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Bioactive compounds and the antioxidant capacity in new kiwi fruit cultivars

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ABSTRACT

The aim of this investigation was to find the best among seven different kiwi fruit cultivars ('Hayward', 'Daheung', 'Haenam', 'Bidan', 'Hort16A', 'Hwamei' and 'SKK12') for human consumption and to classify them as groups. Therefore, the contents of bioactive compounds and the level of antioxidant capacities of these cultivars were determined in four different extracts and compared. It was found that the contents of the bioactive compounds and the level of antioxidant capacities in different extracts differ significantly ($P < 0.05$). Bioactive compounds and the antioxidant capacities were significantly higher in 'Bidan' and 'SKK12' cultivars than in other studied samples. The ethanol and water extracts of these cultivars exhibited high binding properties with human serum albumin (HSA) in comparison with catechin. In conclusion, based on fluorescence profiles the seven new kiwi fruit cultivars can be classified for three groups: 'Hayward' (including 'Daheung', 'Haenam', Hwamei' and 'SKK12'), 'Bidan' and 'Hort 16A'. In MS – profiles some differences in the peaks were found between the cultivar groups. All studied fruits could be a valuable addition to known disease preventing diets.

1. Introduction

Nowadays some authors recommend consumption of fruits with high bioactivity (Proteggente et al., 2002; Sun, Chu, Wu, & Liu, 2002), because only such fruits are effective in prevention and treatment of various diseases (Lansky, & Newman, 2007; Larson, Neumark-Sztainer, Hannan, & Story, 2007; Lindeberg et al., 2007; Duttaroy & Jørgensen, 2004). Most of the used fruits have many cultivars (Fukuda, Suezawa, & Katagiri, 2007; Toledo et al., 2008; Wall et al., 2008). It was shown that even cultivars grown in the same geographic and climatic conditions differ significantly (Ercisli, Ozdemir, Sengul, Orhan, & Gungor, 2007; Toledo et al., 2008). So, Toledo et al. (2008) studied the bioactivity of durian cultivars such as Mon Thong, Chani, Kan Yao, Pung Manee and Kradum 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 concluded that among the studied durian cultivar Mon Thong is preferable (Haruenkit et al., 2010). Widely consumed kiwi fruit has many cultivars (Ercisli et al., 2007). Which of them is preferable for human consumption? In order to answer this question it was decided to investigate seven kiwi fruit cultivars ('Hayward', 'Daheung', 'Haenam', 'Bidan', 'Hort16A', 'Hwamei' and 'SKK12') and to divide them to groups. The content of the bioactive compounds and the level of antioxidant capacity (AC) were determined and compared. In order to receive reliable data the AC was determined by four complementary assays: ABTS, DPPH, FRAP and CUPRAC and the mass-spectra profile. Human serum albumin is the drug carrier's protein and serves to greatly amplify the capacity of plasma for transporting drugs. It is interesting to investigate *in vitro* how this protein interacts with polyphenols extracted from kiwi fruit samples in order to get useful information of the properties of polyphenol–protein complex. Therefore the functional properties

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[2] Prof. Simon Trakhtenberg died in November 2011.
[3] This article was written in memory of Dr. Zeev Tashma, who encouraged our research group and participated in our research.

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of new kiwi fruit cultivars were studied by the interaction of ethanol and water polyphenol extracts with a small protein such as HSA, using 3D-FL.

As far as we know not results of such investigations were published.

2. Material and methods

2.1. Chemicals

Trolox (6-hydroxy-2,5,7,8,-tetramethyl-chroman-2-carboxylic acid); 2,2′-azobis-2-methyl-propanimidamide; 1,1-diphenyl-2-picrylhydrazyl (DPPH), $FeCl_3 \times 6H_2O$; Folin–Ciocalteu reagent (FCR); *Tris, tris(hydroxymethy1)aminomethane;* lanthanum (III) chloride heptahydrate; $CuCl_2 \times 2H_2O$; and 2,9-dimethyl-1,10-phenanthroline (neocuproine), potassium persulfate, quercetin, human serum albumin, 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

Kiwi fruits of seven cultivars were harvested at the optimal stage in orchard, located in Haenam county (longitude 126° 15″ and latitude 34° 18″), Jeonnam province, Korea, 2012. All cultivars, except 'Hort 16A', are were bred in Korea and classified as 'Hort'. 'Hort 16A' is a New Zealand gold kiwi fruit and was purchased in 2012 from farmer, located in Jeju Island. 'Hwaemi' and 'SKK-12' are green kiwi fruit cultivars of 100 g size as 'Hayward'. 'Bidan' has a smaller size of 20 g and its skin is white (flesh is green). The peeled fruits were weighed, chopped and homogenised under 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 capacity

The lyophilized samples of kiwi fruit cultivars were extracted with ethanol, water, acetone and hexane at room temperature. The extracts were filtered in a Buchner funnel. After removal of the solvents in a rotary evaporator at a temperature below 40 °C, and the aqueous solution was freeze-dried. The polyphenols were determined by Folin–Ciocalteu method with measurement at 750 nm with spectrophotometer (Hewlett–Packard, model 8452A, Rockvile, USA). The results were expressed as mg of gallic acid equivalents (GAE) per g DW (Singleton, Orthofer, & Lamuela-Raventos, 1999). The extracts of condensed tannins (procyanidins) with 4% methanol vanillin solution were measured at 500 nm. Flavonoids, extracted with 5% $NaNO_2$, 10% $AlCl_3 \times 6H_2O$ and 1 M NaOH, were measured at 510 nm (Bener, Özyürek, Güçlü, & Apak, 2010). The total flavanols amount was estimated using the *p*-dimethylaminocinnamaldehyde (DMACA) method, and then the absorbance at 640 nm was read. To ensure the presence of flavanols on the nuclei, subsequent staining with the DMACA reagent resulted in an intense blue coloration in plant extract (Feucht & Polster, 2001). As it was mentioned previously, (+)-catechin served as a standard for flavonoids and flavanols, and the results were expressed as catechin equivalents (CE).

The AC was determined by the following assays:

(1) 2, 2-Azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt (ABTS) method for the screening of antioxidant capacity is reported as a decolorization assay applicable to both lipophilic and hydrophilic antioxidants, including flavonoids, hydroxycinnamates, carotenoids, and plasma antioxidants. The influences of both the concentration of antioxidant and duration of reaction on the inhibition of the radical cation absorption are taken into account when determining the antioxidant capacity. ABTS radical cation was generated by the interaction of ABTS (7 mM/L) and $K_2S_2O_8$ (2.45 mM/L). This solution was diluted with methanol until the absorbance in the samples reached 0.7 at 734 nm (Re et al., 1999).

(2) Cupric reducing antioxidant capacity (CUPRAC): This assay is based on utilising the copper (II)-neocuproine [Cu (II)-Nc] reagent as the chromogenic oxidising agent. To the mixture of 1 ml of copper (II)-neocuproine and NH_4Ac buffer solution, acidified and non acidified methanol extracts of fruits (or standard) solution (x, in ml) and H_2O [(1.1-x) ml] were added to make the final volume of 4.1 ml. The absorbance at 450 nm was recorded against a reagent blank (Apak, Guclu, Ozyurek, & Karademir, 2004).

(3) Scavenging free radical potentials were tested in solution of 1, 1-Diphenyl-2-picrylhydrazyl (DPPH). In its radical form, DPPH has an absorption band at 515 nm which disappears upon reduction by an antiradical compounds. DPPH solution (3.9 mL, 25 mg/L) in methanol was mixed with the samples extracts (0.1 mL), then the reaction progress was monitored at 515 nm until the absorbance was stable (Brand-WIlliams, Cuvelier, & Berset, 1995).

(4) Ferric-reducing/antioxidant power (FRAP) assay measures the ability of the antioxidants in the investigated samples to reduce ferric-tripiridyltriazine [Fe (III)-TPTZ] to a ferrous form [(Fe (II)]. FRAP reagent (2.5 mL of a 10 mmol ferric-tripiridyltriazine solution in 40 mmol HCl plus 2.5 mL of 20 mmol $FeCl_3xH_2O$ 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 & Strain, 1996).

2.4. Fluorometric measurements

Fluorometric measurements were used for the evaluation of binding properties of kiwi fruit extracts to human serum albumin. Two dimensional (2D-FL) and three dimensional (3D-FL) fluorescence measurements for all 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 310 to 500 nm; and at excitation of 295 nm. The 3D-FL spectra were collected with subsequent scanning emission spectra from 250 to 500 nm at 1.0 nm increments by varying the excitation wavelength from 200 to 350 nm at 10 nm increments. Catechin was used as a standard. All solutions for protein interaction were prepared in 0.05 mol/l Tris–HCl buffer (pH 7.4), containing 0.1 mol/l NaCl. The final concentration of HSA was 2.0×10^{-6} mol/l. The HSA was mixed with quercetin in the proportions of HSA: extract = 1:1.

2.5. MS analysis

In order to compare the extracted phenolics in additional to the used solvents 50% methanol in water acidified with 1% formic acid; and 50% methanol in water were used. Different extractions were carried out in order to achieve the better phenols recovery using variable ratio of water and methanol, with and without formic acid in mass-spectra profiles (Fracassetti, Costa, Moulay, & Tomás-Barberán, 2013). These extracts were submitted to MS

analysis for determination of bioactive compounds (Sanz et al., 2010). A mass spectrometer, a TSQ Quantum Access Max (Thermo Fisher Scientific, Basel, Switzerland) was used. Analytes were ionised by electrospray ionoization (ESI) in negative mode. Vaporizer temperature was kept at 100 °C. All samples were done by direct infusion in the mass spectrometer by use ESI source at negative ion mode, full scan analysis, range of 100–900 *m*/*z*. For optimisation of the acquisition parameters and for identity confirmation only a part of standards was employed, not for all compounds that were found in the investigated samples. Settings for the ion source were as follows: spray voltage 3000 V, sheath gas pressure 35 AU; ion sweep gas pressure 0 AU; auxiliary gas pressure at 30 AU; capillary temperature at 200 °C, skimmer offset 0 V (Gómez-Romero et al., 2011; Mikulic-Petkovsek, Slatnar, Stampar, & Veberic, 2012).

2.6. Statistical analyses

To verify the statistical significance, mean ± SD of five independent measurements were calculated. Differences between groups were tested by two ways ANOVA. In the assessment of the antioxidant capacity, Spearman correlation coefficients (*R*) were used. Linear regressions were also calculated. *P*-values of <0.05 were considered significant.

3. Results and discussion

3.1. Polyphenols, flavonoids, flavanols and tannins

The combination of determination of bioactive compounds as total phenols, total flavonoids, total flavanols and tannins, determined spectroscopically, and with antioxidant assays, fluorescence and mass spectra can be used in comparison and fingerprinting analysis of new kiwi fruit cultivars. These methods can be used for rapid distinguishing of the cultivars.

The results of the determination of the contents of these bioactive compounds in all seven studied kiwi fruits cultivars are shown in the Table 1. As can be seen, the contents of polyphenols in ethanol and water extracts were significantly higher than in acetone and hexane extracts (P in all cases < 0.05). The contents of flavonoids in ethanol extract were significantly higher in 'Haenam' and 'Bidan', in water extracts – in 'SKK12' and 'Hwamei', in acetone and hexane extracts – in 'Bidan' (P in all cases < 0.05). The contents of flavanols in ethanol and water extracts were significantly higher in 'Haenam', and 'Bidan', in acetone and hexane extracts – in 'Haenam' (P in all cases < 0.05). The contents of tannins in ethanol extracts were significantly higher in 'SKK12', in water and acetone extracts – in 'Bidan', and in hexane extracts – in 'SKK12' (P in all cases < 0.05). As can be seen, the contents of the bioactive compounds extracted by different solvents differ significantly: the content of the main bioactive compound – polyphenols was significantly higher in 'SKK12', 'Hwamei' and 'Bidan' ($P < 0.05$).

3.2. Antioxidant capacity

The results of the determination of the level of antioxidant capacity of seven studied kiwi fruit cultivars are shown in the Table 2. As can be seen: (a) according to all assays the significantly highest level of AC in all extracts was in 'SKK12', following by 'Hwamei' and 'Bidan' ($P < 0.05$). ABTS and CUPRAC are two electron transfer assays and therefore the obtained results are similar. As can be seen, according to all four used assays, the significantly highest level of antioxidant capacity was registered in 'Bidan', 'SKK12' and 'Hwamei' cultivars ($P < 0.05$). As was shown above, these cultivars have also the highest content of polyphenols among studied cultivars (Table 1).

3.3. Fluorometric data

The 3D-FL of kiwi fruit cultivars ethanol extracts differ by the wavelengths of the peaks and their fluorescence intensity (FI), and could be classified according to the fluorescence results to three groups 'Hayward'(including 'Daheung', 'Haenam', Hwamei' and 'SKK12'), 'Bidan' and 'Hort 16A'. The following common peaks appeared in three groups: at λex/em of 290/220, 400/230 and 600/210 nm. 'Hort 16A' showed one big peak at 400/300 nm, which was not found in any of cultivars. 'Hwamei', which is similar to 'Hayward' showed one peak at 300/280, characteristic only for this cultivar. At λex/em of 700/400 nm the biggest prominent peak was in 'Bidan' cultivar, decreasing for 'Hayward', 'Hwamei' and' Hort 16A' (Fig. 1C, B, D and A, respectively). The binding properties of the kiwi fruit samples in comparison with the pure flavonoids such as catechin are shown in two-dimensional fluorescence spectra (2D-FL). One of the main peaks for HSA was found at λex/em of 220/357 nm (Fig. 1E). The interaction of HSA and the ethanol extracts of kiwi fruit cultivars (Fig. 1E) showed slight change in the position of the main peak at the wavelength of 357 nm and the decrease in the fluorescence intensity (FI). The following changes appeared when the ethanol extracts of kiwi fruit were added to HSA [initially the main peak at emission 357 nm and FI of 961.00 (Fig. 1E, the upper line is HSA). The reaction with the kiwi fruit extracts and catechin decreased the FI of HSA (Fig. 1E, the lowest line). The following decrease in the FI (%) occurred during the interaction of ethanol extracts with HSA: HSA + 'Hayward' = 3.86; HSA + 'Haenam' = 6.71; HSA + 'Hort 16A' = 7.63; HSA + 'Bidan' = 10.18; HSA + 'Bidan' = 12.03; HSA + 'Hwamei' = 15.05; HSA + 'SKK 12' = 11.65; HSA + catechin = 15.41. The water extracts showed the results of the decrease (%) of HSA intensity (Fig. 1F): HSA + 'Hayward' = 2.03; HSA+'Hort 16A' = 10.79; HSA + 'Bidan' = 15.47; HSA + catechin = 15.89; HSA + 'Hwamei' = 18.76; HSA + 'SKK 12' = 21.24. These data were slightly higher than with ethanol extracts and such strong binding properties of water extracts are proportional to their amount of polyphenols (Table 1). These results were in direct relationship with the antioxidant capacities of the extracts (Table 2). The synergism of bioactive compounds is shown when to the mixture of HSA and extracts of kiwi fruit catechin was added. Our very recent results showed that the fluorescence is significantly quenched, because of the conformation of proteins, phenolic acids and flavonoids (Namiesnik et al., 2013). This interaction was investigated using tryptophan fluorescence quenching. Our result is in agreement with others that quercetin, as an aglycon, is more hydrophobic and demonstrates strong affinity toward HSA. Other results (Xiao, Chen, Cao, Chen, & Yang, 2011) differ from the reported by us, probably because of the variety of antioxidant abilities of pure flavonoids and different ranges of fluorometry scanning ranges used in a similar study. The strong binding properties of phenolics show that they may be effective in prevention of atherosclerosis under physiological conditions. Quercetin can suppress HSA. Much of the bioactivities of citrus flavanones significantly appear to impact blood and microvascular endothelial cells, therefore it was essential to investigate the interaction between kiwi fruit polyphenols and serum albumin. The binding constants ranked in the following order quercetin > rutin > calycosin > calycosin-7-O-(sup)-D-glucoside [formononetin-7-O-(sup)-D-glucoside (Liu et al., 2010). 3-D fluorescence can be used as an additional tool for the characterisation of the polyphenol extracts of kiwi fruit cultivars and their binding properties.

3.4. MS spectra

The ESI-MS in negative ion mode of studied extracts slightly differ between cultivars. As it was shown previously the cultivars were

Table 1
Bioactive compounds of seven kiwi fruit cultivars in ethanol (Et), water (W), acetone (Ac) and hexane (He) extracts.[1,2,3]

	POL (mg GAE/g)	FLAVON (mg CE/g)	FLAV (μg CE/g)	TAN (mg CE/g)
HaywardEt	4.48 ± 0.44^{a}	1.22 ± 0.12^{a}	37.84 ± 3.67^{de}	2.84 ± 0.26^{c}
Daheung Et	4.18 ± 0.40^{a}	0.99 ± 0.11^{a}	5.82 ± 0.56^{a}	1.63 ± 0.16^{a}
HaenamEt	6.82 ± 0.55^{b}	4.25 ± 0.41^{c}	42.96 ± 0.45^{e}	2.85 ± 0.21^{c}
BidanEt	11.45 ± 1.12^{c}	4.32 ± 0.38^{c}	15.80 ± 1.51^{c}	2.48 ± 0.23^{b}
Hort16AEt	10.23 ± 1.07^{c}	1.23 ± 0.09^{a}	31.88 ± 3.21^{d}	2.88 ± 0.28^{c}
SKK12Et	14.48 ± 1.46^{d}	2.39 ± 0.21^{b}	10.53 ± 1.07^{b}	3.01 ± 0.28^{c}
HwameiEt	13.11 ± 1.29^{cd}	2.23 ± 0.21^{b}	9.46 ± 0.98^{b}	2.81 ± 0.27^{c}
HaywardW	5.30 ± 0.45^{a}	0.57 ± 0.12^{a}	16.35 ± 1.65^{b}	1.17 ± 0.14^{a}
DaheungW	5.50 ± 0.54^{a}	0.55 ± 0.06^{a}	7.90 ± 0.78^{a}	1.57 ± 0.14^{b}
HaenamW	7.69 ± 0.69^{b}	0.70 ± 0.09^{b}	8.87 ± 0.88^{a}	1.17 ± 0.11^{a}
BidanW	13.97 ± 1.32^{d}	1.00 ± 0.11^{b}	39.92 ± 3.83^{d}	3.04 ± 033^{d}
Hort16AW	11.08 ± 1.14^{c}	1.37 ± 0.13^{c}	8.59 ± 0.81^{a}	2.37 ± 2.24^{c}
SKK12 W	16.34 ± 1.11^{e}	1.75 ± 0.07^{d}	19.68 ± 1.94^{c}	1.60 ± 0.03^{b}
HwameiW	14.23 ± 1.39^{d}	1.62 ± 0.11^{d}	14.47 ± 1.44^{ab}	2.50 ± 0.15^{c}
HaywardAc	1.15 ± 0.05^{a}	0.61 ± 0.07^{b}	18.91 ± 1.87^{e}	1.42 ± 0.18^{c}
DaheungAc	0.84 ± 0.07^{a}	0.48 ± 0.06^{a}	2.98 ± 0.27^{a}	0.82 ± 0.09^{a}
HaenamAc	1.82 ± 0.04^{b}	2.11 ± 0.24^{d}	21.43 ± 2.32^{f}	1.43 ± 0.16^{c}
BidanAc	3.39 ± 0.33^{d}	2.17 ± 0.22^{d}	7.84 ± 0.78^{c}	1.25 ± 0.13^{b}
Hort16AAc	2.74 ± 0.21^{c}	0.62 ± 0.08^{b}	15.91 ± 1.58^{d}	1.44 ± 0.15^{c}
SKK12Ac	5.11 ± 0.52^{e}	1.21 ± 0.23^{c}	5.24 ± 0.51^{b}	1.51 ± 0.16^{c}
HwameiAc	4.85 ± 0.48^{e}	1.12 ± 0.12^{c}	4.71 ± 0.47^{b}	1.45 ± 0.14^{c}
HaywardHe	0.49 ± 0.03^{a}	0.42 ± 0.07^{a}	12.63 ± 1.32^{d}	0.95 ± 0.9^{b}
DaheungHe	0.31 ± 0.04^{a}	0.32 ± 0.03^{a}	1.97 ± 0.19^{a}	0.55 ± 1.2^{a}
HaenamHe	1.15 ± 0.13^{b}	1.43 ± 0.16^{c}	14.31 ± 1.34^{e}	0.95 ± 0.7^{b}
BidanHe	2.07 ± 0.25^{c}	1.45 ± 0.14^{c}	5.26 ± 0.52^{c}	0.83 ± 0.6^{b}
Hort16AHe	1.67 ± 0.14^{bc}	0.41 ± 0.04^{a}	10.63 ± 1.13^{cd}	0.96 ± 0.5^{b}
SKK12He	3.42 ± 0.33^{d}	0.81 ± 0.08^{b}	3.49 ± 0.32^{b}	1.03 ± 0.09^{b}
HwameiHe	3.04 ± 0.33^{d}	0.75 ± 0.07^{b}	3.14 ± 0.31^{b}	0.97 ± 0.09^{b}

POL, polyphenols; FLAVON, flavonoids; FLAV, flavanols; TAN, tannins; CE, catechin equivalent; GAE, gallic acid equivalent; HaywardEt, DaheungEt, HaenamEt, HwameiEt, Hort16AEt, SKK12Et and BidanEt, kiwi fruit cultivars extracted with 100% ethanol; HaywardW, DaheungW, HaenamW, HwameiW, Hort16AW, SKK12W and BidanW, kiwi fruit cultivars extracted with water; HaywardAc, DaheungAc, HaenamAc, HwameiAc, Hort16AAc, SKK12Ac and BidanAs, kiwi fruit cultivars extracted with acetone; HaywardHe, DaheungHe, HaenamHe, HwameiHe, Hort16He, SKK12He and BidanHe, kiwi fruit cultivars extracted with hexane.
[1] Values are means ± SD of 5 measurements.
[2] Values in columns for every bioactive compound with the same solvent bearing different superscript letters are significantly different ($P < 0.05$).
[3] Per g dry weight.

Table 2
The antioxidant capacities of seven kiwi fruit cultivars (μmolTE/g DW) in ethanol[A], water[B], acetone[C], and hexane[D] extracts.[1,2,3]

	Hayward	Daheung	Haenam	Bidan	Hort 16A	SKK12	Hwamei
ABTS[A]	18.21 ± 1.65^{a}	17.42 ± 1.65^{a}	22.43 ± 2.18^{a}	34.25 ± 3.23^{c}	31.15 ± 3.11^{b}	37.18 ± 3.65^{c}	33.25 ± 3.31^{b}
ABTS[B]	20.41 ± 2.11^{a}	22.40 ± 2.23^{a}	26.18 ± 2.43^{a}	39.16 ± 3.87^{c}	34.12 ± 3.41^{b}	42.14 ± 4.32^{d}	39.35 ± 3.87^{c}
ABTS[C]	4.82 ± 0.45^{a}	4.05 ± 0.42^{a}	5.42 ± 0.52^{a}	12.41 ± 1.24^{ab}	11.12 ± 1.11^{ab}	14.15 ± 1.43^{b}	13.16 ± 1.31^{b}
ABTS[D]	1.61 ± 0.15^{a}	1.42 ± 0.14^{a}	1.83 ± 0.18^{a}	4.23 ± 0.41^{b}	4.11 ± 0.41^{b}	4.83 ± 0.48^{b}	4.52 ± 0.45^{b}
CUPRAC[A]	20.18 ± 2.04^{a}	19.44 ± 1.87^{a}	24.12 ± 2.32^{ab}	35.42 ± 3.23^{bc}	32.14 ± 2.16^{b}	38.15 ± 3.87^{c}	34.18 ± 3.21^{bc}
CUPRAC[B]	21.14 ± 2.11^{a}	23.40 ± 1.87^{a}	27.41 ± 2.12^{b}	40.18 ± 3.23^{d}	35.61 ± 2.76^{c}	43.27 ± 3.23^{d}	40.91 ± 3.45^{d}
CUPRAC[C]	4.01 ± 0.32^{a}	4.94 ± 0.27^{a}	6.12 ± 0.54^{ab}	13.13 ± 1.21^{c}	12.43 ± 0.85^{b}	15.25 ± 1.32^{d}	14.21 ± 1.34^{c}
CUPRAC[D]	1.51 ± 0.13^{a}	1.38 ± 0.11^{a}	1.73 ± 0.14^{ab}	4.11 ± 0.41^{bc}	3.85 ± 0.34^{b}	4.63 ± 0.43^{c}	4.41 ± 0.27^{bc}
FRAP[A]	6.12 ± 0.56^{a}	5.42 ± 0.54^{a}	10.21 ± 1.01^{ab}	18.44 ± 1.76^{c}	11.25 ± 1.12^{b}	21.15 ± 1.71^{c}	20.14 ± 1.98^{c}
FRAP[B]	7.12 ± 0.65^{a}	7.88 ± 0.67^{a}	11.33 ± 1.08^{ab}	21.32 ± 1.78^{c}	13.12 ± 1.31^{b}	24.55 ± 2.18^{c}	23.11 ± 2.11^{c}
FRAP[C]	1.58 ± 0.15^{a}	1.15 ± 0.09^{a}	2.43 ± 0.18^{ab}	4.75 ± 0.28^{c}	3.81 ± 0.32^{b}	5.36 ± 0.43^{c}	5.05 ± 0.41^{c}
FRAP[D]	0.53 ± 0.04^{a}	0.48 ± 0.03^{a}	0.81 ± 0.07^{ac}	1.65 ± 0.09^{c}	1.31 ± 0.12^{b}	1.98 ± 0.11^{d}	1.79 ± 0.14^{d}
DPPH[A]	6.95 ± 0.54^{a}	5.80 ± 0.45^{a}	7.65 ± 0.45^{ab}	14.41 ± 1.34^{c}	11.18 ± 1.13^{b}	17.23 ± 1.43^{d}	15.42 ± 1.28^{c}
DPPH[B]	6.08 ± 0.56^{a}	6.90 ± 0.43^{a}	9.14 ± 0.41^{ab}	17.15 ± 1.54^{c}	13.24 ± 1.43^{b}	18.42 ± 1.67^{d}	17.85 ± 1.87^{c}
DPPH[C]	1.75 ± 0.17^{a}	1.41 ± 0.12^{a}	2.18 ± 0.15^{ab}	4.15 ± 0.32^{c}	3.18 ± 0.23^{b}	4.87 ± 0.28^{c}	4.37 ± 0.32^{c}
DPPH[D]	0.65 ± 0.07^{a}	0.52 ± 0.05^{a}	0.74 ± 0.08^{ab}	1.48 ± 0.12^{c}	1.21 ± 0.09^{b}	2.03 ± 0.04^{d}	1.74 ± 0.06^{c}

[1] Values are means ± SD of 5 measurements; [2] Values in columns for kiwi fruits with the same solvent bearing different superscript letters are significantly different ($P < 0.05$); [3] per g dry weight. Cupric reducing antioxidant capacity (CUPRAC), 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) Ferric-reducing/antioxidant power (FRAP).
[A,B] Extracted at room temperature in concentration of 25 mg lyophilized sample in 1 mL ethanol, 1 mL water, respectively.
[C] Extracted at room temperature in concentration of 40 mg lyophilized sample in 1 ml acetone.
[D] Hexane.
[1] Values are means ± SD of 5 measurements.
[2] Values in rows with different superscript letters are significantly different ($P < 0.05$).
[3] Per g dry weight.

classified according to fluorometric measurements to three groups: 'Hayward' (including 'Daheung', 'Haenam', Hwamei' and 'SKK12'), 'Bidan' and 'Hort 16A'. There were done all the spectra analyses, but only these groups are presented in Fig. 2 and Table 3. In all cultivars the main peak was at m/z 190.97 (100%) corresponded to quinic acid (Table 3, Fig. 2), but small peaks differ from one group to another (Table 3). 'Hwamei' slightly differ in methanol extracts from the other four cultivars which belong to the 'Hayward' group (Table 3, Fig. 2B). MeOH/water/50/50 showed as well differences in these 3 groups (Table 3, Figs. 2B, F, J). 'Bidan' contained also the main peak with m/z 191(100%) with average peaks different from the first group such as 308.95 and 366.91 (Table 3). MeOH/water/

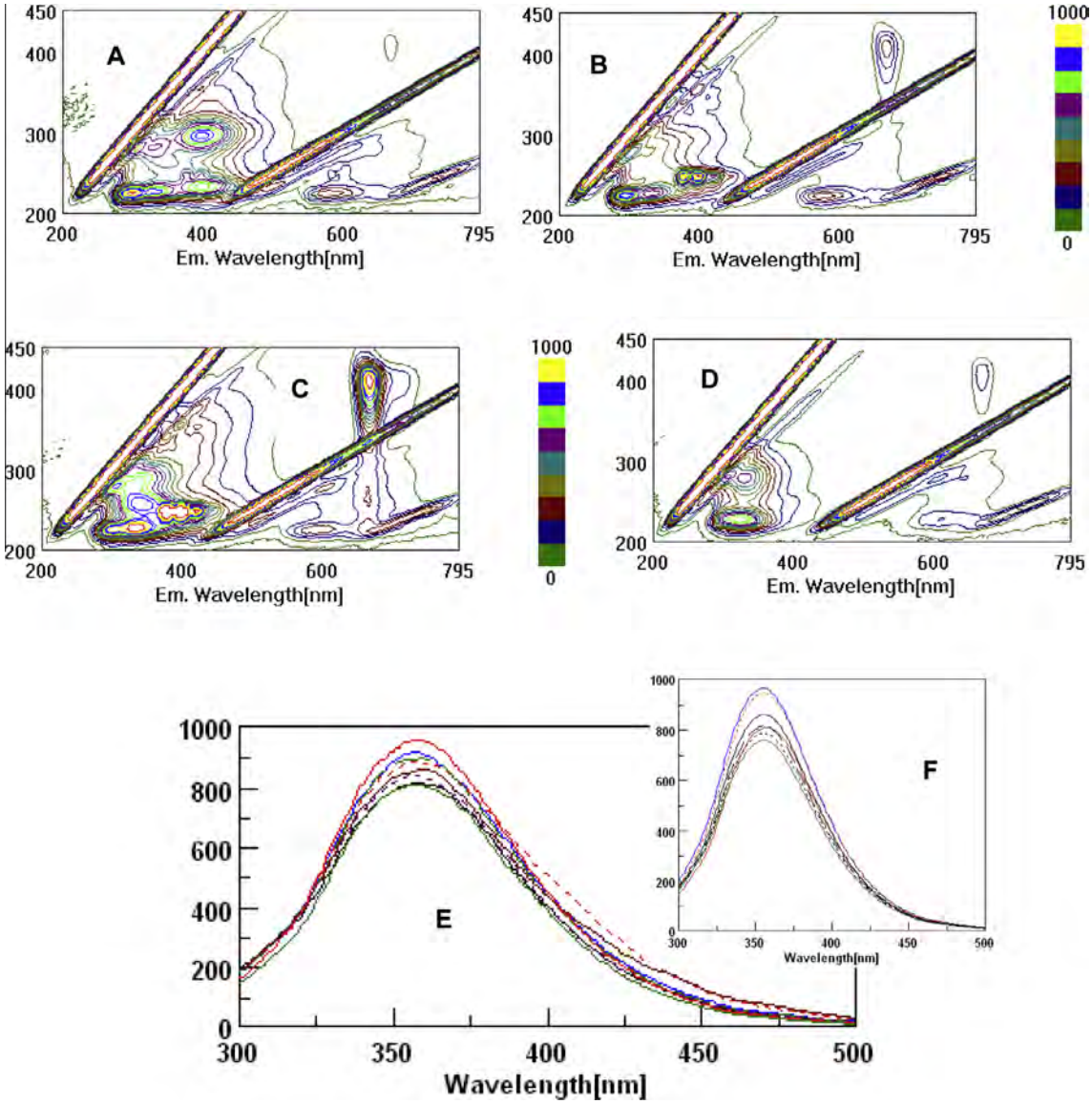

Fig. 1. Contour maps of three dimensional fluorescence (3D-FL) spectra of ethanol extracts of A, B, C, D, 'Hort16A' 'Hayward'; 'Bidan', D, and 'Hwamei'. 2D-FL spectrum illustrate the interaction between humam serum albumin (HSA), catechin, ethanol (E) and water (insert F) extracts of kiwi fruit cultivars. The change in the fluorescence intensity as a result of binding affinity with kiwi fruit extracts: E, HSA [first line from the top with fluorescence intensity (FI) of 961.00]; HSA + 'Hayward' (second line from the top with FI = 923.94), HSA + 'Haenam' (third line, FI = 896.54), HSA + 'Hort16A' (fourth line, FI = 887.66), HSA + 'Bidan' (fifth line, FI = 863.18), HSA + 'Hwamei' (sixth line, FI = 845.40), HSA + 'SKK12' (seventh line, FI = 816.41), HSA + catechin (eighth line, FI = 812.90). Insert F, HSA [first line from the top with fluorescence intensity (FI) of 967.64]; HSA + 'Hayward' (second line from the top with FI = 948.00), HSA + 'Hort16A' (third line, FI = 863.23), HSA + 'Bidan' (fourth line, FI = 817.90), HSA + catechin (fifth line, FI = 813.85), HSA + 'Hwamei' (sixth line, FI = 786.39), HSA + 'SKK12' (seventh line, FI = 762.12). In all reactions were used the following conditions: HSA (2.0×10^{-6} mol/L); catechin (1.7×10^{-6} mol/L); ethanol extracts in concentration of 50 μg/mL. The binding was during 1 h at 25 °C. Fluorescence intensities are on *y*-axis and emission wavelengths – on *x*-axis.

formic acid/50%/49%/1% extracts were different and contained different peaks mostly in 'Hayward' and 'Bidan' groups of *m*/*z* 370.97 and 225.02, respectively (Table 3, Figs. G, K). Acetone fractions of the groups showed one main peak of m/z 191 with a number of small peaks with different masses (Table 3, Figs. 2D, H, L, P). As can be seen all kiwi fruit ethanol extracts characterised by chlorogenic acid of the [M-H] – deprotonated molecule (*m*/*z* 353) and the ion corresponding to the deprotonated quinic acid (*m*/*z* 191), which was consistent with Sun, Liang, Bin, Li, and Duan (2007). The recorded spectra were in the same scale (in the range between 100 and 600 *m*/*z*) for comparison. We choose negative mode for the MS method because in many publications was described that this mode is the best for analysis of low- molecular phenolic compounds (Gómez-Romero et al., 2011; Sun et al., 2007). The main peaks were identified and the recorded MS spectra can be used as a fingerprint for characterisation of different kiwi fruit cultivars, based on the percentage of the main peaks. The most abundant is chlorogenic acid. This is in agreement with Mittelstadt, Negron, Schofield, Marsh, and Parker (2013), who showed that one of the novel aspects of kiwifruit is the presence of a high level of quinic acid which contributes to the flavour of the fruit. Quinic acid metabolism intersects with the shikimate pathway, which is responsible for the de novo biosynthesis of primary and secondary aromatic metabolites. Our results are in accordance with Clifford (2000), Fiorentino et al. (2009) and Sârbu et al. (2012), where fingerprinting of kiwi fruit was suggested. Palafox-Carlos et al. (2012) showed the interactions of four major phenolic compounds (chlorogenic, gallic, protocatechuic and vanillic acid) found in 'Ataulfo' mango pulp. Significant synergism was found in the majority of the all combinations, as well as the combination of the four phenolics. Cultivars

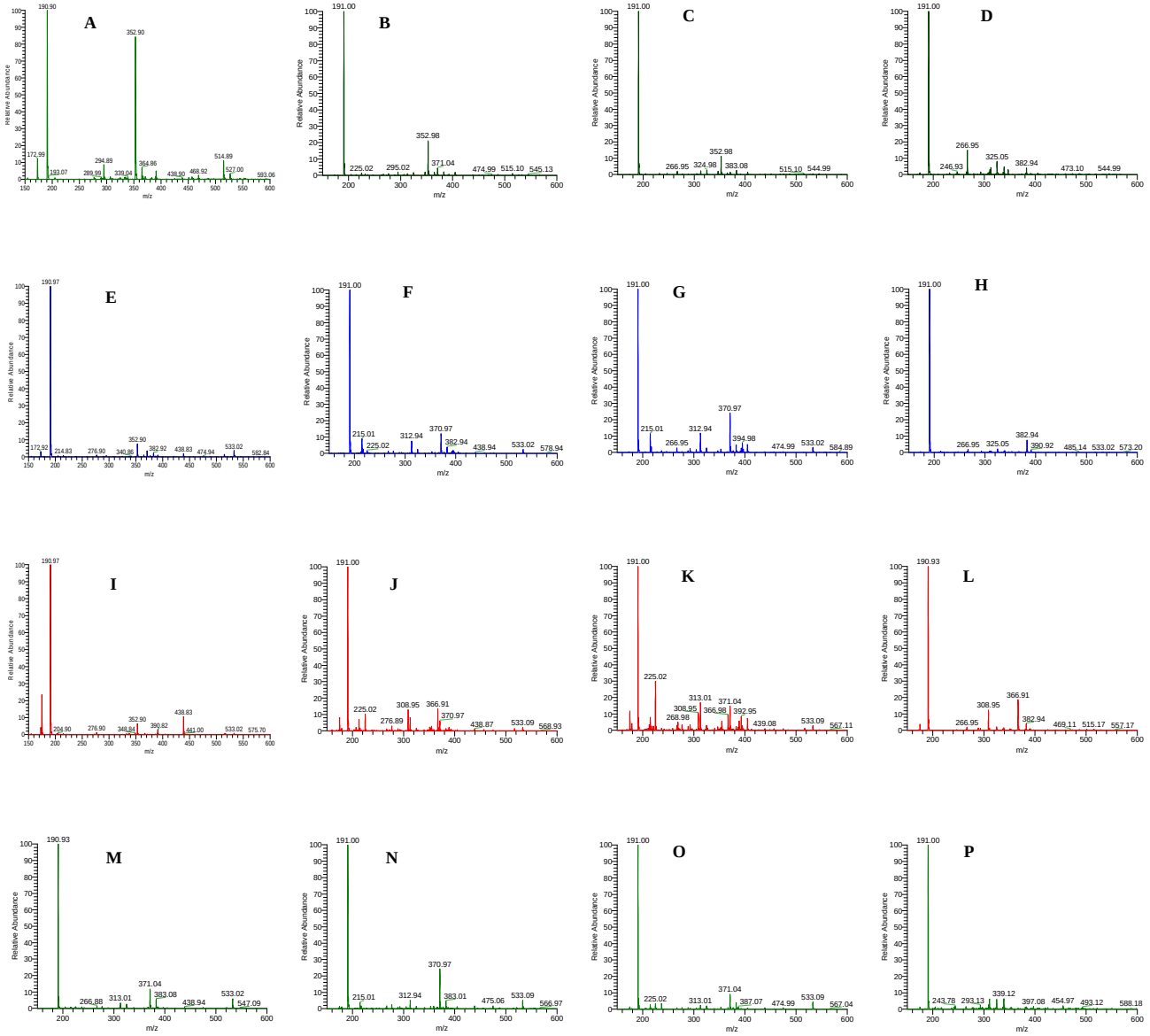

Fig. 2. ESI-MS spectra in negative ion mode of kiwi fruit cultivar groups extracts. A, B, C, D, EtOH, MeOH/water, MeOH/water/acid, acetone of 'Hort 16A'; E, F, G, H, EtOH, MeOH/water, MeOH/water/acid, acetone of 'Hayward'; I, J, K, L, EtOH, MeOH/water, MeOH/water/acid, acetone of 'Bidan'; M, N, O, P, EtOH, MeOH/water, MeOH/water/acid, acetone of 'Hwamei'.

of fruits and vegetables even grown in the same geographic and climatic conditions could different significantly and therefore, it must be taken into consideration (Koh, Wimalasiri, Chassy, & Mitchell, 2009; Toledo et al., 2008). Manolopoulou and Papadopoulou (1998), described such differences in kiwi fruit cultivars. However, in their study were investigated mainly respiratory and physico-chemical changes of four kiwi fruit cultivars during cool-storage. Manolopoulou and Papadopoulou (1998) investigated only four cultivars: Allison, Bruno, Hayward and Monty harvested at the proper stage of maturity. They investigated respiration rates, production of ethylene, shelf-life. Among bioactive compounds only ascorbic acid content was measured. No changes in antioxidant activity were described. Therefore, it was decided to study seven well known kiwi fruit cultivars, determine and compare contents of main bioactive compounds and the level of the antioxidant capacity in order to find the best for human consumption. It must be underlined once again that these fruits were at the same stage of ripening and grown in the same geographic and climatic conditions. Therefore, no doubt, the determined data must be reliable. The results of present investigation show that all kiwi fruit cultivars contain high quantities of bioactive compounds. Also our previous data (Park et al., 2008) and of others (Amodio, Colelli, Hasey, & Kader, 2007; Jeong, Lee, Bae, & Choi, 2007; Tavarini, Degl'Innocenti, Remorini, Massai, & Guidi, 2008) are in agreement with our present results. However, the results are different for different cultivars (Castaldo, Lo Voi, Trifiro, & Gherardi, 1992; Du, Li, Ma, & Liang, 2009; Samadi-Maybodi, & Shariat, 2003). So, the contents of the main bioactive compound – polyphenols was significantly higher in 'SKK12', 'Bidan' and 'Hwamei' ($P < 0.05$). The obtained results depend on the year of collection and the extraction procedure, therefore our recent published results differ from the presently reported (Park et al., 2011). Also the significant highest level of antioxidant capacity and binding abilities were registered in the same cultivars: 'SKK12', 'Bidan' and 'Hwamei' ($P < 0.05$).

4. Conclusions

Seven relatively new cultivars were divided to three groups mostly based on fluorometric measurements and supported by MS-spectra. The contents of bioactive compounds, antioxidant

Table 3
Mass spectral data (molecular ion and the major fragment ions of polyphenols extracted from kiwi fruit).

Extracts		[M–H–] and fragmentation in ESI, (% in MS)	Compound
Ethanol	Hort 16A	190.90(100)	Quinic acid
		352.9(85)	Caffeoylquinic acid
	Hayward	191.0(100)	Quinic acid
		352.9(10)	Caffeoylquinic acid
	Bidan	190.97(100)	Quinic acid
		352.9(10)	Caffeoylquinic acid
		438.83(15)	n-Triacontanol
	Hwamei	191.0(100)	Quinic acid
		371.04(12)	Sinensetin or tangeretin
Methanol:water (50:50)	Hort16A	191.0(100)	Quinic acid
		352.98(22)	Caffeoylquinic acid
	Hayward	191.0(100)	Quinic acid
		312.94(8)	Cirsimaritin
		370.97(11)	Sinensetin or tangeretin
	Bidan	191.0(100)	Quinic acid
		308.95(14)	Cinnamoyl glucose
		366.91(14)	Feruloylquinic acid
	Hwamei	191.0(100)	Quinic acid
		370.97(24)	Sinensetin or tangeretin
Methanol:water:acid	Hort16A	191.0(100)	Quinic acid
		352.98(12)	Caffeoylquinic acid
	Hayward	191.0(100)	Quinic acid
		215.01(14)	Bergapten
		312.94(14)	Cirsimaritin
		370.97(25)	Sinensetin or tangeretin
	Bidan	191.0(100)	Quinic acid
		225.02(30)	Uknown
		308.95(12)	Cinnamoyl glucose
		313.01(16)	Cirsimaritin
		371.04(15)	Sinensetin or tangeretin
	Hwamei	191.0(100)	Quinic acid
		371.04(10)	Sinensetin or tangeretin
Acetone	Hort16A	191.0(100)	Quinic acid
		266.95(15)	Genistin
	Hayward	191.0(100)	Quinic acid
		382.94(10)	Uknown
	Bidan	190.93(100)	Quinic acid
		308.95(15)	Cinnamoyl glucose
		366.91(18)	Feruloyquinic acid
	Hwamei	191.0(100)	Quinic acid
		339.12(8)	Phenylnaringenin

capacity and binding properties are significantly higher in SKK12', 'Bidan' and 'Hwamei' cultivars. The SKK12', 'Bidan' and 'Hwamei' and to less degree other four studied cultivars could be a valuable addition to known disease preventing diets.

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