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Original Article

Comparison of the contents of bioactive compounds and the level of antioxidant activity in different kiwifruit cultivars

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ABSTRACT

The aim of this investigation was to find the best among four different cultivars of kiwifruit ('Hayward', 'Daeheung', 'Haenam' and 'Bidan') for human consumption. The bioactive compounds and the level of antioxidant activity of these cultivars were determined and compared. The presence of polyphenols (flavonoids and phenolic acids) in the investigated samples was studied by Fourier transform infrared (FT-IR) spectroscopy. By far the highest levels ($P < 0.05$) of polyphenols and ascorbic acids were found in 'Bidan' (25.9 ± 1.3 mg GAE/g and 152 ± 10.4 mg/g DW, respectively), which also contained the highest levels of protocatechuic and vanillic acids. In addition, the level of antioxidant activity (μM TE/g DW) was significantly higher ($P < 0.05$) in 'Bidan' (121 ± 5.8, 109 ± 11.2, 102 ± 6.6 and 94 ± 4.7 for CUPRAC, ABTS, DPPH and FRAP radical scavenging assays, respectively). Pattern-recognition techniques (cluster analysis, principal component analysis, factor analysis, and canonical discriminant analysis) were used to compare the cultivars. A high correlation was found among the polyphenols ($R^2 = 0.99$), ascorbic acid ($R^2 = 0.99+$) and the antioxidant activity in the studied cultivars. In conclusion, the overall bioactivity of the cultivars was: 'Bidan' > 'Haenam' > 'Daeheung' = 'Hayward'. 'Bidan', a relatively new cultivar, can be recommended for consumption.

1. Introduction

Consumption of fruits and vegetables plays a special role in prevention and treatment of various diseases (Haruenkit et al., 2007; Koh et al., 2009; Sun et al., 2002; Mertz et al., 2009). The health benefits of fruits are attributable in part to their bioactive components such as phenolics and pectic polysaccharides (Franke et al., 2004; Sun-Waterhouse et al., 2009; Río Segade et al., 2008; Cano et al., 2008). One of these fruits is the fruit of the kiwi tree (Jeong et al., 2007; Park et al., 2008a,b, 2009; Samadi-Maybodi and Shariat, 2003). The kiwifruit is the edible berry of a cultivar group of the woody vines of several *Actinidia* species. The most common commercially available, green-fleshed kiwifruit is the 'Hayward' cultivar, which belongs to the *Actinidia deliciosa* species (Fiorentino et al., 2009). It has been shown that kiwifruit is effective even in the prevention of coronary atherosclerosis (Duttaroy and Jørgensen, 2004). These authors reported that consumption of two or three kiwifruits daily reduces the level of triglycerides in the blood by 15% and platelet aggregation response by 18%, compared with controls ($P < 0.05$). Different indices in kiwifruits were investigated (Park et al., 2008a,b, 2009; Du et al., 2009; Fiorentino et al., 2009; Koutouvela et al., 2009). Not all investigators in the past have used a wide range of assays to determine the antioxidant activity, and they did not study kiwifruit cultivars. Fiorentino et al. (2009), for example, studied one cultivar, 'Hayward', and the antioxidant activity was assayed only by DPPH. 'Bidan', is a new, large-fruited kiwifruit cultivar, 'White', selected from *Actinidia eriantha* and widely cultivated in Korea (Nishiyama, 2007; Park et al., 2008b; Wu et al., 2009). This cultivar could be the fourth generation of kiwifruit to be commercialized (Jo et al., 2007). Therefore it was interesting to compare the bioactivity of this cultivar with the well-known and widely consumed type 'Hayward' with that of two others, 'Daeheung' and 'Haenam', using four generally accepted assays for the determination of their antioxidant activity.

As far as we know, results of such investigations have not been published.

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2. Materials and methods

2.1. Chemicals

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent (FCR), lanthanum (III) chloride heptahydrate, $FeCl_3 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$, 2,9-dimethyl-1 and 10-phenanthroline (neocuproine) were purchased 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. Deionized and distilled water was used throughout.

2.2. Samples

All kiwifruit cultivars that reached commercial maturity stage were harvested in an orchard located in Heanam County, Jeonnam Province, Korea in 2009. One kilogram of each cultivar was used for the analyses (Greenfield and Southgate, 2003). The samples were washed with tap water and dried. Their edible parts were prepared manually without using steel knives. The peeled fruits were weighed, chopped and homogenized in liquid nitrogen in a high-speed blender (Hamilton Beach Silex professional model) for 1 min. A weighed portion (50–100 g) was then lyophilized for 48 h (Virtis model 10–324), and the dry weight was determined. The samples were ground to pass through a 0.5 mm sieve and stored at −20 °C until the bioactive substances were analysed.

2.3. Determination of bioactive compounds and antioxidant activity

The contents of minerals, dietary fibers, polyphenols, tannins, flavonoids and flavanols in methanol extracts of the studied cultivars were determined as previously described (Gorinstein et al., 2009; Haruenkit et al., 2007). Each analysis was replicated five times, using five extracts from each cultivar.

The presence of polyphenols (flavonoids and phenolic acids) in the investigated samples was examined by Fourier transform infrared (FT-IR) spectroscopy. A Bruker Optic GMBH Vector FT-IR spectrometer (Bruker Optic GMBH, Attingen, Germany) was used to record IR spectra. A potassium bromide microdisc was prepared from finely ground lyophilized powder of 2 mg of fruit samples with 100 mg of KBr (Edelmann and Lendl, 2002; Sinelli et al., 2008).

Phenolic acids were extracted as described elsewhere (Cvikrová et al., 1991).

Free, methanol soluble ester-bound (released after alkaline hydrolysis) and methanol soluble glycoside-bound (released after acid hydrolysis) phenolic acids were obtained from a methanolic extract of tissue ground in liquid nitrogen. The 2,6-di-*terc* butyl β-cresol was added, as an antioxidant, and nitrogen was immediately bubbled through the sample, after adding NaOH, to minimize the oxidation of phenolic acids during alkaline hydrolysis. Phenolic acids were analysed by HPLC using a Dionex (Dionex Sofron GmbH, Germering, Germany) Liquid Chromatography system consisting of a P660-HPLC Pump, ASI-100 Automated Sample Injector, TCC-100 Thermostated Column Compartment and PDA-100 Photodiode Array Detector, equipped with a C18 column (Waters Spherisorb ODS-2 5 μm, 250 mm × 4.6 mm, Supelco, Ballefonte, PA, USA) and controlled by Chromeleon Software 6.5. The analytes (in 10 μL or 20 μL portions of the samples) were eluted with a mobile phase (flow rate, 0.5 mL min^{-1}) at 45 °C consisting of the following gradient of 0.01 M citric acid, 0.01 M $Na_2HPO_4 \cdot 2H_2O$, adjusted to pH 2.4 with H_3PO_4 (solvent A) and 80% (v/v) methanol (solvent B), in percentages of solvent A: 0–40 min, 100–85%; 40–60 min, 85–65%; 60–75 min, 65–0%; 75–83 min, isocratic 0%; 83–90 min, 0–100%. The column was washed with 100% solvent A. Eluted phenolic acids were quantified by monitoring their absorption maxima and comparing them with those of authentic reference compounds (Sigma–Aldrich, Prague, Czech Republic).

Flavonoid compounds were extracted with 80% methanol accordingly to the method of glycoside-bound phenolic acids extraction (released after acid hydrolysis). Compounds were eluted using a gradient of acetonitrile (ACN) with phosphoric acid by modified method of Peifeng (Xue et al., 2007). Epicatechin, esculetin, quercetin, kaempferol and apigenin were used as standards.

Total polyphenols were extracted from a 50 mg aliquot of lyophilized fruit samples with 5 mL of 100% methanol. Then polyphenols were determined by the Folin-Ciocalteu method with measurement at 750 nm using spectrophotometer (Hewlett-Packard, model 8452A, Rockvile, USA). The results were expressed as milligrams of gallic acid equivalents (GAE) per gram DW. Flavonoids, extracted with 5% $NaNO_2$, 10% $AlCl_3 \cdot 6H_2O$ and 1 M NaOH, were measured at 510 nm. The total flavanols were estimated using the *p*-dimethylaminocinnamaldehyde (DMACA) method, and then the absorbance at 640 nm was read. The extracts of condensed tannins (procyanidins) with 4% methanol vanillin solution were measured at 500 nm. (+)-Catechin served as a standard for flavonoids, flavanols, and tannins, and the results were expressed as catechin equivalents (CE).

For determination of antioxidant activity, four complementary assays were used: ABTS, DPPH, FRAP and CUPRAC (Apak et al., 2004; Ozgen et al., 2006).

Ferric-reducing antioxidant power (FRAP) was used to measure the ability of the antioxidants contained in the samples to reduce ferric tripiridyltriazine (Fe^{3+}-TPTZ) to a ferrous form (Fe^{2+}), which absorbs light at 593 nm. The antioxidant activity was determined at constant concentration and also with different concentrations of the fruits from 5 to 25 mg/mL. 2,2-Azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid) diamonium salt ($ABTS^{\bullet+}$) was generated by the interaction of ABTS (mmol/L) and $K_2S_2O_8$ (2.45 mmol/L). This solution was diluted with methanol until the absorbance reached 0.7 at 734 nm. 1-Diphenyl-2-picrylhydrazyl method (DPPH) solution (3.9 mL, 25 mg/L) in methanol was mixed with the samples extracts (0.1 mL). The reaction progress was monitored at 515 nm until the absorbance was stable. Cupric-reducing antioxidant capacity (CUPRAC) 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 NH_4Ac buffer

Table 1
The contents of minerals (mg 100 g^{-1} DW) and dietary fibers (mg g^{-1} DW) in four kiwifruit cultivars.

	'Hayward'	'Daeheung'	'Haenam'	'Bidan'
P	244 ± 10.9[a]	243 ± 10.8[a]	244 ± 10.8[a]	365 ± 14.3[b]
K	1683 ± 21.1[a]	1683 ± 21.1[a]	1683 ± 21.1[a]	1997 ± 27.1[b]
Ca	146 ± 7.02[a]	146 ± 7.08[a]	146 ± 7.09[a]	187 ± 8.91[b]
Mg	74.3 ± 3.61[a]	74.4 ± 3.62[a]	74.3 ± 3.58[a]	94.5 ± 4.31[b]
Na	22.6 ± 1.12[a]	22.5 ± 1.11[a]	22.6 ± 1.12[a]	34.5 ± 1.71[b]
Fe	1.14 ± 0.05[a]	1.12 ± 0.05[a]	1.13 ± 0.05[a]	2.11 ± 0.11[b]
Mn	1.94 ± 0.11[a]	1.91 ± 0.11[a]	1.93 ± 0.11[a]	2.92 ± 0.14[b]
Cu	0.09 ± 0.00[a]	0.09 ± 0.00[a]	0.09 ± 0.00[a]	0.20 ± 0.01[b]
Zn	1.00 ± 0.05[a]	1.00 ± 0.05[a]	1.01 ± 0.05[a]	1.68 ± 0.08[b]
B	0.54 ± 0.03[a]	0.54 ± 0.03[a]	0.54 ± 0.03[a]	0.94 ± 0.04[b]
S	13.3 ± 0.61[a]	13.4 ± 0.61[a]	13.3 ± 0.61[a]	22.2 ± 1.11[b]
TDF	80.4 ± 0.3[a]	80.5 ± 0.3[a]	80.3 ± 0.3[a]	80.6 ± 0.3[a]
IDF	56.3 ± 0.2b[a]	56.4 ± 0.2b[a]	56.3 ± 0.2b[a]	56.4 ± 0.2b[a]
SDF	24.1 ± 0.1[a]	24.1 ± 0.1[a]	24.0 ± 0.1[a]	24.2 ± 0.1[a]

Values are means ± SD per gram dry weight (DW); $n = 5$ samples per cultivar, each subsampled and analysed 5 times. Values in rows with different superscript letters are significantly different ($P < 0.05$).
Abbreviations: TDF, total dietary fiber; IDF, insoluble dietary fiber; SDF, soluble dietary fiber.

solution, extract of fruit sample (or standard) solution (x mL) and H_2O [$(1.1 - x)$ mL] were added to produce the final volume of 4.1 mL. The absorbance at 450 nm was recorded against a reagent blank.

2.4. Statistical analysis

Values are means ± SD per gram dry weight (DW) of 25 measurements representing commercial maturity status of fruits and their replicates. Five replications of five extracts from each cultivar were done. Differences between groups were tested by two ways ANOVA. In the assessment of the antioxidant activity, Spearman correlation coefficients (R) were used. Linear regressions were also calculated. P-values of <0.05 were considered significant. For pattern recognition techniques, the cluster analysis (CA), principal component analysis (PCA), factor analysis (FA), and canonical discriminant analysis (CDA) were used. CA was used to arrange a set of cases into clusters. PCA reduced dimensionality of the data (but retain most of the original variability in the data set). Linear combinations of original dependent variables to a smaller set of new uncorrelated variables (called principal components), or in the case of factor analysis to a new set of variables (called factors) based on patterns of correlation among the original variables was used. CDA generated canonical variables, which are linear combinations of the original variables that described the variation between pre-specified classes in a manner analogous to the way in which PCA summarizes the variation among individual samples. Pattern recognition techniques were realized by the statistical program Unistat® (Unistat Ltd., 4 Shirland Mews, London W9 3DY, England).

3. Results

3.1. Minerals and dietary fibers

The results of mineral composition and the contents of dietary fibers in four cultivars are shown in Table 1. As can be seen, between the studied cultivars only mineral composition in 'Bidan' differ significantly ($P < 0.05$). Total, insoluble and soluble

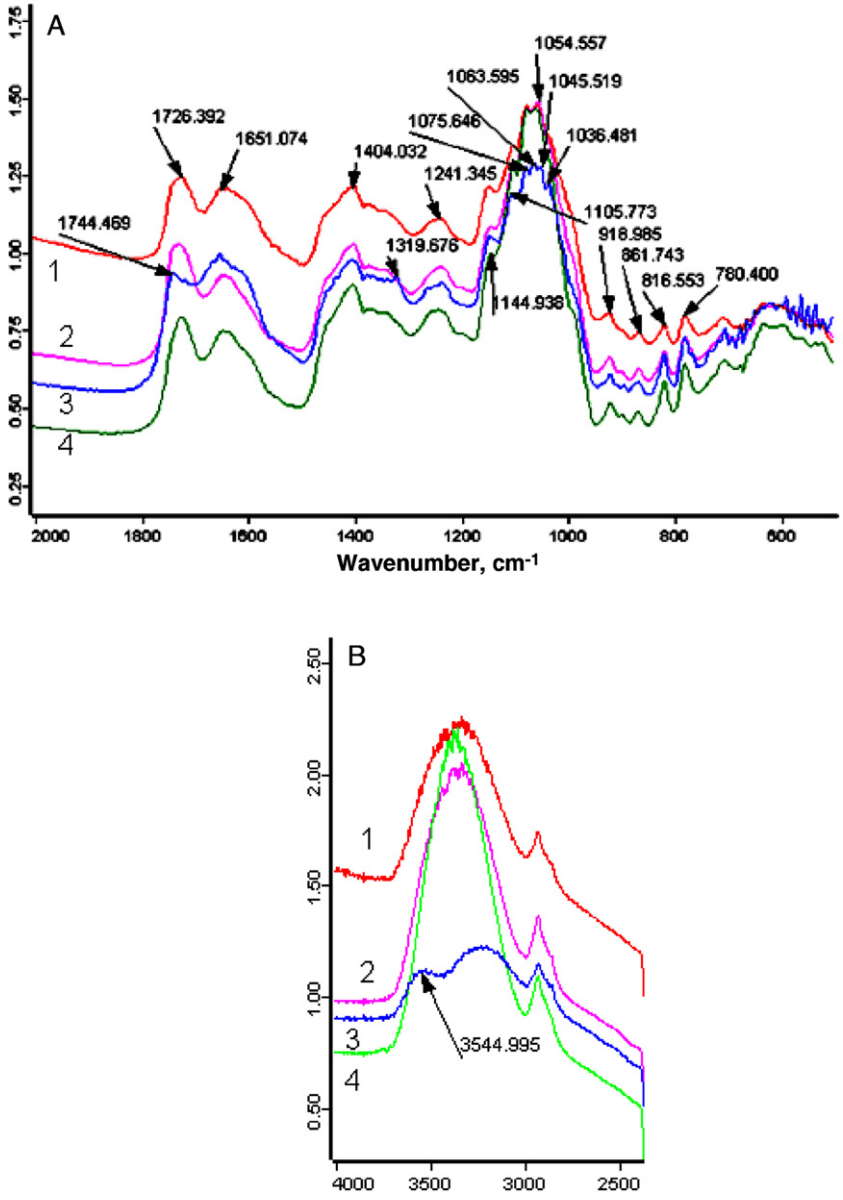

Fig. 1. FTIR spectra of polyphenols from kiwifruit cultivars: (A) from 2000 to 600 cm^{-1}: 1, 'Hayward'; 2, 'Daeheung'; 3, 'Bidan'; 4, 'Haenam'. (B) From 4000 to 2500 cm^{-1}: 1, 'Hayward'; 2, 'Daeheung'; 3, 'Bidan'; 4, 'Haenam'.

dietary fibers in kiwifruits cultivars do not significantly differ ($P > 0.05$).

3.2. Phenolic acids, polyphenols, tannins, ascorbic acid, flavonoids and flavanols

Kiwifruit samples (Fig. 1A, lines 1–4) in the region of polyphenols showed slightly different bands than the standards, but the wavelengths of the bands were similar in this group of fruits. The wavelength numbers of FTIR spectra for catechin at 831, 1040, 1112, 1144, 1285, 1478, 1512 and 1611 cm^{-1} were assigned to –C–H alkenes, –C–O alcohols, C–O–H alcohols, –OH aromatic, C–O alcohols, C–H alkanes, C=C aromatic ring and C=C alkenes, respectively. Gallic acid showed the following wavelength numbers (cm^{-1}): 866, 1026, 1237, 1451, 1542 and 1619 (Gorinstein et al., 2010). The absorption band at 1744 cm^{-1} was present in 'Daeheung' (Fig. 1A, line 2), 1145 and 1106 cm^{-1} in 'Bidan' (Fig. 1, line 3), 1036 cm^{-1} in 'Hayward' (Fig. 1A, line 1). Absorption bands at 1726, 1651, 1404, 1320, 1241, 1064, 1055, 1046, 919, 862, 817 and 780 cm^{-1} were similar in all kiwifruit cultivars. The main absorption band was only in 'Bidan' of about 3545 cm^{-1} (Fig. 1B, line 3).

Phenolic acids and flavonoid compounds were extracted from another cultivar *A. chinensis* Planch. 'Hort16A' and also from organically grown 'Hayward' followed by HPLC determination in order to evaluate the differences in the bioactive compounds in similar to standard kiwi cultivar 'Hayward'. In addition, in two cultivars, 'Hayward' organic and 'Hort16A', the amount of phenolic acids ($\mu g\ g^{-1}$ DW) was the following: protocatechuic (23.4 ± 1.10 and 25.7 ± 1.30), *p*-hydroxybenzoic (0.44 ± 0.03 and 0.38 ± 0.03), vanillic (6.18 ± 0.40 and 4.65 ± 0.30), caffeic (45.3 ± 2.80 and 17.1 ± 0.70), syringic (1.10 ± 0.03 and 1.78 ± 0.06), *p*-coumaric (5.15 ± 0.20 and 2.61 ± 0.05), ferulic (1.50 ± 0.08 and 1.58 ± 0.04), anisic (0.42 ± 0.03 and 1.05 ± 0.05). Protocatechuic, *p*-hydroxybenzoic, vanillic, caffeic, syringic, *p*-coumaric, ferulic and anisic acids were detected in all fruit samples. The most abundant were protocatechuic and vanillic acids in 'Bidan'. *p*-Hydroxybenzoic, syringic, ferulic and anisic acids were not abundant in the samples (Fig. 2 and Table 2). From five flavonoids ($\mu g\ g^{-1}$ DW) that were tested in the experiment, kaempferol was detected in 'Daeheung' (0.32 ± 0.05), 'Bidan' (0.76 ± 0.07) and 'Hort16A' (0.78 ± 0.08) and in 'Hayward', organic-quercetin (0.66 ± 0.05). The contents of polyphenols and ascorbic acids were significantly higher in 'Bidan' cultivar, flavonoids in 'Hayward' and flavanols in 'Haenam' (*P* in all cases < 0.05, Table 3).

3.3. Antioxidant activity

The results of the determination of the level of antioxidant activity of four studied kiwifruit cultivars are shown in Table 3. According to all four assays used, the significantly highest level of antioxidant activity was registered in 'Bidan' cultivar ($P < 0.05$). As was shown above, this cultivar has the highest contents of polyphenols and ascorbic acid. It was of interest to know which of the two bioactive compounds mentioned is the main contributor to the antioxidant activity of the studied cultivars.

As was calculated, a very good correlation between the antioxidant activity as determined by all antioxidant assays and polyphenols and ascorbic acid appeared:

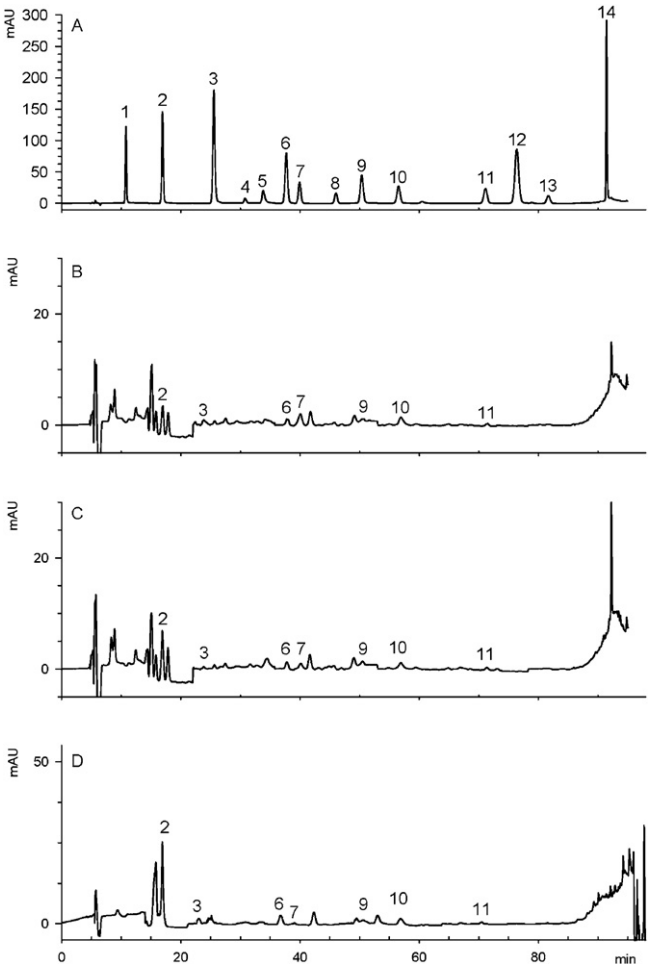

Fig. 2. HPLC analysis of methanol soluble ester-bound phenolic acids extracted from kiwifruits. Each profile represents an equivalent amount of extract, normalized on a volume of extract per 5 mg of tissue basis. Chromatograms are showing the separation: A – standard mixture; B – 'Hayward'; C – 'Hort16A'; D – 'Bidan'. 1 – gallic acid; 2 – protocatechuic acid; 3 – *p*-hydroxybenzoic acid; 4 – *m*-hydroxybenzoic acid, 5 – 2,3-dihydroxybenzoic acid, 6 – vanillic acid; 7 – caffeic acid; 8 – chlorogenic acid; 9 – syringic acid; 10 – *p*-coumaric acid; 11 – ferulic acid; 12 – anisic acid; 13 – sinapic acid; 14 – cinnamic acid.

Table 2
Contents of phenolic acids in kiwifruits ($\mu g\ g^{-1}$ DW).

Compounds	'Hayward'	'Haenam'	'Daeheung'	'Bidan'
Protocatechuic acid	14.1 ± 0.70^a	23.4 ± 1.10^c	17.66 ± 1.30^b	60.1 ± 3.20^d
p-Hydroxybenzoic acid	0.66 ± 0.02^c	0.49 ± 0.03^b	0.33 ± 0.03^a	0.53 ± 0.05^b
Vanillic acid	5.41 ± 0.30^a	6.09 ± 0.28^a	5.11 ± 0.24^a	7.15 ± 0.50^b
Caffeic acid	21.7 ± 1.10^c	10.2 ± 0.52^b	7.18 ± 0.33^a	11.9 ± 0.40^b
Syringic acid	0.66 ± 0.02^b	0.62 ± 0.03^b	0.48 ± 0.02^a	0.81 ± 0.05^c
p-Coumaric acid	4.05 ± 0.30^b	3.12 ± 0.12^a	2.88 ± 0.11^a	3.74 ± 0.07^b
Ferulic acid	1.50 ± 0.05^c	0.39 ± 0.02^a	0.37 ± 0.02^a	0.49 ± 0.03^b
Anisic acid	0.73 ± 0.03^c	0.41 ± 0.02^a	0.39 ± 0.02^a	0.45 ± 0.02^b

Methanol soluble individual phenolic acids (represented by the sum of free, ester- and glycoside-bound forms) were extracted from the lyophilized fruits. Anisic acid was found in free and glycoside – bound forms only; $n = 5$ samples per cultivar, each sub-sampled and analysed 5 times. Values in rows with different superscript letters differ significantly ($P < 0.05$).

Table 3
The contents of bioactive compounds and the antioxidant activity (μM TE/g) in four kiwifruit's cultivars (DW).

Samples	'Haenam'	'Daeheung'	'Hayward'	'Bidan'
Polyph, mg GAE/g	4.00 ± 0.2^{a}	4.86 ± 0.3^{a}	9.60 ± 0.5^{b}	25.9 ± 1.3^{c}
Tannins, mg CE/g	2.40 ± 0.1^{b}	1.20 ± 0.06^{a}	$3.12 \pm 0.\ 2^{b}$	2.40 ± 0.1^{b}
Ascorbic acid, mg/g	6.56 ± 0.2^{a}	13.6 ± 0.2^{b}	30.4 ± 0.4^{c}	152 ± 2.4^{d}
Flavonoids, μg CE/g	162 ± 8.1^{c}	53.8 ± 2.7^{a}	92.1 ± 4.6^{b}	54.5 ± 2.7^{a}
Flavanols, μg CE/g	509 ± 25.1^{c}	347 ± 17.1^{b}	624 ± 31.1^{d}	266 ± 13.2^{a}
ABTS	38.1 ± 1.5^{b}	27.0 ± 1.2^{a}	22.9 ± 0.9^{a}	109 ± 11.2^{c}
DPPH	16.5 ± 1.8^{b}	9.98 ± 0.8^{a}	8.49 ± 0.4^{a}	102 ± 6.6^{c}
FRAP	40.7 ± 2.1^{c}	18.9 ± 0.9^{b}	11.0 ± 0.5^{a}	94.4 ± 4.7^{d}
CUPRAC	44.4 ± 2.2^{b}	25.0 ± 1.2^{a}	23.0 ± 1.1^{a}	121 ± 5.8^{c}

Values are means ± SD per gram dry weight (DW); $n = 5$ samples per cultivar, each sub-sampled and analysed 5 times. Values in rows with different superscript letters are significantly different ($P < 0.05$).
Abbreviation: Polyph, polyphenols.

Polyphenols (PF) vs. the scavenging radical methods showed high correlation:

PF vs. ABTS $y = 3.97x + 5.35$, $R^2 = 0.99$;
PF vs. DPPH $y = 4.75x - 16.5$, $R^2 = 0.97$;
PF vs. FRAP $y = 3.69x + 0.45$, $R^2 = 0.99$;
PF vs. CUPRAC $y = 4.53x + 3.14$, $R^2 = 1.00$.

Ascorbic acid (AA) vs. scavenging radical methods showed also high correlation:

AA vs. ABTS $y = 0.59x + 19.3$, $R^2 = 1.00$;
AA vs. DPPH $y = 0.67x + 0.68$, $R^2 = 1.00$;
AA vs. FRAP $y = 0.54x + 13.9$, $R^2 = 0.96$;
AA vs. CUPRAC $y = 0.67x + 19.3$, $R^2 = 1.00$.

Following four scavenging antioxidant assays, the average correlation coefficient was found to be R^2av = 0.99 for polyphenols and R^2av = 0.99 for ascorbic acid with their antioxidant activities.

The following results arose from the pattern-recognition analysis of the antioxidant data (all the absorbance readings at 0, 5, 10, 15, 20, 25 and 30 min) as characteristics of scavenging of free DPPH radical examined by kiwifruit extract at concentration of 100 mg/ml. The calculated data of relative antioxidant activity of DPPH and FRAP determinations (μM TE/g DW) were used for the statistical evaluation. Fig. 3 (cluster analysis, CA) illustrates the arrangement of all the kiwifruit cultivar samples in the clusters produced by a clustering algorithm with method computing an unweighted average distance within groups and the square Euclidean distance between objects. Graphic representations of this agglomerative tree plot reveal the presence of four clusters well corresponding to four kiwifruit cultivars according to their antioxidant variables. Dendrogram exposes two very similar groups of kiwifruit – 'Hayward' and 'Daeheung' – with a very different cluster of 'Bidan' cultivar, which has a higher antioxidant activity. Differences and similarities of four kiwifruit cultivars are very well visualized by a principal component analysis of these fruits by DPPH and FRAP antioxidant activity (Fig. 4A and B). 'Haenam' and especially 'Bidan' kiwifruit cultivars significantly differ in these properties from the 'Daeheung' and 'Hayward' kiwifruits. Scores of the first two principal components (Fig. 4A) cumulatively represent up to 99% of the total variance and is related mainly to the absorbance readings in the first component and to the calculated FRAP and DPPH values in the second principal

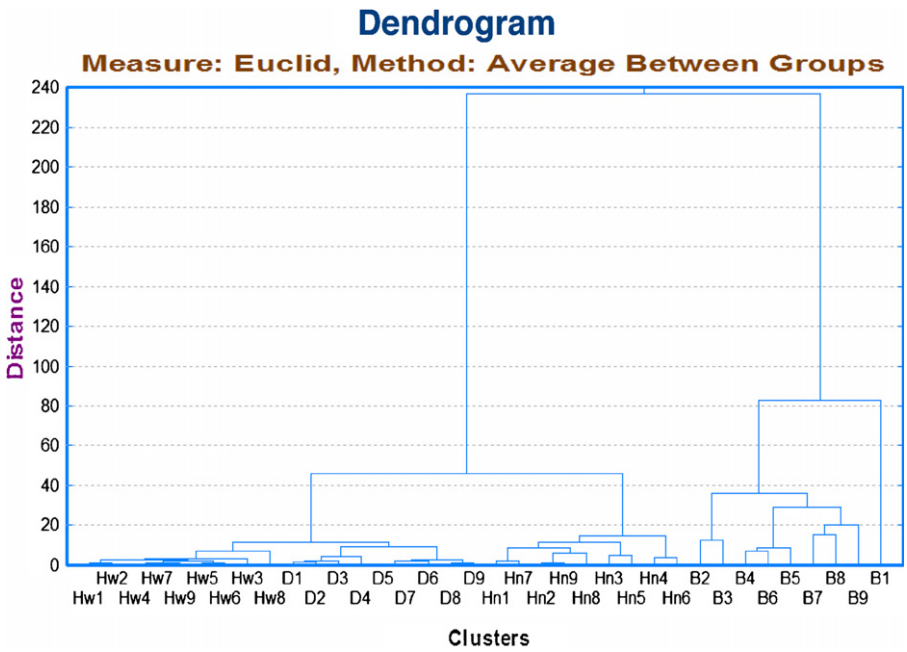

Fig. 3. Dendrogram depicting the relationship between four kiwifruit cultivars (B – 'Bidan', D – 'Daeheung', Hn – 'Haenam', Hw – 'Hayward') according to their antioxidant activity variables. Abbreviations: DPPH, FRAP and absorbance readings of three replicates at three concentrations, samples 1–9. DPPH, 1,1-diphenyl-2-picrylhydrazyl; FRAP, ferric reducing antioxidant power.

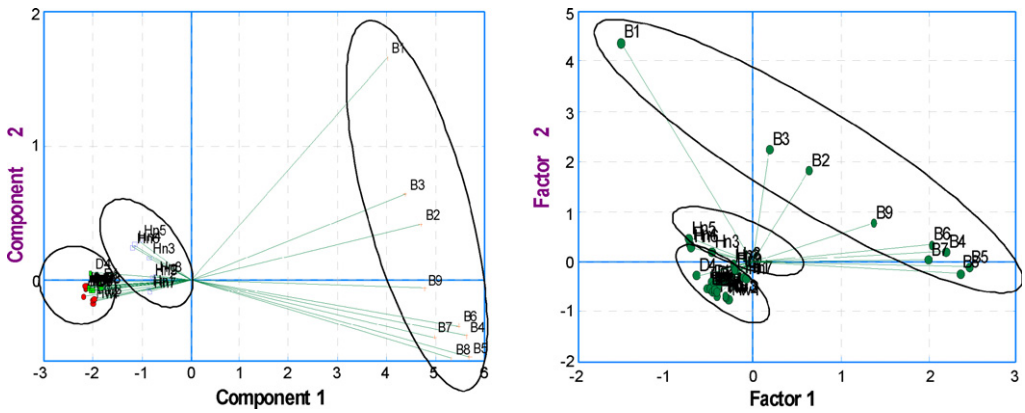

Fig. 4. Principal component analysis of four kiwifruit cultivars (B – 'Bidan', D – 'Daeheung', Hn – 'Haenam', Hw – 'Hayward'): A/Plot of principal component, B/Plot of factor score with Varimax rotation using the antioxidant activity variables (DPPH, FRAP and absorbance readings of the three replicates at three concentrations, samples 1–9).

component. Principal component factoring with the Equimax rotation (Fig. 4B) led to similar results. All antioxidant variables were elaborated by following stepwise and canonical discriminant-analysis procedures. Stepwise discriminant analysis selected absorbance readings at time 0 and 25 min as well as the antioxidant activity calculated at DPPH assay as the most important variables on the discrimination procedure (Table 4), where a model of discrimination is built step-by-step. Specifically, at each step, all variables are reviewed and evaluated to determine which one will contribute most to the discrimination between groups. At the canonical discriminant analysis, first discrimination function – explaining up to 99% of the total variance – was totally sufficient for discrimination among all examined kiwifruit cultivars. Absorbance reading at 15 min of DPPH antiradical activity measurement was found to be the best variable for 209 kiwifruits' canonical discrimination. Besides the 100% success in sample classification and differentiation, the plot of discriminant score (Fig. 5) clearly shows similarities of 'Daeheung' and 'Hayward' kiwifruits in their antiradical and ferric reducing abilities. Kiwifruit 'Bidan', with very strong antioxidant properties, significantly differs from the other fruit cultivars.

Table 4
Results of stepwise discriminant analysis of 'Haenam', 'Bidan', 'Daeheung' and 'Hayward' kiwifruit cultivars according to their antioxidant descriptors.

Variable	Entered at step	F-to-Enter	Significance	Wilks' Lambda
A0	1	516	0.00	0.02
Antiox	2	279	0.00	0.00
A25	3	13.3	0.00	0.00

Abbreviations: A0, absorbance readings at 0 min; Antiox, antioxidants (μM TE/g DW); A25, absorbance reading at 25 min; entered at step, step of discriminant analysis; F-to-Enter, stepwise selection criteria; Wilks' Lambda, a reflectance of the variables importance; the smaller the Wilks' Lambda, the more important the variable.

4. Discussion

Consumption of fruits and vegetables with high contents of bioactive compounds and high antioxidant activity guarantees the best nutritional results (Franke et al., 2004; Haruenkit et al., 2007; Vinson et al., 2002). Therefore, most scientists recommend consumption only of such fruits (Proteggente et al., 2002; Sun

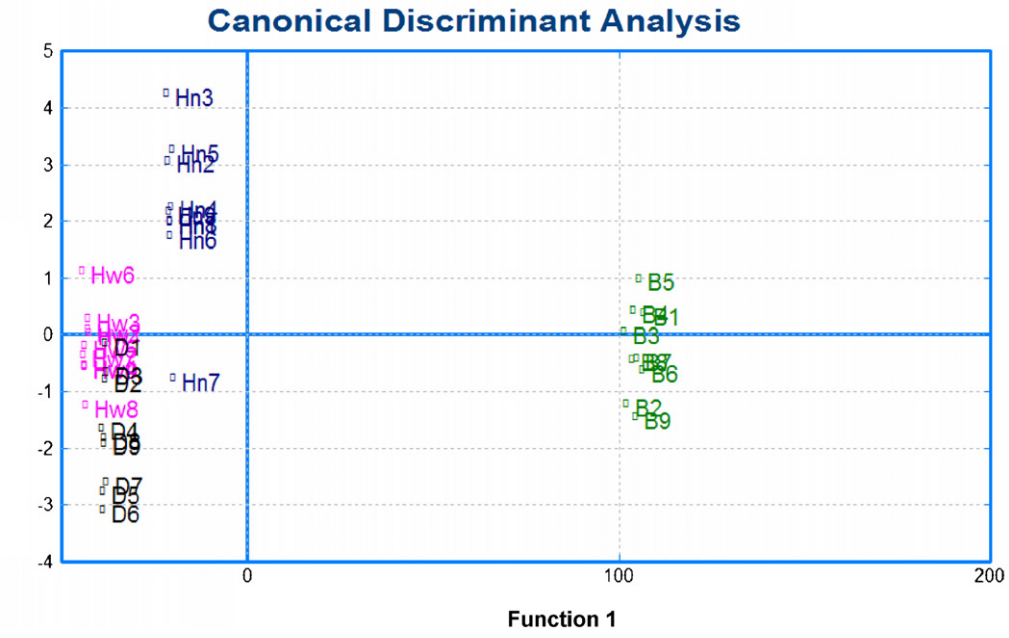

Fig. 5. Canonical discriminant analysis of four kiwifruit cultivars (B – 'Bidan', D – 'Daeheung', Hn – 'Haenam', Hw – 'Hayward'), according to their antioxidant activity variables (DPPH, FRAP and absorbance readings of the three replicates at three concentrations, samples 1–9).

et al., 2002). It was shown that cultivars of natural products grown in the same geographic and climatic conditions could differ significantly (Koh et al., 2009; Toledo et al., 2008). So, Toledo et al. (2008) studied the bioactivity of Mon Thong, Chani, Kan Yao, Pung Manee and Kradum durian cultivars at the same stage of ripening from the same geographic region grown in the same climatic conditions in order to find the best among them for human consumption. It was found that total polyphenols (mg gallic acid equivalent (GAE)/100 g fresh weight (FW)) and flavonoids (mg catechin equivalent (CE)/100 g FW) in Mon Thong (361 ± 23.2 and 93.9 ± 7.4) were significantly higher ($P < 0.05$) than in Kradum (272 ± 11.2 and 69.2 ± 5.3) and Kan Yao (283 ± 16.5 and 72.1 ± 6.8). Toledo et al. (2008) concluded that among the studied durian cultivar Mon Thong is preferable, based on the content of the bioactive compounds. Different fruit cultivars were studied based on different indices through seed phenolic maturity and total polyphenols (Río Segade et al., 2008; Cano et al., 2008). The cultivars studied were characterized on the basis of their phenolic composition (anthocyanins, proanthocyanidins, catechins) in order to know their oenological potential. Principal component analysis allowed a first approximation to the characterization and differentiation of the red cultivars studied on the base of their phenolic composition in vintage, as measured by spectrophotometric methodologies (Río Segade et al., 2008). The used methods are similar to ours in the investigation of the main phenolic compounds and then in application of the statistical analysis.

As previously stated, in our present investigation, four different kiwifruit cultivars ('Bidan', 'Haenam', 'Daeheung' and 'Hayward') were studied with the purpose of identifying the best for human consumption. It must be stressed that these fruits were at the same stage of ripening and grown in the same geographic and climatic conditions. Therefore, there is no doubt that the resulting data were reliable.

It was found that the mineral contents in all cultivars were high, comparable and similar to the data of others (Samadi-Maybodi and Shariat, 2003; Jeong et al., 2007). The content of K was significantly higher than of other minerals ($P < 0.05$), as was reported by Castaldo et al. (1992). Calcium, one of the main mineral nutrients representing fruit quality, was also determined by Montanaro et al. (2007), and our results corresponded with the cited data.

In addition, the content of dietary fibers in all four cultivars was high and comparable ($P > 0.05$), as was shown by other investigators (Jeong et al., 2007).

The bioactive compounds were extracted with methanol. Based on a number of investigations, it was shown that the highest yield of polyphenols can be carried out with 96% (v/v) of methanol or ethanol (Sun-Waterhouse et al., 2009). In another report the major compounds (phenolic compounds and carotenoids) were analysed in the extracts of the edible part of three tropical fruits. Antioxidant values were estimated in different solvent extracts and results were compared with published data in common fruits (Mertz et al., 2009). The obtained results corresponded with other investigations. So, different citrus varieties have been analysed for narirutin, hesperidin and total vitamin C. The influence of variety on the content of bioactive constituents and its contribution to taxonomy at the varietal level is studied (Cano et al., 2008).

However, the results for different cultivars differed. So, the contents of polyphenols and ascorbic acids were significantly higher in 'Bidan' cultivar, flavonoids – in 'Hayward' and flavanols – 'Haenam' (P in all cases < 0.05). Other authors found high amount of phenolics (mg GAE/g DW) in 'Hayward' kiwifruit – 2.19 (Tavarini et al., 2008) and 2.94 (Jeong et al., 2007). Our results were similar to others, showing that the new cultivar 'Bidan' contains a high content of vitamin C and minerals (Nishiyama, 2007; Wu et al., 2009). The physiological activity of methanol extracts of 'Bidan' was relatively high compared to 'Daeheung', 'Jecygold' and 'Hayward' (Park et al., 2008b). Some new varieties such as 'Tsachilidi' were compared with the predominant kiwifruit 'Hayward'. The phenolic and ascorbic acid contents and the total antioxidant activity of fruit extracts were higher in 'Tsachilidi' than 'Hayward' during shelf-life (Koutouvela et al., 2009). Varietal differences in the total phenolic content in flesh were determined among the cultivars, and local collections of hardy kiwifruit had average values of 0.18 g/100 g FW. The major components of phenolics in the flesh were (+)-catechin, chlorogenic acid, rutin, (−)-epicatechin and quercetin (Kim et al., 2009). Other researchers found that the predominant phenolic compounds were hydroxycinnamic acids, flavonols and the flavan 3-ol epicatechin (Montanaro et al., 2007).

The amount of ascorbic acid (mg/g DW) in kiwifruit cultivars differs significantly ($P < 0.05$): from 6.56 ± 0.2 for 'Hayward' and 152 ± 10.4 for 'Bidan'. Our results were higher than those reported by other authors: 1.88–3.00 mg/g DW, according to Tavarini et al. (2008) and 6.67 mg/g DW – Castaldo et al. (1992), and the lowest value was about 1.69 mg/g DW – Jeong et al. (2007). Our data were similar with others showing that the cultivar 'Bidan' has very high vitamin C and mineral content compared to the standard *A. deliciosa* cultivar 'Hayward' (Jo et al., 2007; Park et al., 2008b).

The significant highest level of antioxidant activity was registered in 'Bidan' cultivar by all four assays ($P < 0.05$). Also the highest contents of polyphenol and ascorbic acid were registered in the same cultivar.

The role of ascorbic acid in total antioxidant activity of fruits is controversial: Wang et al. (1996), Vinson et al. (2002), and Du et al. (2009). We wanted to know which of the two mentioned bioactive compounds is the main contributor to antioxidant activity. A very good correlation between the antioxidant activity determined by four assays and polyphenols was found. Other researchers have found that strong correlations between antioxidant activity as assessed by both DPPH and FRAP and the total phenolics (Yuka et al., 2003). Our results can be compared with Du et al. (2009), where eight *Actinidia* genotypes were evaluated. The antioxidant potential was tested by the same assays as in our study (DPPH, ABTS, FRAP and others) for their polyphenol composition and vitamin C contents. The significance analysis demonstrated that the antioxidant capacity of *A. eriantha* and *Actinidia latifolia* fruits was significantly higher than that of other genotypes, which was about 3.3–8.7-fold higher than the *A. deliciosa* cv. 'Hayward' assayed in ABTS, DPPH, ORAC and FRAP methods. In our results the antioxidant capacity by ABTS, DPPH, FRAP and CUPRAC in 'Bigan' was about 5.2–12.06.

Our study showed that the total polyphenol and vitamin C contents showed great variation among *Actinidia* genotypes and high correlation with the total antioxidant capacity. We conclude that significant genotypic difference exists in the total antioxidant capacity of *Actinidia* fruits. The wild *A. eriantha* and *A. latifolia* species have significantly higher antioxidant capacity than the cultivars of *A. chinensis* and *A. deliciosa*. Both total polyphenols and vitamin C are major contributors to the total antioxidant capacity in *Actinidia* fruit and have high correlation between these two variables. We found that the content of the ascorbic acid was high in all cultivars of kiwifruit, and the contribution of ascorbic acid to the total antioxidant activity of the studied fruits was equal to that of the polyphenols.

5. Conclusions

All of the cultivars of kiwifruit that were studied here contain high comparable quantities of minerals and dietary fibers. The contents of polyphenols and ascorbic acid and the antioxidant activity are significantly higher in 'Bidan' cultivar. Polyphenols and ascorbic acid contribute equally to the high total antioxidant

activity. Statistical analysis showed the similarities and differences in new kiwifruit cultivars. The relatively newly commercialized Korean cultivar 'Bidan' and – to a lesser degree, 'Hayward', 'Daeheung' and 'Haenam' – could be a valuable addition to known disease-preventing diets.

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References

Apak, R., Guclu, K., Ozyurek, M., Karademir, S.E., 2004. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. Journal of Agricultural and Food Chemistry 52, 7970–7981.

Cano, A., Medina, A., Bermejo, A., 2008. Bioactive compounds in different citrus varieties. Discrimination among cultivars. Journal of Food Composition and Analysis 21, 377–381.

Castaldo, D., Lo Voi, A., Trifiro, A., Gherardi, S., 1992. Composition of Italian kiwi (*Actinidia chinensis*) puree. Journal of Agricultural and Food Chemistry 40, 594–598.

Cvikrová, M., Meravý, L., Macháčková, I., Eder, J., 1991. Phenylalanine ammonia-lyase, phenolic acids and ethylene in alfalfa (*Medicago sativa* L.) cell cultures in relation to their embryogenic ability. Plant Cell Report 10, 251–255.

Du, G., Li, M., Ma, F., Liang, D., 2009. Antioxidant capacity and the relationship with polyphenol and vitamin C in *Actinidia* fruits. Food Chemistry 113, 557–562.

Duttaroy, A.K., Jøorgensen, A., 2004. Effects of kiwifruits consumption in human volunteers on platelet aggregation and plasma lipids *in vitro*. Platelets 15, 287–292.

Edelmann, A., Lendl, B., 2002. Toward the optical tongue: flow-through sensing of tannin-protein interactions based on FTIR spectroscopy. Journal of American Chemical Society 24, 14741–14747.

Fiorentino, A., D'Abrosca, B., Pacifico, S., Mastellone, S., Scognamiglio, M., Monaco, P., 2009. Identification and assessment of antioxidant capacity of phytochemicals from kiwifruits. Journal of Agricultural and Food Chemistry 57, 4148–4155.

Franke, A.A., Custer, L.J., Arakaki, C., Murphy, S.P., 2004. Vitamin C and flavonoid levels of fruits and vegetables consumed in Hawaii. Journal of Food Composition and Analysis 17, 1–35.

Gorinstein, S., Haruenkit, R., Poovarodom, S., Park, Y.-S., Vearasilp, S., Suhaj, M., Ham, K.-S., Heo, B.-G., Cho, J.-Y., Jang, H.-G., 2009. The comparative characteristics of snake and kiwi fruits. Food Chemistry and Toxicology 47, 1884–1891.

Gorinstein, S., Haruenkit, R., Poovarodom, S., Vearasilp, S., Ruamsuke, P., Namiesnik, J., Leontowicz, M., Leontowicz, H., Suhaj, M., Sheng, G.P., 2010. Some analytical assays for the determination of bioactivity of exotic fruits. Phytochemical Analysis 21, 355–362.

Greenfield, H., Southgate, D.A.T., 2003. Food Composition Data: Production, Management and Use, 2nd ed. FAO, Rome, Chapter 5, pp. 74–76, Chapter 9, pp. 165–168.

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., Gorinstein, S., 2007. Health properties and nutritional value of Durian (*Durio zibethinus* cv Mon Thong): experiments *in vitro* and *in vivo*. Journal of Agricultural and Food Chemistry 55, 5842–5849.

Jeong, C.H., Lee, W.J., Bae, S.H., Choi, S.G., 2007. Chemical components and anti-oxidative activity of Korean gold kiwifruit. Han'guk Sikp'um Yongyang Kwahak Hoechi 36, 859–865.

Jo, Y.S., Cho, H.S., Park, M.Y., Bang, G.P., 2007. Selection of a sweet *Actinidia eriantha*, 'Bidan'. Acta Horticulturae 753, 253–258.

Kim, J.G., Beppu, K., Kataoka, I., 2009. Varietal differences in phenolic content and astringency in skin and flesh of hardy kiwifruit resources in Japan. Scientia Horticulturae 120, 551–554.

Koh, E., Wimalasiri, K.M.S., Chassy, A.W., Mitchell, A.E., 2009. Content of ascorbic acid, quercetin, kaempferol and total phenolics in commercial broccoli. Journal of Food Composition and Analysis 22, 637–643.

Koutouvela, E., Giannousis, K., Ziogas, V., Molassiotis, A., 2009. A comparative study of shelf-life performance of two kiwifruit cultivars in relation to maturity and nutritional quality. Acta Horticulturae 825, 583–588.

Mertz, C., Gancel, A.-L., Gunata, Z., Alter, P., Dhuique-Mayer, C., Vaillant, F., Mercedes Perez, A., Ruales, J., Brat, P., 2009. Phenolic compounds, carotenoids and antioxidant activity of three tropical fruits. Journal of Food Composition and Analysis 22, 381–387.

Montanaro, G., Treutter, D., Xiloyannis, C., 2007. Phenolic compounds in young developing kiwifruit in relation to light exposure: implications for fruit calcium accumulation. Journal of Plant Interactions 2, 63–69.

Nishiyama, I., 2007. Fruits of the *Actinidia* genus. Advances in Food and Nutrition Research 52, 293–324.

Ozgen, M., Reese, R.N., Tulio Jr., A.Z., Scheerens, J.C., 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. Journal of Agricultural and Food Chemistry 54, 1151–1157.

Park, Y.-S., Jung, S.-T., Kang, S.-G., Heo, B.-G., Arancibia-Avila, P., Toledo, F., Drzewiecki, J., Namiesnik, J., Gorinstein, S., 2008a. Antioxidants and proteins in ethylene-treated kiwifruits. Food Chemistry 107, 640–648.

Park, Y.S., Kim, B.W., Kim, T.-C., Jang, H.G., Chon, S., Cho, U.K., Ja, Y., Jiang, S.H., Heo, B.G., 2008b. Physiological activity of methanol extracts from Korean kiwifruits. Weon'ye Gwahag Gi'sulji 26, 495–500.

Park, Y.-S., Jung, S.-T., Kang, S.-G., Heo, B.-G., Lee, S.-H., Toledo, F., Arancibia-Avila, P., Drzewiecki, J., Gorinstein, S., 2009. Radical scavenging capacity of ethylene treated kiwifruit. Journal of Food Biochemistry 33, 674–692.

Proteggente, A.R., Pannala, A.S., Paganga, G., Van Buren, L., Wagner, E., Wiseman, S., Van De Put, F., Dacombe, C., Rice-Evans, C.A., 2002. The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition. Free Radical Research 36, 217–233.

Río Segade, S., Soto Vázquez, E., Díaz Losada, E., 2008. Influence of ripeness grade on accumulation and extractability of grape skin anthocyanins in different cultivars. Journal of Food Composition and Analysis 21, 599–607.

Samadi-Maybodi, A., Shariat, M.R., 2003. Characterization of elemental composition in kiwifruit grown in Northern Iran. Journal of Agricultural and Food Chemistry 51, 3108–3110.

Sinelli, N., Spinardi, A., Di Egidio, V., Mignani, I., Casiraghia, E., 2008. Evaluation of quality and nutraceutical content of blueberries (*Vaccinium corymbosum* L.) by near and mid-infrared spectroscopy. Postharvest Biology and Technology 50, 31–36.

Sun, J., Chu, Y.F., Wu, X.Z., Liu, R.H., 2002. Antioxidant and anti proliferative activities of common fruits. Journal of Agricultural and Food Chemistry 50, 7449–7454.

Sun-Waterhouse, D., Wen, I., Wibisono, R., Melton, L.D., Wadhwa, S., 2009. Evaluation of the extraction efficiency for polyphone extracts from by-products of green kiwifruit juicing. International Journal of Food Science and Technology 44, 2644–2652.

Tavarini, S., Degl'Innocenti, E., Remorini, D., Massai, R., Guidi, L., 2008. Antioxidant capacity, ascorbic acid, total phenols and carotenoids changes during harvest and after storage of 'Hayward' kiwifruit. Food Chemistry 107, 282–288.

Toledo, F., Arancibia-Avila, P., Park, Y.-S., Jung, S.-T., Kang, S.-G., Gu Heo, B., Drzewiecki, J., Zachwieja, Z., Zagrodzki, P., Pasko, P., Gorinstein, P.S., 2008. Screening of the antioxidant and nutritional properties, phenolic contents and proteins of five durian cultivars. International Journal of Food Sciences and Nutrition 59, 415–427.

Vinson, J.A., Liang, X.Q., Proch, J., Hontz, B.A., Dancel, J., Sandone, N., 2002. Polyphenol antioxidants in citrus juices: in vitro and in vivo studies relevant to heart disease. Flavonoids in cell function. Advances in Experimental Medicine and Biology 505, 113–122.

Wang, H., Cao, G., Prior, R.L., 1996. Total antioxidant capacity of fruits. Journal of Agricultural and Food Chemistry 44, 701–705.

Wu, Y.J., Xie, M., Zhang, Q.C., Jiang, G.H., Zhang, H.Q., Long, Q.J., Han, W.J., Chen, J.W., Shong, G.H., 2009. Characteristics of 'White': a new easy-peel cultivar of *Actinidia eriantha*. New Zealand Journal of Crop and Horticultural Science 37, 369–373.

Yuka, I., Yumiko, K., Miyo, N., Takashi, K., 2003. Antioxidative activity of tropical fruit. Feijoa sellowiana berg. Nippon Kasei Gakkaishi 54, 945–949.

Xue, P., Zhao, Y., Wang, B., Hong, L.H., 2007. Simultaneous determination of seven flavonoids in *Potentilla multifida* by HPLC. Journal of Chromatographic Science 45, 216–219.