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Antioxidants and proteins in ethylene-treated kiwifruits

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Abstract

Ethylene-treated kiwifruit (*Actinidia deliciosa*) cultivar 'Hayward' was compared with the air-treated one. The correlation coefficients between total polyphenols and the antioxidant capacities measured by [2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] (ABTS) with Trolox equivalent antioxidant capacity (TEAC), 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), and cupric reducing antioxidant capacity (CUPRAC) assays for ethylene-treated kiwifruits were as followed: 0.74; 0.93 and 0.98, in comparison with air-treated samples of 0.72, 0.88 and 0.97, respectively. CUPRAC produced the most consistent measurements for ethylene-treated kiwifruit. In extracted and separated, by electrophoresis, kiwifruit proteins differences were found in the sodium dodecyl sulfate–protein bands, in the region of 32 kDa, in samples after the first days of treatment. Based on antioxidant activity and the protein profiles it can be concluded that the ethylene treatment shortened the ripening process of the fruits.

Keywords: *Actinidia deliciosa*; Polyphenols; Flavonoids; Antioxidant capacity; Proteins; Ethylene treatment

1. Introduction

The beneficial effects of fruits and vegetables may be explained by the antioxidants (Imeh & Khokhar, 2002; Moyer, Hummer, Finn, Frei, & Wrolstad, 2002; Jung et al., 2005; Rush et al., 2006). The polyphenol compounds may function individually in order to protect lipoproteins and vascular cells from oxidation, or by other mechanisms, such as reducing plasma lipid levels: LDL cholesterol and triglycerides, a significant increase in the ability of leukocytes to repair DNA breakage by free radicals (Jayaprakasam, Vareed, Olson, & Nair, 2005; Jung et al., 2005; Rush et al., 2006). These benefits are stimulating research to investigate the contents of the bioactive compounds of natural products and their total antioxidant capacity (Dawes & Keene, 1999; Guo et al., 2003; Halvorsen et al., 2002; Katsube et al., 2004; Lim, Lim, & Tee, 2007; Nenadis, Wang, Tsimidou, & Zhang, 2004; Parejo et al., 2002). Many authors investigated mostly common fruits: apples, pears and peaches (Apak, Guclu, Ozyurek, & Karademir, 2004; Nilsson et al., 2005). However, at the fruit markets of North America and Europe appeared different kinds of tropical and subtropical fruits such as mango, guava, papaya, kiwifruit and many others (Leontowicz et al., 2007; Lim et al., 2007; Sarni-Manchado, Le Roux, Le Guerneve, Lozano, & Cheynier, 2000). The antioxidant

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activity of kiwifruit can be compared with mangosteen, avocado, papaya, mango and cempedak (Leong & Shui, 2002; Lim et al., 2007; Nishiyama, 2007). Now most investigators recommend the use of fruits, including kiwifruits, with high antioxidant activity (Jayaprakasam et al., 2005; Jung et al., 2005; Leontowicz et al., 2007; Scalzo, Politi, Pellegrini, Mezzetti, & Battino, 2005).

In the recent reviewed reports (DeEll, Ayres, & Murr, 2007; De Morais, Lima, Alves, Alves, & Alves, 2006; Hayama, Shimada, Fujii, Ito, & Kashimura, 2006; Mao, Wang, & Que, 2007; Rodrigo & Zacarias, 2007) ethylene and 1-methylcyclopropene treatments in kiwifruit, peach, sapodilla,'empire' and 'delicious' apples and oranges and their quality were studied.

The parameters which were analysed for postharvest quality were: weight loss, external appearance, firmness, colour, 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, 2006b) the comparison was done on the bioactive compounds. In this report the results of the previous harvest will be compared with the present results based on the extraction of total polyphenols. For the first time the protein profiles during the ripening are discussed.

This report was conducted to study the kinetic changes of antioxidant capacity of the ethylene-treated kiwifruit and to find the optimal parameters of this treatment. 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavengers, reduction of ferric-tripiridyltriazine [Fe(III)-TPTZ] to a ferrous form Fe(II) and utilisation of copper(II)–neocuproine [Cu(II)–Nc] reagent as the chromogenic oxidising agent assays were applied and the most suitable radical scavenging method for the process of ripening was chosen.

As far as we know there are no published reports.

2. Materials and methods

2.1. Chemicals

Trolox(6-hydroxy-2,5,7,8,-tetramethyl-chroman-2-carboxylic acid); butylated hydroxyanisole (BHA); 2,2′-azobis-2-methyl-propanimidamide; $FeCl_3 \times 6H_2O$; Folin–Ciocalteu reagent; 1,1-diphenyl-2-picrylhydrazyl radical (DPPH); $CuCl_2 \times 2H_2O$ and neocuproine (2,9-dimethyl-1,10-phenanthroline), potassium persulfate, sodium dodecyl sulfate (SDS), β-mercaptoethanol (β-ME), acrylamide, polyacrylamide, Coomassie Brilliant Blue R and molecular weight marker (20–97 kDa) were obtained from Sigma Chemical Co., St. Louis, MO, USA. 2,4,6-Tripyridyl-*s*-triazine (TPTZ) was purchased from Fluka Chemie, Buchs, Switzerland. All reagents were of analytical grade. Deionised and distilled water were used throughout.

2.2. Samples and preparation

'Hayward' kiwifruits (*Actinidia deliciosa*) harvested in October 2006 were from Muan county and were purchased from the same farmer. Fruits with defects were discarded and good fruits of average weight of 100 g were cleaned with tap water, and placed in glass jar. The fruits were randomly divided into two groups: air (AT) and ethylene treated (ET) and were ripened immediately after harvest. Kiwifruit samples of the ET group were treated with 100 ppm of 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 (AT) or air mixed with ethylene (ET) at 300 ml min^{-1}. Then the ethylene and air-treated kiwifruits were ripened separately using the same conditions, at 20 °C, in a growth chamber (Percival, USA) for 10 days.

2.3. Extraction and hydrolysis of total polyphenols

A 50 mg aliquot of lyophilised 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 90 °C for 3 h with vortexing every 30 min. The samples were cooled, diluted to 10 ml with methanol and centrifuged for 5 min, at 4000*g*, with a benchtop centrifuge to remove solids. This procedure was described in details (Park et al., 2006b).

2.4. UV–visible spectrophotometric analyses

All spectra were measured on an Uvikon 930 (Bio-Teck-Kontron) and were recorded from 250 to 600 nm. All solutions of phenols were prepared in methanol at a concentration of 1 mM (Sarni-Manchado et al., 2000).

2.5. Total polyphenols determination

The Folin–Ciocalteu method was used and the measurement was performed at 765 nm with gallic acid as the standard (Singleton, Orthofer, & Lamuela-Raventos, 1999).

2.6. Total flavonoid determination

Flavonoids (extracted with 5% $NaNO_2$, 10% $AlCl_3 \times 6H_2O$ and 1 M NaOH) were measured at 510 nm with known (+)-catechin concentration as a standard and expressed as milligrams of catechin equivalents per g dry weight (Singleton et al., 1999).

2.7. Determination of the antioxidant capacity

The following assays were used in this report:

2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS) scavenging assay: the $ABTS^{\cdot+}$ 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 $ABTS^{\cdot+}$ 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 (Pellegrini et al., 2007).

Ferric-tripiridyltriazine [Fe(III)-TPTZ] is reduced to a ferrous form Fe(II) which absorbs light at 593 nm in the FRAP (ferric-reducing/antioxidant power) assay. The ferro- and ferric-iron form complexes with TPTZ reagent are the main products of this reaction. Antioxidant level was calculated by plotting a standard curve of absorbance against concentration of Fe(II) standard solution or Trolox.

1,1-Diphenyl-2-picrylhydrazyl radical (DPPH) of 0.1 mM methanolic solution was added (5 μl) to kiwifruit extracts. The control was prepared as above without any extract, and MeOH was used for the baseline correction. Changes in the sample's absorbance were measured at 517 nm. BHA was used for comparison (Ozgen, Reese, Tulio, Scheerens, & Miller, 2006).

Copper(II)–neocuproine [Cu(II)–Nc] reagent is used as the chromogenic oxidising agent in cupric reducing antioxidant capacity (CUPRAC). To the mixture of 1 ml of Cu(II), Nc and NH_4Ac buffer solution, antioxidant sample (or standard) solution (x ml) and H_2O [$(1.1 - x)$ ml] were added to make the final volume of 4.1 ml. The absorbance at 450 nm was recorded against a reagent blank (Apak et al., 2004).

The antioxidant activities of standards such as synthetic antioxidants, phenolic acids and flavonoids were determined by the above methods.

Three antioxidant assays [DPPH, ABTS and Fe(III)-TPTZ] were compared at the same periods of time duration (20, 30, 60, 90 and 120 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).

2.8. SDS-polyacrylamide gel electrophoresis

Kiwifruit juice was used for SDS-PAGE, which was carried out according to Laemmli (1970) using a Hoeffer SE-600 apparatus. The resolving gel was 13.7% T and 1.7% C and the stacking gel was 3.8% T and 1.8% C, as it was described in details in Park et al. (2006a).

2.9. Statistical analyses

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

3. Results

3.1. Polyphenols

The contents of phenolic compounds (mg GAE/g DW) in the ET kiwifruit extracts during 10 days treatment were in the range from 14.92 ± 1.51 to 26.70 ± 2.87 and for flavonoids (mg catechin equivalents/g) from 2.09 ± 0.31 to 3.25 ± 0.38 and in the AT kiwifruit extracts for phenolics (mg GAE/g DW) were in the range from 25.17 ± 2.49 to 24.37 ± 1.77 and for flavonoids (mg catechin equivalents/g) from 2.27 ± 0.27 to 1.99 ± 0.19.

The UV spectra of the studied extracts had the maximum absorption in a broad range from 278 to 286 nm and 325 nm (Fig. 1), indicating the differences in the composition of total phenols. These spectra suggest that flavonoids predominate in the phenolic compounds of the extract and the absorption at 325 nm suggests the presence of phenolic acids (Waterman & Mole, 1994).

There were various contents of phenolic compounds in the extracts of hydrolysed total polyphenols. UV spectra of the extracts from kiwifruit, air and ethylene treated, samples were recorded and then compared. The differences were found at the first, sixth and tenth days. The following peaks were measured in kiwifruit AT (air treated) extracts (Fig. 1a) – first day: peak (1) at wavelength λ (nm) = 213.1 with absorbance units (AU) of 1.893; and peak (6) at λ (nm) = 322.6 and AU = 1.625; sixth day: peak (2) at λ (nm) = 211.1, AU = 1.698; and peak (7) at λ (nm) = 319.7 and AU = 1.677; tenth day: peak (3) at λ (nm) = 217.3, AU = 2.301; peaks (4) and (5) = minimal; peak (8) at λ (nm) = 323.4, AU = 2.243.

The following peaks were measured in kiwifruit ET (ethylene treated) extracts (Fig. 1B) – first day: peak (1) at λ (nm) = 219.2 and AU = 2.217; and peak (6) at λ (nm) = 319.7 nm and AU = 1.357; sixth day: peak (2) at λ (nm) = 219.2, AU = 2.043; and peak (7) at λ (nm) = 319.2 nm and AU = 1.712; tenth day: peak (3) at λ (nm) = 212.7, AU = 1.950; peaks (4) and (5) = minimal; peak (8) at λ (nm) = 319.8, AU = 1.989.

The comparison of the peaks, in the extracts of total polyphenols of AT (Fig. 1a) and ET (Fig. 1b), in their wavelengths and absorbances showed that the main peak (1) after the first and sixth days of treatment shifted about 6 nm to the right with an increase in absorbance of 1.2 times; at the tenth day the main peak (1) shifted to the left about 5 nm with decrease in absorbance of 1.2 times.

The peaks estimated in the standard phenolic acids (caffeic, ferulic and *p*-coumaric, Fig. 1c) corresponded with the peaks of investigated kiwifruit samples (Fig. 1a and b).

3.2. Antioxidant capacities and correlation

The content of total polyphenols and related total antioxidant capacities by Fe(III)-TP, ABTS and Cu(II)–Nc significantly increased in ethylene treated (Fig. 2b) than in the

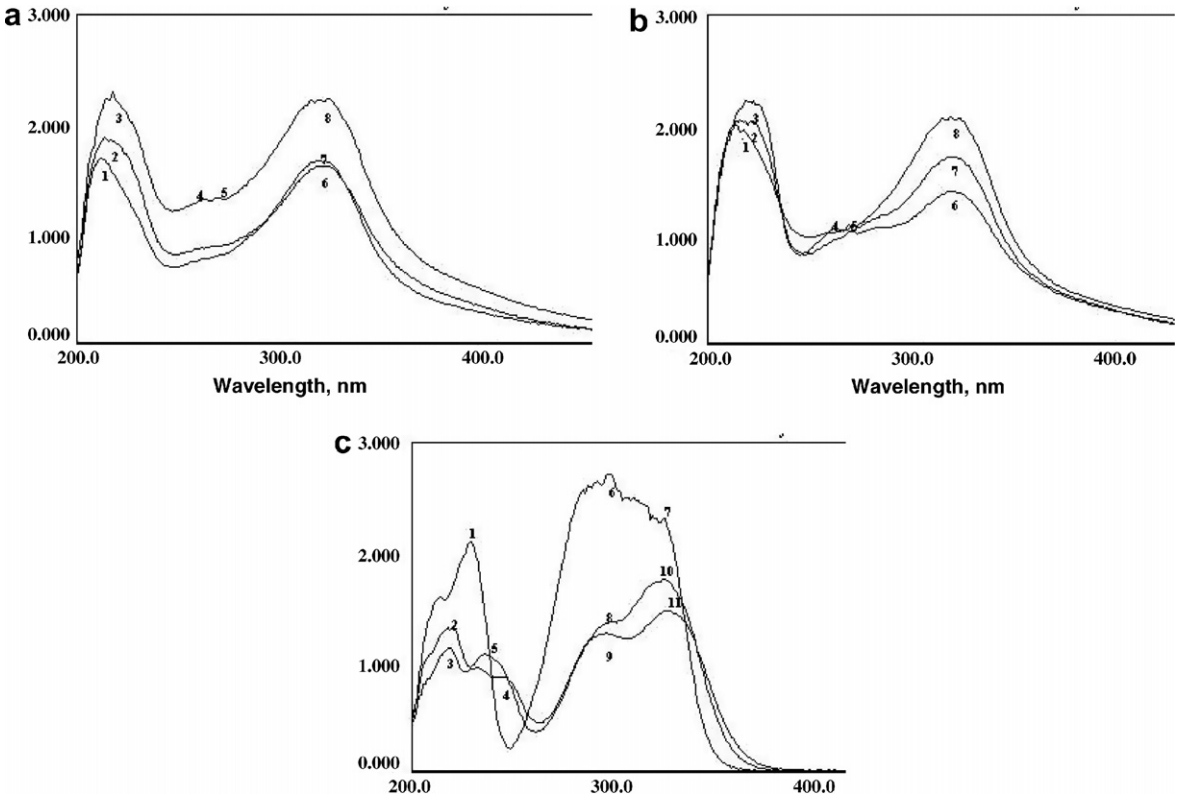

Fig. 1. UV–visible spectrum of the major compounds from total polyphenol kiwifruit extracts detected at the corresponding wavelength: (a) total polyphenols of TA (treated with air) samples at 0.5 mg ml^{-1} with major peaks (nm): (1), 213.1; (2), 211.1 and (3), 217.3, corresponding to 1, 6, and 10 days; (b) total polyphenols of ethylene-treated (ET) samples at 0.5 mg ml^{-1} with major peaks (nm): (1), 219.2; (2), 219.2 and (3), 212.7, corresponding to 1, 6 and 10 days; (c) standard phenolic acids at 0.1 mM with the following major peaks (nm): *p*-coumaric, (1), 228.5; (6), 296.8; (7), 324.0; caffeic, (2), 217.0; (4), 242.7; (8), 295.1; (10), 325.0 and ferulic acids (3), 217.7; (5), 234.9; (9), 296.8; (11), 323.5.

air-treated kiwifruit samples (Fig. 2a) after the middle of the treatment, starting from the fifth day ($P < 0.05$).

The Fe(III)-TP, ABTS and Cu(II)–Nc values for each extract were compared and correlated with the total phenolic contents (Fig. 3). The relationship between the values of total polyphenol concentrations, air-treated samples vs. antioxidant capacities (Fig. 2a), for Fe(III)-TP, ABTS and Cu(II)–Nc were 0.8845, 0.7229 and 0.9738, respectively. For ethylene-treated samples the calculations showed (Fig. 3b) the following order between the polyphenols and the antioxidant capacities determined by the three methods: 0.9321, 0.7423 and 0.9842, respectively. The results are rather interesting as there is an excellent linear response, especially for the ethylene-treated samples only for Cu(II)–Nc ($R^2 = 0.98$). The overall estimation of the presented data showed that after ethylene treatment the correlation coefficients were higher than in the air-treated kiwifruits.

The expected correlation between the polyphenols and Fe(III)-TP, ABTS and Cu(II)–Nc values is known, because all these assays are similar and working by the same mechanism (single electron transfer). These assays take into account the wide variety and range of action of antioxidant compounds presented in actual kiwifruit (Pellegrini et al., 2007; Scalzo et al., 2005). The antioxidant activities of fruit extracts depend on the time of assays used, therefore, the samples were measured at the same concentration and the same periods of time: 20, 30, 60, 90 and 120 min (Figs. 4–6). As it was explained above ABTS, DPPH and Fe(II)-TP were used also as a comparison for a longer time and with different pH for ABTS. The obtained results by these methods were higher for Fe(II)-TP than in the same method, but for a short time (Figs. 2–4). The data for ABTS did not change as much as for Fe(II)-TP (Figs. 2, 3 and 5).

3.3. Protein profiles

The protein profiles of the sampling dates of the experiment are shown, the comparison between the electrophoretic bands (Fig. 7) of air-treated fruits (lanes A1–A10) and the ethylene-treated ones (lanes E1–E10) during the 10 days period did not show drastic changes. After separation with SDS-PAGE electrophoresis, 4 bands were detected at the molecular weights of 16 (average bands) 20 and 24 (diffused and minor) and the major ones with sharpness in the range of 32 kDa.

4. Discussion

As was mentioned in Section 1, there are many recent reports dealing with the treatment of fruits. The data

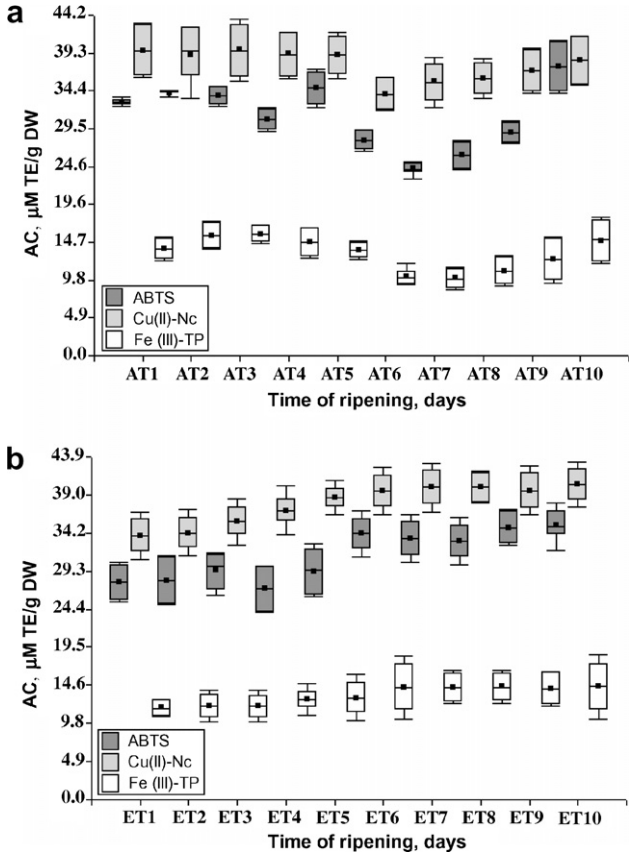

Fig. 2. Changes in kiwifruit antioxidant capacity (μM TE/g) determined by three different radical scavenging assays with the means and standard deviations: ABTS, Fe(III)-TP and Cu(II)–Nc during 10 days of ripening: (a), in air-treated (AT) kiwifruit. (b) in ethylene-treated (ET) kiwifruits. Abbreviations: AC, antioxidant capacity; ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); Fe(III)-TP, ferric-tripiridyltriazine; Cu(II)–Nc, copper(II)–neocuproine; AC expressed as μM TE (Trolox equivalent)/g DW. The mean values are shown with the standard deviations.

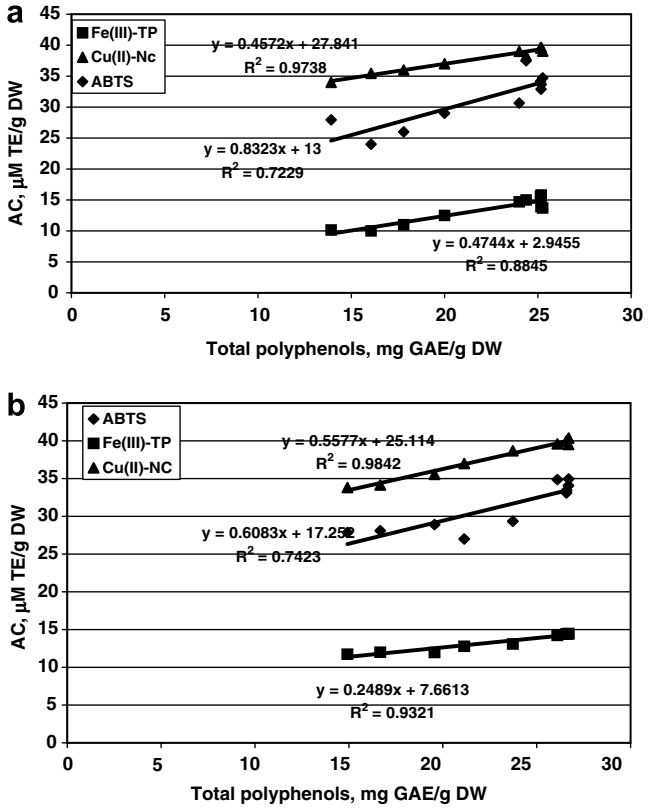

Fig. 3. Relationships, calculated by linear regression analysis between the total polyphenols (mg GAE/g DW) and their antioxidant capacity (μM TE/g DW) determined by three different radical scavenging assays: ABTS, Fe(III)-TP and Cu(II)–Nc during 10 days of ripening: (a) in air-treated (AT) kiwifruit. (b) in ethylene-treated (ET) kiwifruits: (■) FC (mg GAE/g DW, *X*) to Fe(III)-TP (μM TE/g DW, *Y*); (▲) FC (mg GAE/g DW, X) to Cu(II)–Nc (μM TE/g DW, *Y*) and B, (♦) FC (mg GAE/g DW, *X*) to ABTS (μM TE/g DW, Y). Abbreviations: AC, antioxidant capacity; ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); Fe(III)-TP, ferric-tripiridyltriazine; Cu(II)–Nc, copper(II)–neocuproine; AC expressed as μM TE (Trolox equivalent)/g DW.

obtained from the present investigation were in agreement with Hayama et al. (2006), De Morais et al. (2006), DeEll et al. (2007), Mao et al. (2007) and Rodrigo and Zacarias (2007), who showed the best results extending the postharvest life of sapodilla for 6 days, for peach with the time dependent ethylene treatment as well as the other fruits. Possible roles of the ethylene receptors in regulating fruit development and ripening were also discussed in Pang et al. (2007). The obtained results of the last harvest were similar to the previous one. The results of polyphenols in the last harvest were higher that in the previous (Park et al., 2006b) because only free polyphenols were extracted, therefore, the presented UV peaks in this report were slightly different and the maximum wavelength was shifted in average of about 5–6 nm. In relation to the findings reported here the content of polyphenols was similar to data of other investigators (Scalzo et al., 2005). Based on our recent results about the kiwifruits and published in relation to other fruits (with the same extraction procedure) this comparison was expected: the methanolic extracts contain significantly lower amount of polyphenols than the hydrolysed ones extracted with the solvent with the combination of methanol and acid (Park et al., 2006a, 2006b). As the polarities of antioxidant components from individual samples are likely to be different, the choice of extraction solvents is critical. In our previous reports different extracts of free and total polyphenols (Park et al., 2006b) were compared and it was shown that the total polyphenol extract expressed higher values than the free one.

The highest content of total polyphenols was registered in the air-treated sample, on the first day, and decreased significantly on the last day. Oppositively, in the ethylene-treated samples it was a slight increase of polyphenols after one day and then it was an increase of about 44.1%. The patterns of the changes in the content of the flavonoids were slightly different from those of total polyphenols and the increase was about 35.7%. According to our data, the correlation coefficient between the Folin–Ciocalteu assay and the DPPH radical scavenging assay is higher than others. These results correspond with the data of others

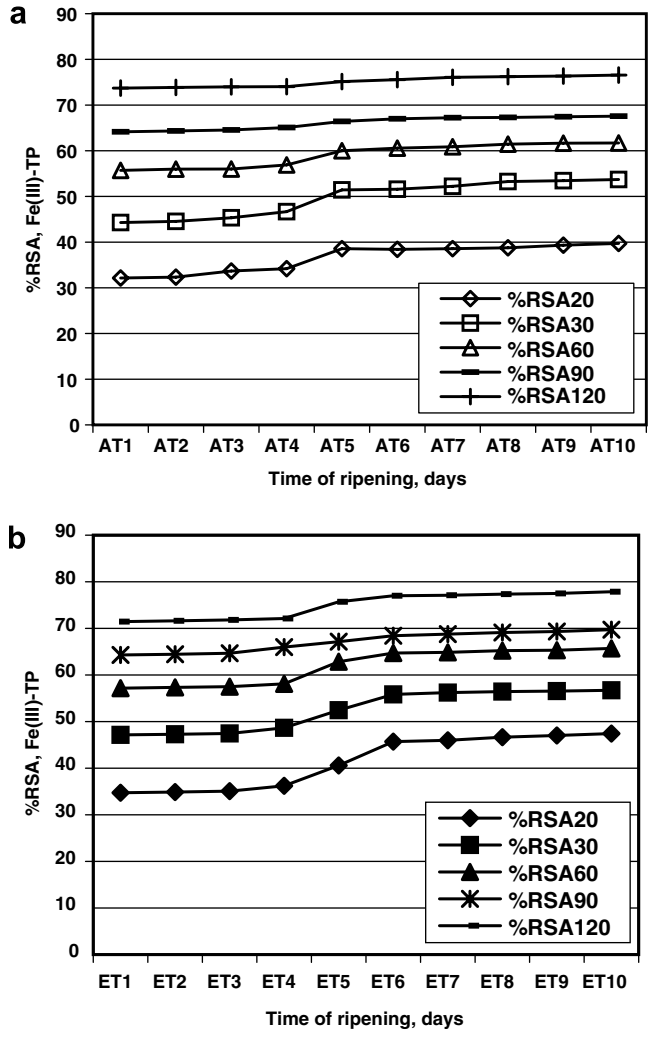

Fig. 4. Changes in radical scavenging activity (RSA, %) of total polyphenol extracts determined by Fe(III)-TP assay: (a) in air-treated (AT) kiwifruit. (b) in ethylene-treated (ET) kiwifruits during 10 days of ripening. Abbreviations: RSA determined after 20, 30, 60, 90 and 120 min by Fe(III)-TP, ferric-tripiridyltriazine.

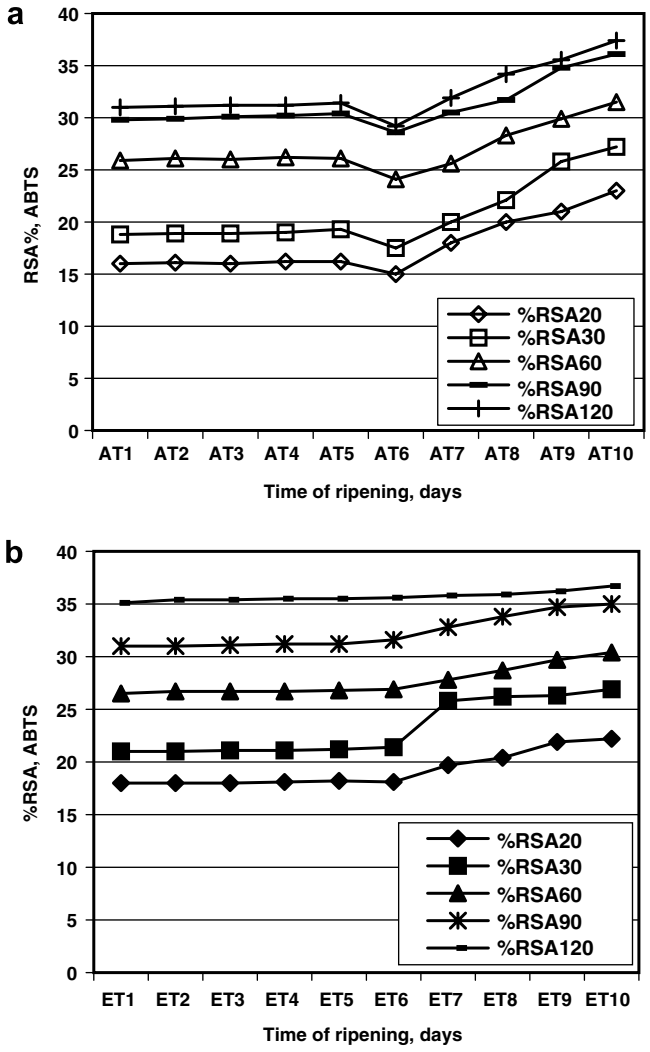

Fig. 5. Changes in radical scavenging activity (RSA, %) of total polyphenol extracts determined by ABTS assay: (a) in air-treated (AT) kiwifruit. (b) in ethylene-treated (ET) kiwifruits during 10 days of ripening. Abbreviations: RSA determined after 20, 30, 60, 90 and 120 min of the reaction by ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid).

(Katsube et al., 2004; Parejo et al., 2002), who reported that the correlation between DPPH radical scavenging activity and total phenol content as estimated by the Folin–Ciocalteu method was significantly high and varied from 0.70 to 0.90. Such results indicate that DPPH radical scavenging activity can be credibly predicted on the basis of the Folin–Ciocalteu assay and that these two methods depend on a similar mechanism: the propensity to donate hydrogen as well as all other applied methods (Katsube et al., 2004; Lim et al., 2007; Ozgen et al., 2006; Parejo et al., 2002).

Antioxidant activities varied among the kiwifruit samples as determined by various used assays: the highest was with FRAP, intermediate with DPPH, 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 (Apak et al., 2004; 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 and DPPH (Ozgen et al., 2006; Pellegrini et al., 2007).

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), in Fe(II)-TP (24.9 ± 0.7) and in DPPH (15.9 ± 0.1). 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. For ET1–ET10 samples by ABTS the range was from 6.01 ± 0.76 to 6.28 ± 0.87 and for AT1–AT10 samples: from 5.31 ± 0.53 to 6.25 ± 0.75, respectively. For ET1–ET10 samples determined by Fe(II)-TP the range

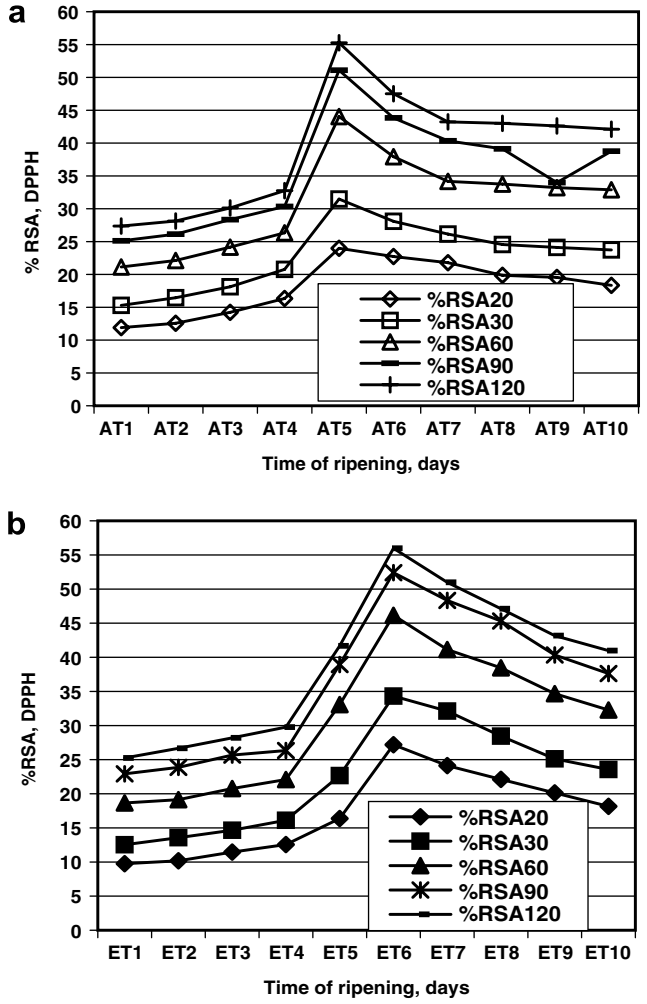

Fig. 6. Changes in radical scavenging activity (RSA, %) of total polyphenol extracts determined by DPPH assay: (a) in air-treated (AT) kiwifruit. (b) in ethylene-treated (ET) kiwifruits during 10 days of ripening. Abbreviations: RSA determined after 20, 30, 60, 90 and 120 min of scavenging reaction by DPPH, 1,1-diphenyl-2-picrylhydrazyl radical.

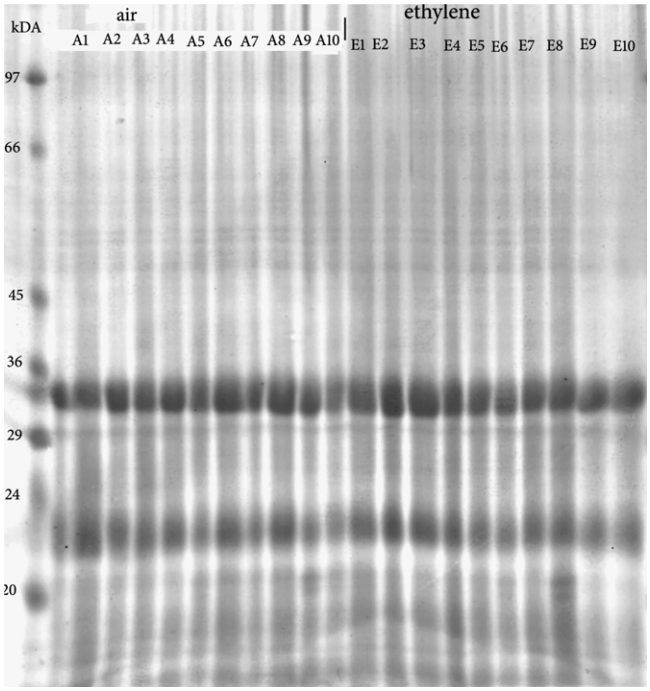

Fig. 7. Band intensities of proteins extracted from kiwifruits and separated by SDS-PAGE: lanes A1–A10, air-treated; E1–E10, ethylene-treated samples, respectively, from 1 to 10 days. Molecular weight markers: (kDa): 97 – phosphorylase b; 66 – albumin; 45 – ovalbumin; 36 – glyceralaldehyde-3-phosphate dehydrogenase; 29 – carbonic anhydrase; 24 – trypsinogen; 20 – trypsin inhibitor, loading 2 μl.

was from 5.45 ± 0.76 to 5.95 ± 0.87 and for AT1–AT10 samples: from 5.63 ± 0.56 to 5.85 ± 0.61, respectively. The results obtained can be compared with the data of Scalzo et al. (2005), where for kiwifruit *Actinidia* the results of total ABTS were of 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. Jung et al. (2005) have shown that the antioxidant activity of aqueous and 70% EtOH extracts with the concentration of 10 mg/ml by DPPH (RSA%) was about 21.55 and 21.80, in comparison with the obtained data of 18%.

The obtained results have shown that major components of antioxidant activity in kiwifruits are dietary natural antioxidant polyphenols. This was in line with other reports (Halvorsen et al., 2002; Katsube et al., 2004) showing that in edible plants and fruits, polyphenols play the main role and contribution to the overall antioxidant activity. As 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 were in a wide range of reported literature data (Fisk, McDaniel, Strik, & Zhao, 2006; Jung et al., 2005; Leong & Shui, 2002) as well as within our recent published data (Park et al., 2006a, 2006b). The data depended on the extraction procedure of the kiwifruit: solvent used (acetone, methanol and water), duration and the temperature of extraction. Leong and Shui (2002) measured the antioxidant activity of various fruits using the DPPH method and reported that strawberry had 4.72 mg/g ascorbic acid equivalents (AAE) and kiwifruit had 1.36 mg/g AAE. We could not find data on the different extraction procedures of kiwifruit and the measuring of the antioxidant activity. Pellegrini et al.

(2007) showed that the antioxidant activity on the example of orange and tomato is strongly affected by the solvents during 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 air-treated samples.

Harvest maturity and storage condition had no significant ($P > 0.05$) effect on the antioxidant activity. 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 (Fisk et al., 2006). 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. One study (Dawes & Keene, 1999) on fuzzy kiwifruit juice reported that phenolic compounds, present in clarified kiwifruit juice, were at levels <1.7 mg/l whereas 3.0 mg/g FW of total phenols in the edible portion of commercial kiwifruit was reported (Imeh & Khokhar, 2002). Moyer et al. (2002) explored total phenolic content for a variety of berry crops and reported 1.7–9.6 mg GAE/g *Vaccinium* blueberries and huckleberries, 1.3–10.8 mg GAE/g *Rubus* blackberries, raspberries and black raspberries, and 1.9–17.9 mg GAE/g *Ribes* gooseberries, currants and jostaberries.

Other studies have measured vitamin C content in kiwifruit. Nishiyama (2007) indicated that there was a wide variation in vitamin C content in *Actinidia arguta* fruit, ranging from 0.37 to 1.85 mg/g FW, and fruit from *A. arguta* cultivar Gassan, Issai and Mitsuko had much higher vitamin C contents than 'Hayward', suggesting that some *A. arguta* cultivars may be useful genetic resources. The levels of whole fruit mean ascorbic acid in six genotypes of *Actinidia chinensis* ranged from 0.98 to 1.63 mg/g FW and mean oxalic acid varied between 0.18 and 0.45 mg/g FW. 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 the 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 protein profiles of ethylene- and air-treated samples were presented for the first time. It was impossible to find some data connected with the same fruit during the ripening, but our data were comparable with Möller, Kayma, Steinhart, and Paschke (1997), Zhu, Lu, Zhang, Zhao, and Liu (2000) and Gavrović-Jankulović et al. (2002), who showed that the main protein band was concentrated in 24 and 30 kDa. It was also shown that some bands at around 30 kDa were apparently observed during the development of the fruit. The highest protease activity in a gradient polyacrylamide gel was found around the 13 kDa protein (Gavrović-Jankulović et al., 2002; Zhu et al., 2000). Möller et al. (1997) also used the SDS-electrophoretic separation of proteins which were extracted with acetone/diethylether. There was only a slight difference in the protein profile of our samples; the 32 kDa protein band was more distinguished in our experiment and the extraction of proteins was done with a sample buffer. As was mentioned in Park et al. (2006a), 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 qualities of both protein groups were either without significant differences or with a small decrease. The ripening of kiwifruit was accelerated by ethylene treatment and the protease activity in the fruit slightly declined. The same discussion was in Pang et al. (2007), showing that the amount of protein decreased gradually towards maturation and reached the lowest level at the ripening stage.

5. Conclusion

An ethylene treatment of kiwifruit is preferable: the bioactivity of the studied kiwifruit as determined by different scavenging antioxidant assays, increased during the ethylene treatment and its maximum was registered on the sixth day of the treatment. Total polyphenols contribute most to the overall antioxidant activity of the kiwifruit. The antioxidant activity of treated kiwifruits increased during ripening and ethylene treatment.

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