHITA, Y., YAMANAKA, M., SHIMOHASHI, A., FUKUDA, T. and OOTA, T. 2004. Varietal difference in vitamin C content in the fruit of kiwifruit and other Actinidia species. J. Agric. Food Chem. *52*, 5472–5475.

OKAMOTO, G. and GOTO, S. 2005. Juice constituents in Actinide argot fruits produced in Shinto, Okayama. Okayama Deg. Nog. Gak. Hoko. *94*, 9–13.

OZGEN, M., REESE, R.N., TULIO, A.Z., SCHEERENS, J.C. and MILLER, A.R. 2006. Modified 2, 2-azino-bis-3-ethylbenzothiazoline-6-sulfonic

acid (ABTS) method to measure antioxidant capacity of selected small fruits and comparison to ferric reducing antioxidant power (FRAP) and 2, 2′-diphenyl-1-picrylhydrazyl (DPPH) methods. J. Agric. Food Chem. *54*, 1151–1157.

OZYUREK, M., GUCLU, K., BEKTASOGLU, B. and APAK, R. 2007. Spectrophotometric determination of ascorbic acid by the modified CUPRAC method with extractive separation of flavonoids-La(III) complexes. Anal. Chim. Acta *588*, 88–95.

PARK, Y.-S., JUNG, S.-T., KANG, S.-G., DRZEWIECKI, J., NAMIESNIK, J., HARUENKIT, R., BARASCH, D., TRAKHTENBERG, S. and GORINSTEIN, S. 2006a. In vitro studies of polyphenols, antioxidants and other dietary indices in kiwifruit (Actinidia deliciosa). Int. J. Food Sci. Nutr. *57*, 107–122.

PARK, Y.-S., JUNG, S.-T., KANG, S.-G., DELDADO-LICON, E., KATRICH, E., TASHMA, Z., TRAKHTENBERG, S. and GORINSTEIN, S. 2006b. Effect of ethylene treatment on kiwifruit bioactivity. Plant Foods Hum. Nutr. *61*, 151–156.

PRIOR, R.L., GU, L., WU, X., JACOB, R.A., SOTOUDEH, G., KADER, A.A. and COOK, R.A. 2007. Plasma antioxidant capacity changes following a meal as a measure of the ability of a food to alter in vivo antioxidant status. J. Am. Coll. Nutr. *26*, 170–181.

RASSAM, M. and LAING, W. 2005. Variation in ascorbic acid and oxalate levels in the fruit of Actinidia chinensis tissues and genotypes. J. Agric. Food Chem. *53*, 2322–2326.

RASSAM, M. and LAING, W.A. 2006. The interaction of the 11S globulin-like protein of kiwifruit seeds with pepsin. Plant Sci. *171*, 663–669.

SCALZO, J., POLITI, A., PELLEGRINI, N., MEZZETTI, B. and BATTINO, M. 2005. Plant genotype affects total antioxidant capacity and phenolic contents in fruit. Nutrition *21*, 207–213.

SCHRÖDER, R. and ATKINSON, R.G. 2006. Kiwifruit cell walls: Towards an understanding of softening? N. Z. J. For. Sci. *36*, 112–129.

SINGLETON, V.L., ORTHOFER, R. and LAMUELA-RAVENTOS, R.M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. *299*, 152–178.

TATSUKI, M. 2007. Regulation of ethylene biosynthesis in ripening fruit. Gek. Fudo Kemikaru *23*, 23–27.

VILAS-BOAS, E.V.D.B. and KADER, A.A. 2007. Effect of 1-methylcyclopropene (1-MCP) on softening of fresh-cut kiwifruit, mango and persimmon slices. Postharvest Biol. Technol. *43*, 238–244.

YAMAZAKI, M., UEDA, H. and YUI, S. 2000. Antioxidative function of kiwi fruit and oxidative damage in host. Biol. Ind. *17*, 24–31.