ETHYLENE TREATED KIWI FRUITS DURING STORAGE. 
PART I: POSTHARVEST BIOACTIVE, ANTIOXIDANT AND 
BINDING PROPERTIES

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Received for Publication February 19, 2016
Accepted for Publication April 17, 2016

doi:10.1111/jfpp.13084

†This article was written in memory of my 
dear brother Prof. Simon Trakhtenberg, 
who died in November 2011, 
who encouraged me and our research 
group during all his life.

ABSTRACT

Kiwi fruits “Hayward” were submitted to ethylene treatment during 24 h, follow-
ing by storage at 20°C for 10 days. Significant differences were found in polyphen-
ols and in the antioxidant capacities in conventional, low chemical and organic 
kiwi fruits. Ethylene treatment increased the bioactivity of organic, low chemical 
and conventional kiwi fruit. The antioxidant values for organic fruits were signifi-
cantly higher than for conventional and nontreated samples. All investigated kiwi 
fruits showed a high level of correlation between the contents of phenolic com-
ounds, their antioxidant and binding values. The statistical evaluation of bioac-
tivity demonstrated that cultivation system and ethylene treatment following by 
storage have the potential to enhance the accumulation of health-beneficial food 
compounds in kiwi fruit and changes of the binding properties.

PRACTICAL APPLICATIONS

The article describes the impact of three treatments including different agricul-
tural systems, ethylene treatment and storage and biochemical changes in kiwi 
fruit. Agricultural system produced three different kiwi fruits: organic, semi-
organic and conventional. Different cultivation systems affect the polyphenols 
and antioxidant status of kiwi fruit. During the ethylene treatment and storage 
the antioxidant status and binding properties have increased. In general these 
treatments affect the contents of the main bioactive compounds which have high 
nutritional value. In practice we can select the best treatment and its duration in 
connection with the best quality of the final product. This could be of economical 
importance for the kiwi fruit industry in development of new products with long 
shelf life and high quality of the final product.

INTRODUCTION

Fruit and vegetable intake is associated with lower risk of 
chronic diseases as a result of consumption of antioxidant 
substances. Increased consumption of fruits protects car-
diovascular diseases, because of the unique composition 
of green kiwi fruit, which has the potential to benefit 
cardiovascular disease risk (Lee et al. 2010; Gammon 
et al. 2012). Organic foods have higher antioxidant 
capacity, because this form of agricultural management 
could induce synthesis of secondary compounds such as
polyphenols (Faller and Fialho 2010). Interest in organically produced food is increasing in response to concerns about intensive agricultural practices and their potential effect on human health as well as on the environment (Roghelia and Patel 2013). The effect of cultivation system on phenolic profile and antioxidant capacity in black and red currants, blueberries, tomatoes was evaluated (Wang et al. 2008; Wojdylo et al. 2013; Pataro et al. 2015). Kiwi fruit (Actinidia deliciosa) “Hayward,” grown under different conditions, is one of the most known cultivars. The effect of cultivation systems and fruit postharvest management on the antioxidant properties of fruits and vegetables was investigated (Migliori et al. 2012). Postharvest performance of organic and conventional “Hayward” kiwi fruits grown on the same farm in Marysville, California, and harvested at the same maturity stage was compared (Amodio et al. 2007). “Hayward” treated with ethylene or 1-methylcyclopropene (1-MCP) was compared with nontreated in a number of reports (Park et al. 2006; Jhalegar et al. 2011; Vieira et al. 2010). CaCl₂ sprays significantly enhanced fruit Ca concentration and improved fruit quality during cold storage and shelf life (Gang et al. 2015). However, the main properties of treated and storing kiwi fruit samples were not investigated. The subject of this research was a comparison of the main kiwi fruit properties after ethylene treatment, following storage. To answer these questions, advanced analytical methods were used in combination with statistical calculations.

MATERIALS AND METHODS

Fruit Samples

Kiwi fruit cultivar “Hayward” was grown under conventional, low chemical and organic conditions in the orchard Heanam County (longitude 126° 15’ and latitude 34° 18’), Jeonnam province, Korea. The harvest date was October 30, 2014 and October 18, 2015. Fruits with defects were discarded and 50 good fruits (80–100 g) were placed in five replicated glass jars. These healthy fruits were divided into two groups, the first group was treated with 100 ppm ethylene for 24 h at 20°C, and the second was not treated (control). The fruits were put into an 18 L glass jar and ventilated with humidified flow of air or air mixed with ethylene of 300 mL/mL. Thereafter, the ethylene treated fruits were ripened separately at 20°C growth chamber (Percival Scientific, Inc., 505 Research Drive Perry, IA) for 10 days. Control fruits were immediately ripened at 20°C in the same growth chamber as above. The samples were treated with liquid nitrogen in order to prevent oxidation of phenolic compounds and then lyophilized as previously described (Gorinstein et al. 2011; Park et al. 2012).

Chemicals

Trolox; phenolic standards; Tris, tris (hydroxymethyl) aminomethane; Folin–Gioacalteu reagent; FeCl₃·6H₂O; were purchased from Sigma Chemical Co., St Louis, MO, 4, 6-Tripyridyl-s-triazine (TPTZ) was from Fluka Chemie, Buchs, Switzerland.

Determination of Phenolic Acids and Flavonoids

Chlorogenic, hydroxybenzoic, caffeic and p-coumaric acids, catechin and epicatechin (mg/100g FW) were determined in the sample methanol extract which was filtered through Sepac C18 Cartridge with HPLC solvent. 5mM of hexade-cyltrimethylammonium bromide and 5 mM KH₂PO₄ (pH around 4.6) were added to the sample. The standard and extracted samples were analyzed using HPLC pump system (Waters, Model 510), connected with a UV detector (Waters) set at 365 nm. The Waters Symmetry C₁₈ column (0.5 μm, 4.6x250 mm) was pre-equilibrated with a mobile phase consisting of methanol: water (5:95, v/v) at a flow rate of 1.5 mL/min on the 25°C column temperature (Korsak and Park 2010; Jhalegar et al. 2011). Determination of bioactive compounds and total antioxidant capacities: The phenols were extracted with methanol (concentration 20 mg/mL) during 1 h in a cooled ultrasonic bath. Total polyphenols (TP, mg gallic acid equivalents (GAE)/100 g FW) were determined by Folin–Gioacalteu method (Singleton et al. 1999) with absorbance measurements at 750 nm using spectrophotometer (Hewlett-Packard, model 8452A, Rockville).

Antioxidant Assay/Activity

Ferric reducing/antioxidant power (FRAP, mM Fe (II) equivalent)/100 g FW) assay measures the ability of the antioxidants in the investigated samples to reduce ferric-tripiridyltriazine (Fe³⁺ TPTZ) to a ferrous form (Fe²⁺). FRAP reagent (2.5 mL of a 10 mmol ferric-tripiridyltriazine solution in 40 mmol HCl plus 2.5 mL of 20 mmol FeCl₃ xH₂O and 25 mL of 0.3 mol/L acetate buffer, pH 3.6) of 900 μL was mixed with 90 μL of distilled water and 30 μL of kiwi fruit extract samples as the appropriate reagent blank. The absorbance was measured at 595 nm (Benzie and Strain 1996).

Fluorometric Measurements

Two dimensional (2D–FL) fluorescence measurements for kiwi fruit extracts at a concentration of 0.01 mg/mL were recorded on a model FP-6500, Jasco spectrofluorometer, serial N261332, Japan, equipped with 1.0 cm quartz cells.
and a thermostat bath. The 2D–FL was taken at emission wavelengths from 300 to 750 nm; and at excitation of 280 nm. The three-dimensional spectra (3D-FL) were collected with subsequent scanning emission spectra from 200 to 795 nm at 1.0 nm increments by varying the excitation wavelength from 200 to 500 nm at 10 nm increments (Gorinstein et al. 2011; Park et al. 2015).

**Statistical Analysis**

The data were processed by statistical analysis in order to assess the effects of production conditions as well as the effects of ethylene treatment and long-term storage on the content of selected phenolic acids, flavonoids and polyphenols on “Hayward” kiwi fruit extracts. Two-way ANOVA...
(Friedman test) was used to consider the significance of the differences resulting from the above-mentioned treatments. The differences were recognized as significant at the level of significance of $P \leq 0.05$, and the differences in means of individual compared characteristics were recognized as highly significant at $P < 0.001$. Multivariate statistics was used to reduce the dimensionality of the space of experimental characteristics and to visualize the effects of treatments on the properties of kiwi fruit samples. From multidimensional pattern recognition techniques, the principal component analysis (PCA) and canonical discriminant analysis (CDA) were used to define, interpret and visualize the significance of individual ways of treatment on kiwi fruit antioxidant characteristics. In order to find possible tendencies in the samples and the discriminant power of individual antioxidant characteristics, principal component factoring was effectively applied. All the statistical calculations and data visualizations were performed using the statistical package Unistat v. 6.0 (Unistat, London, United Kingdom). Origin (Microcal, Northampton, MA) was also used for data visualization in plots, if not specified otherwise.

**RESULTS AND DISCUSSION**

**Phenolic Acids and Flavonoids**

The fresh "Hayward" kiwi fruit samples cultivated by conventional, low chemical and organic methods, treated with ethylene for 24 h at 20°C and then stored were examined for bioactivity quality parameters: hydroxybenzoic, chlorogenic, caffeic and $p$-coumaric acids, catechin, epicatechin, antioxidant activity (FRAP) and total phenolic content (TPC). Phenolic compounds in kiwi fruit pulp were separated and characterized by reversed-phase HPLC. During ethylene treatment and then storage different changes appeared in the bioactivity of the investigated fruits. Strongly acidic compounds were identified as derivatives of $p$-coumaric and caffeic acids (CAs), including chlorogenic acid, protocatechuic acid, and a derivative of 3, 4-dihydroxybenzoic acid. The hydroxybenzoic acid (mg/100g FW) has changed for the organic treated fruits (from $10.03 \pm 0.10$ to $12.66 \pm 0.63$) in comparison with the conventional (from $5.77 \pm 0.29$ to $7.76 \pm 0.39$, Fig. 1a). The chlorogenic acid (mg/100g FW) has changed for the
organic treated fruits (from 36.22 ± 2.27 to 49.11 ± 2.46) in comparison with the conventional (from 25.49 ± 0.84 to 35.48 ± 2.27, Fig. 1b). The CA (mg/100g FW) has changed for the organic treated fruits (from 37.33 ± 1.87 to 47.52 ± 2.38) in comparison with the conventional (from 20.46 ± 1.02 to 27.19 ± 1.36, Fig. 1c). The p-coumaric acid (mg/100g FW) has changed for the organic treated fruits (from 13.51 ± 0.68 to 17.11 ± 0.86) in comparison with the conventional (from 8.89 ± 0.39 to 12.60 ± 0.54, Fig. 1d).

As it can be compared from the presented data that all organic kiwi fruit samples significantly differ from conventional associates. This is in line with Doverspike et al. (2015) about the importance of some phenolic acids which are involved in fruit metabolism. Chlorogenic acid (CGA) is a secondary plant metabolite with chemotherapeutic and chemopreventative potentials along with other health benefits and the amount of this acid is the highest. CGA and CA, a product of the decomposition of CGA, found in skins and flesh of various fruits, and comparison of concentrations in organically and conventionally grown fruits is important. Our results were in agreement with Doverspike et al. (2015), but these phenolic acids were found in tomatoes and peaches/nectarines. CA in organic kiwi fruit was 1.75 times higher than in conventional (Fig. 1c), and chlorogenic acid in 1.38 times higher (Fig. 1b). The low chemical fruits were 1.49 and 1.20 times higher than conventional. Our results were agreed with the fact that phenolic compounds were present at levels of <1-7 mg/L in clarified kiwi fruit juice (Dawes and Keene 1999). In spite of many variables studied in this report the results of bioactivity of organic kiwi fruit during two seasons were significantly higher than in conventional fruits (Park et al. 2015).

Some variables are connected with and harvest dates, post-harvest processing, and storage methods and the production quality of completely different systems. Other reports were dealing with the contents of soluble and total phenolic acids in 29 berries and berry products, 24 fruits and fruit peels, and 12 beverages. Among fruits, the highest contents of phenolic acids (28 mg/100 g) were determined in dark plum, cherry, and one apple variety, and lower amount was found in kiwi fruit as it was determined in this study (Mattila et al. 2006; Leontowicz et al. 2016). The weakly acidic fraction contained epicatechin, catechin, and procyanidins (B3, B2 or B4 and oligomers). The content of catechin (mg/100g FW) has changed for the organic treated fruits (from 36.88 ± 0.1.64 to 50.09 ± 2.50) in comparison with the conventional (from 26.19 ± 1.31 to 35.9 ± 1.80, Fig. 2a). Epicatechin (mg/100g FW) has changed for the organic treated fruits (from 34.86 ± 1.39 to 46.96 ± 2.35) in comparison with the conventional samples (from 24.66 ± 1.39 to 33.78 ± 2.05, Fig. 2b). Catechin and epicatechin increased as much as 1.40 times higher in organic fruits than in conventional.

**Antioxidant Activity/Phenolic Acids**

The final results of total phenolics and the antioxidant activities after all treatments differed for conventional and organic kiwi fruit and were significantly higher in organic

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**FIG. 3. EFFECTS OF ETHYLENE TREATMENT (C-CONTROL VERSUS T-TREATED), GROWING CONDITIONS AND STORAGE AT 20°C ON:**

(A) FRAP VALUES (mM Fe\(^{3+}\)/100 g FW) AND (B) TOTAL POLYPHENOLS CONTENT (mg GALLIC ACID EQUIVALENTS (GAE)/100 g FW) OF KIWI FRUIT “HAYWARD”

The depicted data are the averages of 10 measurements.
kiwi fruit than in conventional samples (Fig. 3). The antioxidant activity by FRAP values (mM Fe$^{2+}$/100 g FW) has changed for the organic treated fruits (from 4.05 ± 0.15 to 4.51 ± 0.22) in comparison with conventional (from 2.25 ± 0.11 to 2.56 ± 0.32, Fig. 3a). The total polyphenols (mg GAE/100g FW) have changed for the organic treated fruits (from 145.256 ± 4.225.04 to 163.11 ± 2.96, Fig. 3b) in comparison with conventional (from 137.14 ± 4.22 to 154.18 ± 7.92, Fig. 3b). Previously we showed that not always the quality of organic plants was higher than the normally grown (Park et al. 2012). Antioxidant values found in this research were different from the ones shown by Park et al. (2006). Our results were in line with Wojdylo et al. (2013), where was found that Ribes fruit grown in organic system possess significantly higher total phenolics and antioxidant activity (FRAP) than fruit grown in conventional system. The mean value of total polyphenol content in organically grown currants was similar but statistically higher compared with the conventional cultivation. All currants from organic cultivation possess higher ferric reducing capacity than conventionally grown fruits. The results showed that organic fruits tend to have higher hydrolysable polyphenol contents than conventional ones with values being 11.5% in orange peels, 72.6% in papaya peels, and for organic kiwi fruit about 5.5%. The extraction of phenolic acids in fruits depends on the temperature and the time of extraction. The temperatures of 41–60°C were suitable for major extraction of bioactive compounds and antioxidant capacity from apple pomace. The phenolic compounds were mostly extracted at 60°C in orange peel, as well as the optimization indicated that flavonoids, and antioxidant capacity (DPPH and FRAP assays) were extracted during 0.5 h at 60°C (Hernández-Carranza et al. 2016). Polyphenol content and antioxidant capacity varied among organic and conventional vegetables with no prevalence from either agricultural type. In general, organic agriculture results in food products with similar or slightly higher polyphenol content and antioxidant capacity (Faller and Fialho 2010) showing that blueberry fruit grown from organically culture yielded significantly higher total phenolics and antioxidant activity than fruit from the conventional culture. In organically cultured fruit, the average values for antioxidant activity and polyphenols in comparison with the same indices in conventional were 1.49 and 1.68 times higher (Wang et al. 2008) and in this study the values were 1.76 and 1.06 times. These results indicate that significant differences between two cultivation practices were evident (Wang et al. 2008). Organic kiwi fruits have high levels of total phenol content, resulting in a high antioxidant activity. Their changes were measured after each storage duration at 0, 35, 72, 90 and 120 days of
storage at 0°C and after 1 week of shelf-life simulation at 20°C. In a recent report, as it was mentioned previously, “Hayward” kiwi fruits were treated with 100 ppm ethylene at 20°C for 24 h and then immediately ripened at 20°C for 10 days (Korsak and Park 2010). Comparing antioxidant profile, organically grown fruits showed significantly higher contents of total phenols, flavonoids (catechin and epicatechin) and total antioxidant activities than conventionally grown fruits. Our results (Park et al. 2012) were also in line with Anton et al. (2014), where it was shown that the content of polyphenols was more dependent on year and cultivar than on cultivation conditions. Generally, the cultivation system had minor impact on polyphenols content, and only a few compounds were influenced by the mode of cultivation in all tested cultivars during 2 years. Therefore it is difficult to measure the differences in all variables even during two collection seasons. Recent investigations of nutritional quality in organic versus conventional produce also indicate that soil nitrogen delivery rates strongly affect nutritional quality. Pal et al. (2015) showed a strong correlation between polyphenols and antioxidant activity. Positive relationship between total phenol-flavonoids, total phenol-total antioxidant capacity and flavonoids-total antioxidant capacity among organically grown fruits is presented in this study. Hence, the study concludes that organically grown fruits offer higher amount of nutritional as well as antioxidant properties with inclusion of organically grown fruits in daily diet which could lead to a positive health effects (Roghelia and Patel 2013).

Processing of Data by Multivariate Statistics

Canonical Discriminant Analysis. When the data are processed by CDA separately for the control group of samples and samples treated by ethylene, in both cases absolute differentiation of organically, low-chemically and conventionally grown samples was reached. While for the control group, the dominant role in discrimination had the polyphenols content and FRAP in the first discriminant function and CA and catechin concentrations in the second one. In the ethylene treated group, the dominant role in discrimination played the concentration of hydroxybenzoic acid and FRAP values for the first and hydroxybenzoic and p-coumaric acids concentrations for the second discriminant function construction. As expected on the basis of statistical tests performed with respect on ethylene treatment, the discrimination of kiwi fruit samples remain absolute also when this factor has been ignored, as is clearly depicted on Fig. 4a.
Values of the standardized discriminant coefficients, the dominant role in discrimination exhibited the FRAP values/TPC content and the concentration of \( p \)-coumaric/hydroxybenzoic acids, as regards the first and the second discrimination function construction. It is also apparent from the Fig. 4a, that the properties of conventionally grown samples are closer to that low-chemically grown than those, grown under the organic conditions. This visual tendency is also supported by the calculated distances between the centroids (C-LC = 14.97; C-O = 47.74; LC-O = 34.05). Looking on...
these data it can be also concluded, that the kiwi fruit (in terms of their acids content and antioxidant properties) are significantly influenced by the growing conditions. The classification of kiwi fruit samples according to the time of storage brings only partially successful discrimination and classification, reaching thus the average classification score of 50%. It should be mentioned, that all the other factors (growing conditions, ethylene treatment) were ignored. When the variability of the experimental characteristics is decreased (discrimination is performed separately for the control group of samples nontreated by ethylene, and for the ethylene treated samples), then the results of discrimination are much more promising. In both cases, samples were classified into separate groups according to the time of storage with 100% correctness, as is illustrated on Fig. 4a. However, as the signs of the discrimination coefficients in individual functions are opposite, when discriminated altogether, the discrimination score decreases as indicated above. From the above-presented results, it is apparent that ethylene treatment influences the properties of kiwi fruit samples, as well.

Principal Component Factoring. All the factors lay in the same sector of the graph practically in one cluster, indicating the potential correlation of individual characteristics. Only slight vector differences for FRAP and polyphenols content are obvious (Fig. 5). However, such result can be satisfactorily explained by the fact, that all these characteristics can be considered as antioxidants or revealing the antioxidant properties. Thus, they can be partially used for characterization of antioxidant properties of the complex system. Results of factor analysis fully supported also the values of Pearson's correlation coefficients. For majority of cases, very high correlations (> 0.9) were calculated; with exception for just FRAP and polyphenols content. Last two mentioned are characterized by still high but slightly lower correlation coefficients (≈ 0.8).

Principal Component Analysis. PCA explains the variability of the data set of experimental characteristics and is used to reduce the number of potentially correlating variables via their transformation to noncorrelating group of principal components. In case of PCA of eight experimental

**TABLE 1. FLUORESCENCE DATA OF KIWI FRUIT METHANOL EXTRACTS, HUMAN SERUM ALBUMIN (HSA) AND STANDARD: A, 2D- FLUORESCENCE; B, 3-D FLUORESCENCE**

A

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B

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Abbreviations: line numbers shown in Fig. 1A, Emission mode, λ<sub>ex</sub> = 280/λ<sub>em</sub> = 300; *HSA in buffer; **HSA in methanol; All kiwi fruit methanol extracts and standard were used after interaction with HSA; ConvC, conventional control; OrgC, organic control; ConvTr, conventional treated; LChemTr, low chemical treated; OrgTr, organic treated; CaffAc, caffeic acid; λ<sub>ex</sub>/λ<sub>em</sub>, (nm/nm); Fl, fluorescence intensity, A. U., arbitral units; binding2D/3D is determined in %. In 2D the binding properties were found by the decrease of the fluorescence of the main peak (Fig. 6A, Table 1). In 3D, the binding properties were determined by the decrease of fluorescence intensities in peaks a and b Figs. 6 and 7, Table 1).
characteristics related to antioxidant properties of kiwi fruit, it can be concluded that the PCA explains the variability very effectively – even the 1st principal component is capable of explanation of more than 92% of the whole system variability, while the 2nd component itself explains 5.5% of the remaining variability, reaching thus very promising almost 98% explanation of the whole system variability. 1st PC is constructed equally from all the experimental characteristics, while in the 2nd PC, the dominant role of FRAP value was identified on the basis of eigenvalues comparison. Plot of principal components clearly differentiated the eigenvectors according to the samples growing conditions (conventional - low-chemical –...
organic) but also the differentiation according to the ethylene treatment (control versus treated group) can be performed, as indicated by the dashed line in the Fig. 5b. All eight characteristics were utilized for PCs construction. Scores (in %) indicate the contribution of individual components to the explanation of the properties (variability) of the data set of experimental characteristics and the dominant parameter in PC.

**Fluorescence Measurements**

The changes in the kiwis’ bioactivities during different treatments were interesting to investigate by fluorescence. The 2D fluorescence spectra showed the results described below. At emission of the wavelengths from 347 to 355 nm the recording of the peaks in the fluorescence spectra for conventional, low chemical and organic ethylene treated kiwi fruit after interaction with HSA were with fluorescence intensities (Fls) 683.07, 592.58 and 306.12 arbitral units (Fig. 6A, lines 5–7 from the top) in comparison with nontreated of 815.38 and 814.44 (Table 1, Fig. 6A, lines 3, 4 from the top). The changes in the fluorescence intensity of treated and untreated samples corresponded with the changes of polyphenol compounds (Table 1). The decrease in the intensity of the main peak during binding of polyphenols to HSA was the smallest for organic treated kiwi fruit (Table 1, Fig. 7F, 7Ff) in comparison with conventional control (Table 1, Fig. 6D, 6Df). Conventionally, semi-organically (low chemical) and organically grown kiwi fruit was stored at 20°C for 10 days. Fluorescence measurements showed a difference between the investigated samples, especially in their antioxidant status. The quenching properties of these samples are correlated with their antioxidant activities and the amounts of polyphenols. The decrease in fluorescence intensity (Park et al. 2015) was proportional to the binding properties of extracted polyphenols.

**CONCLUSIONS**

From all the statistical analyses, it can be stated here that the antioxidant properties and selected phenolic acids contents of kiwi fruit significantly influenced by both ethylene treatment and the growing conditions. The effect of long term storage is apparent, as well, however, it can only be evaluated in dependence on ethylene treatment. The binding properties of organically grown kiwi fruit were higher than in conventional.

**ACKNOWLEDGMENTS**

This research was partly supported by the Rural Development Administration (RDA), Korea. The authors are thankful to Dr. Elena Katrich (School of Pharmacy, Hebrew University of Jerusalem) for her technical assistance in determination of antioxidant activity and 3D fluorescence.

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