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Antioxidants and proteins in ethylene-treated kiwifruits

Yong-Seo Park [a], Soon-Teck Jung [b], Seong-Gook Kang [b], Buk Gu Heo [c], Patricia Arancibia-Avila [d], Fernando Toledo [d], Jerzy Drzewiecki [e], Jacek Namiesnik [f], Shela Gorinstein [g,*,1]

[a] Department of Horticultural Science, Mokpo National University, Muan, Jeonnam, South Korea
[b] Department of Food Engineering, Mokpo National University, Muan, Jeonnam, South Korea
[c] Naju Foundation of Natural Dyeing Cultural Institute, Naju, South Korea
[d] Department of Basic Sciences, Universidad del Bio-Bio, Chillan, Chile
[e] Plant Breeding and Acclimatization Institute, Radzikow, Poland
[f] Department of Analytical Chemistry, Gdansk University of Technology, Gdansk, Poland
[g] Department of Medicinal Chemistry and Natural Products, School of Pharmacy, The Hebrew University – Hadassah Medical School, Jerusalem, Israel

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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

* Corresponding author. Tel.: +972 2 6758690; fax: +972 2 6757076.
E-mail address: gorin@cc.huji.ac.il (S. Gorinstein).
[1] S.G. is affiliated with the David R. Bloom Center for Pharmacy.

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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