



Effect of Ethylene Treatment on Kiwifruit Bioactivity

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Abstract. The consumption of fruits and vegetables with high antioxidant activities leads to best healthful results. Therefore, in the present investigation we tried to find the peak of the kiwifruits antioxidant activity during the first 10 days of ethylene treatment (100 ppm at 20 °C).

In order to receive the most reliable data five different antioxidant assays were used: ferric-reducing/antioxidant power (FRAP); cupric reducing antioxidant capacity (CUPRAC); trolox equivalent antioxidant capacity (TEAC); 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH); and Folin-Ciocalteu. It was found by all applied methods that kiwifruit samples have the highest contents of polyphenols and flavonoids and the highest antioxidant activity on the 6-th day of the ethylene treatment. The correlation coefficients between polyphenols, flavonoids and antioxidant activities of kiwifruit methanol extracts with TEAC and CUPRAC, were as followed: 0.81 and 0.63, and 0.23 and 0.17, respectively, and showed that the free polyphenols correlation coefficients were higher than that of the flavonoids.

In conclusion: during ethylene treatment the bioactivity of kiwifruit is increasing and reaches its maximum at the 6th day and therefore it is the optimum time for kiwifruit consumption; total polyphenols were the main contributor to the overall antioxidant activity of kiwifruit; the most sensitive test for antioxidant activities determination is FRAP.

Key words: Kiwifruit, Antioxidants, Antioxidant activity, Antioxidant assays

Introduction

Fruits and vegetables have considerable health properties due to their main antioxidant compounds—phenolics [1]. It was shown that diets containing these natural products prevent and treat various diseases [2, 3]. Therefore, in the last years many investigations of fruits *in vitro* and *in vivo* were conducted [4–6]. It was found that all fruits contain different quantities of antioxidant compounds and have different levels of antioxidant activity [1]. Most researchers investigated traditional fruits [7–9]. However, now we are witnesses of growing interest in tropical and subtropical fruits [10–13]. Among these fruits a special place is reserved for kiwifruit [14–16]. Apart of very good taste, kiwifruit possesses a wide range of important properties. The edible part of kiwifruit is about 90–95%. This fruit contains 10% of total sugars, 0.11–1.2% of proteins, 0.1% of ascorbic acid, minerals and trace metals (copper, iron, manganese, zinc,

calcium, magnesium, potassium, and sodium) and also vitamin E, riboflavin and niacin [17, 18].

This fruit has high antioxidant activity, which is comparable with mangosteen, avocado, papaya, mango and cempedak. It was shown that kiwifruit possesses also heart protective properties [14, 15].

Best health protective results can be achieved from the consumption of fruits with high antioxidant activities [19–22]. Therefore, in the present study we decided to find out when the antioxidant activity of the ethylene treated kiwifruit achieves its maximum. In our previous investigations on kiwifruit were applied only two antioxidant assays: 2, 2'-azino-bis-(3-ethyl-benzothiazoline-6-sulfonate) radical cation (ABTS⁺/K₂S₂O₈) and the Folin-Ciocalteu method [16]. In order to receive the most reliable data five different antioxidant assays were used: (a) Trolox equivalent antioxidant capacity (TEAC); (b) Ferric-reducing/antioxidant power (FRAP); (c) 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH); (d) Cupric reducing antioxidant capacity (CUPRAC); and (e). Folin-Ciocalteu.

As far as we know there are not such published reports, which include results of determination of the antioxidant activity of kiwi fruits by five tests.

Materials and Methods

Chemicals

Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid); butylated hydroxyanisole (BHA); catechin; 2,2'-azobis-2-methyl-propanimidamide; FeCl₃·6H₂O; Folin-Ciocalteu reagent; 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH); CuCl₂·2H₂O and neocuproine (2, 9-dimethyl-1, 10-phenanthroline) were obtained from Sigma Chemical Co., St. Louis, MO, USA. 2, 4, 6-tripryridyl-s-triazine (TPTZ) was purchased from Fluka Chemie, Buchs, Switzerland. All reagents were of analytical grade. Deionized and distilled water were used throughout.

Sample Preparation

'Hayward' kiwifruits harvested in October 2005 were from the Muan county of Republic of Korea 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. They were randomly divided into two groups and named Control (C) and ethylene treated (E) and were ripened immediately after harvest. Kiwifruit samples of the E 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 (C) or air mixed with ethylene (E) at 300 mL min⁻¹. Then the ethylene treated and control (untreated) kiwifruits were ripened separately at the same conditions at 20 °C in a growth chamber (Percival, USA) for 10 days [16].

Extraction of Polyphenols

Defatted samples were extracted from a 50-mg aliquot with 5 mL of 50% methanol/water with heating at 90 °C for 3 h for not hydrolyzed free polyphenols (FP). 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 [1].

UV-Visible Spectrophotometric Analyses

All spectra were measured on an Uvikon 930 spectrophotometer (Kontron Instruments, Watford, UK) and were recorded from 250 to 600 nm. All solutions of phenols were prepared in methanol at a concentration of 1 mM [23].

Polyphenols Determination

The Folin-Ciocalteu method was used and the spectrophotometric measurement was performed at 765 nm with gallic acid as the standard. The results were expressed as mg gallic acid equivalent (GAE)/g DW [24].

Total Flavonoid Determination

Flavonoids (extracted with 5% NaNO₂, 10% AlCl₃·6H₂O and 1 M NaOH) were measured spectrophotometrically at 510 nm with known (+)-catechin concentration as a standard and expressed as milligrams of catechin equivalents per g dry weight [24].

Determination of the Antioxidant Activity

1. Trolox equivalent antioxidant capacity (TEAC). The ABTS^{•+} [2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] radical cation was generated by the in-

teraction of ABTS (250 μM) and K₂S₂O₈ (40 μM). The absorbance measured spectrophotometrically 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). 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 [25]. For the modified assay, ABTS was dissolved in 20 mM acetate buffer (pH 4.5) and prepared with potassium persulfate as described above.

2. Ferric-reducing/antioxidant power (FRAP) assay measures the ability of the antioxidants contained in the fruit samples to reduce ferric-tripiridyltriazine (Fe³⁺-TPTZ) to a ferrous form (Fe²⁺) which absorbs light at 593 nm spectrophotometrically. The ferro- and ferric-iron form complexes with TPTZ reagent are the main products of this reaction. FRAP level was calculated by plotting a standard curve of absorbance against concentration of Fe²⁺ standard solution or Trolox [26].
3. The 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) assay. The volume of kiwifruit extracts in different test tubes was adjusted to 100 μL by adding MeOH. A 0.1 mM methanolic solution of DPPH was added (5 μL) to these tubes. 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 [27, 28].
4. Cupric reducing antioxidant capacity (CUPRAC). This assay is based on utilizing the copper (II)-neocuproine [Cu (II)-Nc] reagent as the chromogenic oxidizing agent. To the mixture of 1 mL of Cu (II), Nc, and NH₄Ac buffer solution, antioxidant sample (or standard) solution (x mL) and H₂O [(1.1-x) mL] were added to make the final volume of 4.1 mL. The absorbance at 450 nm was recorded spectrophotometrically against a reagent blank [29].

Three antioxidant assays (DPPH, ABTS and FRAP) were compared at the same periods of time duration (10, 30, 60, and 120 min) and the same concentration of the investigated fruit's methanolic extracts. For each individual antioxidant assay, a trolox aliquot [28] was used to develop a standard curve. All data were then expressed as trolox equivalents (TE).

Results and Discussion

The results of the determined free polyphenols and flavonoids in kiwifruit extracts at different stages of their treatment show that polyphenols (mg GAE/g) and for flavonoids (mg catechin/g) for untreated samples were in

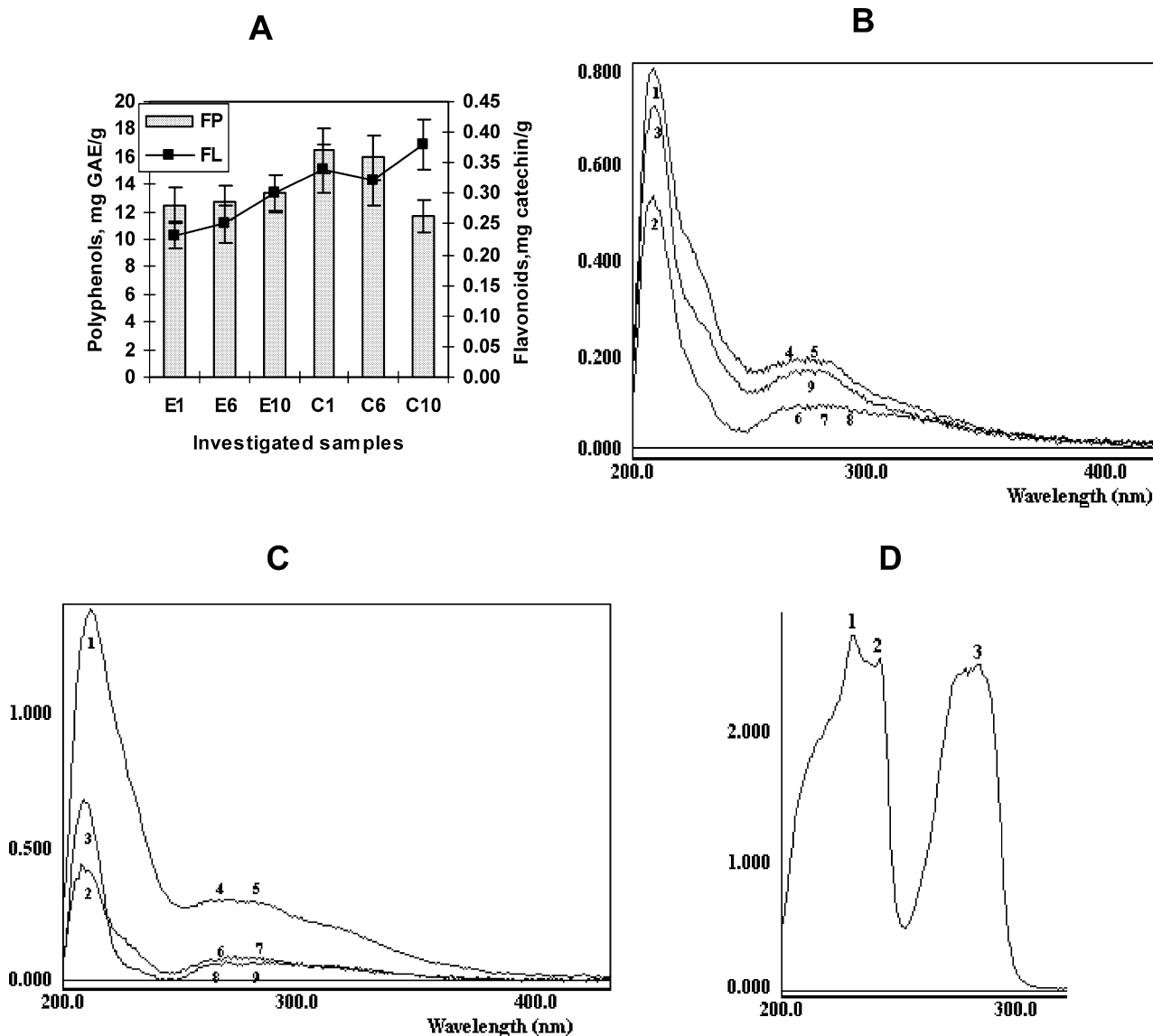


Figure 1. A, polyphenols and flavonoids in kiwifruit extracts; B, C, D, UV-visible spectrum of the free polyphenol compounds detected at the corresponding wavelength: B, free polyphenols of Control (C) samples at 0.5 mg mL⁻¹ with major peaks (nm): (1), 207.4; (2), 206; and (3), 209.1, and minors ones (4–9), corresponding to 1, 6, and 10 days without ethylene treatment; C, free polyphenols of ethylene treated (E) samples at 0.5 mg mL⁻¹ with major peaks (nm): (1), 210.6; (2), 206.4; and (3), 208.8, and minors ones (4–9), corresponding to 1, 6, and 10 days with ethylene treatment; D, catechin at 1mM with the following major peaks (nm): (1), 229.5; (2), 240.6; (3), 282.5.

the range of 16.43 to 11.70 and for treated ones of 13.39 to 12.48 and 0.38 to 0.32 and 0.30 to 0.23, respectively (Figure 1A). These results are in accordance with the data of others [1, 3, 10, 22] and were expected: the methanolic extracts contain significantly lower amount of polyphenols than the hydrolyzed samples [1]. The highest content of free polyphenols was registered in the control sample at the first day and decreased significantly at the last day. Opposite, in the treated samples it was registered an immediate decrease of polyphenols after one day of about 76% and then it was an increase of about 7.3%. As can be seen, the patterns of

the changes in the content of the flavonoids are similar to the total polyphenols.

Different results of phenolic compounds contents were registered, depending on the extraction solvent. Methanolic extracts had maximum absorptions of their UV spectra in a broad range between 278 and 286 nm, which indicated that flavonoids predominated in the phenolic compounds (Figure 1B, C, D). Absorption at 325 nm showed the presence of phenolic acids in that kiwifruit extract [23].

In order to receive reliable data of the possible changes in the antioxidant activity a combination of DPPH, FRAP,

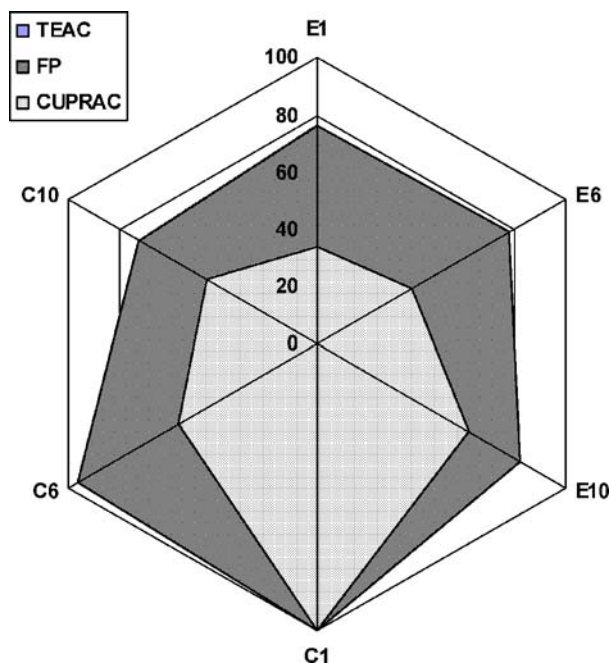


Figure 2. Relative antioxidant activity determined by TEAC and CUPRAC and polyphenols (%) in extracts from kiwifruits at different stages of treatment. Abbreviations: FP, free polyphenols; TEAC, trolox equivalent antioxidant capacity; CUPRAC, cupric reducing antioxidant capacity; C1, C6, C10, untreated kiwifruit samples after 1, 6 and 10 days; E1, E6, E10, treated with ethylene kiwifruit after 1, 6 and 10 days.

TEAC, CUPRAC and Folin assays were used. These assays take into account the wide variety and range of action of antioxidant compounds presented in actual kiwifruit [22, 25], but all these assays are based on electron transfer.

The content of total polyphenols and related antioxidant activity as determined by TEAC and CUPRAC assays was significantly higher in treated (Figure 2) than in the control kiwi samples ($P < 0.05$). In relation to the findings reported here the content of polyphenols and antioxidant activities was similar to data of other investigators [22].

The results of the determined antioxidant activities of the studied kiwifruit samples were in a wide range of reported literature data [1, 3, 15, 21, 22], as well as within our recent published data [16]. The reported data depended on the extraction procedure of the fruits: solvent used (acetone, methanol and water), duration and the temperature of extraction [1, 3, 5]. The expected correlation between the polyphenols and the DPPH, TEAC and FRAP is known, because all these assays are similar and working by the same mechanism (single electron transfer).

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: 10, 30, 60 and 120 min (Figure 3). The antioxidant activities of fruit extracts depend on the time of assays used and therefore the highest was with FRAP (Figure 3C), intermediate with

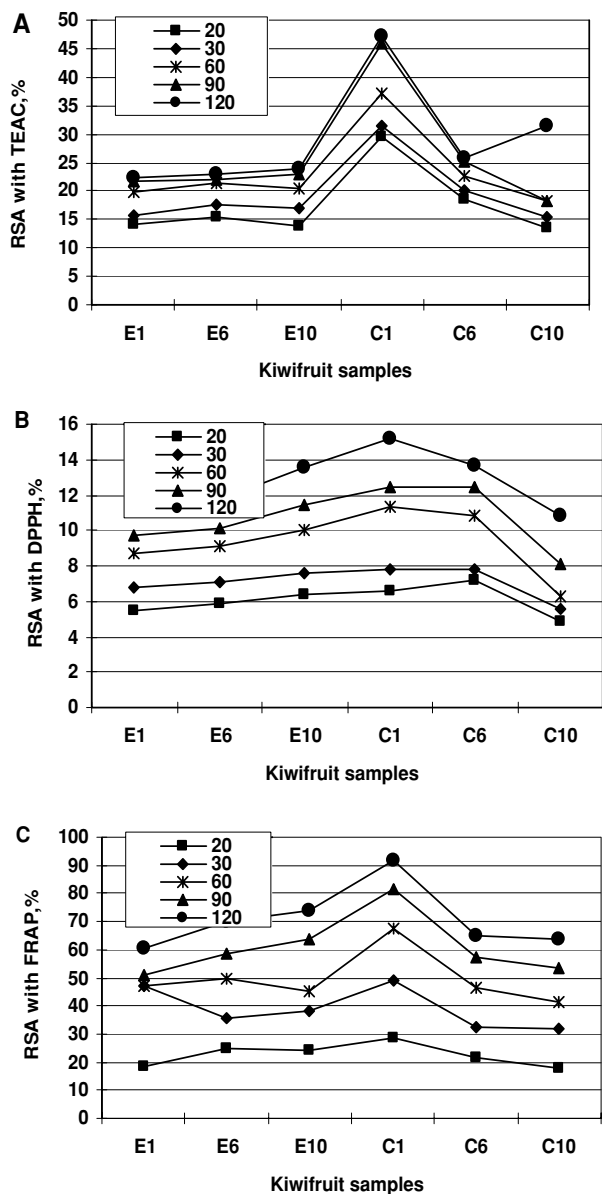


Figure 3. Radical scavenging activity (RSA) of kiwifruit extracts during 10, 30, 60 and 120 min. A, TEAC; B, DPPH; C, FRAP. Abbreviations: TEAC, trolox equivalent antioxidant capacity; DPPH, 1, 1-diphenyl-2-picrylhydrazyl radical; FRAP, ferric-reducing/antioxidant power.

TEAC (Figure 3A), and the lowest with DPPH (Figure 3B), regardless of reaction time, which was slightly different than in another report [28]. The initial values (reaction time about 6–10 min) are comparable to those reported in the presented reviewed articles [25, 26].

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 [25, 26, 28].

The TEAC and CUPRAC values for each extract were similar and well-correlated with the free phenolic contents.

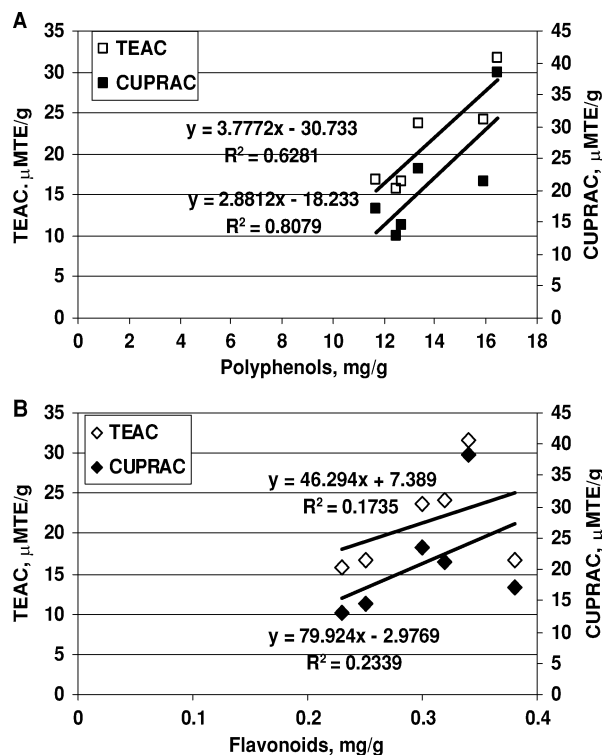


Figure 4. Relationship, calculated by linear regression analysis for kiwifruit extracts between: A, (\square) polyphenol (mg/g DW, X) to TEAC ($\mu\text{MTE/g}$ DW, Y) and (\blacksquare) polyph (mg/g DW, X) to CUPRAC ($\mu\text{MTE/g}$ DW, Y₂); B, (\diamond) flavonoids (mg/g DW, X) to TEAC ($\mu\text{MTE/g}$ DW, Y) and (\blacklozenge) flavonoids (mg/g DW, X) to CUPRAC ($\mu\text{MTE/g}$ DW, Y₂). Polyphenols expressed as mg gallic acid/g dry weight (DW); TEAC, Trolox equivalent antioxidant coefficient; CUPRAC, cupric reducing antioxidant capacity.

The relationship between the values of free polyphenol concentrations vs. antioxidant activities (Figure 4A and B) are rather interesting, as there is an average linear response ($R^2 = 0.81\text{--}0.63$) and with flavonoids is low ($R^2 = 0.23\text{--}0.17$).

Conclusions

During ethylene treatment the bioactivity of kiwifruit is increasing and reaches its maximum at the 6th day. Therefore it is the best time for kiwifruit consumption. Total polyphenols were the main contributor to the overall antioxidant activity of kiwifruit. The antioxidant activities varied among the kiwifruit samples as determined by various used assays: the highest are with FRAP.

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