

Fluorescence and Ultraviolet Spectroscopic Evaluation of Phenolic Compounds, Antioxidant and Binding Activities in Some Kiwi Fruit Cultivars

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Spectrophotometric assays (Folin–Ciocalteu, ferric-reducing/antioxidant power, cupric-reducing antioxidant capacity, and β -carotene linoleate model system) have been used to evaluate the total phenolics and flavonoids in kiwi fruit cultivars in ethanol and water extracts. Fluorescent and infrared measurements were correlated with the obtained spectroscopic data. It was found that the contents of the bioactive compounds and the level of antioxidant activity in different extracts differ significantly ($p < 0.05$). Bioactive compounds and antioxidant activities were significantly higher in two kiwi fruit cultivars (“Bidan” and “SKK12”) than in other studied samples. To our knowledge this is the first report showing the differences and similarities in new kiwi fruit cultivars, using these spectroscopic data. The ethanol extracts of these cultivars exhibited high binding properties with human serum albumin compared with rutin. In conclusion, the applied analytical methods showed the main compounds in the kiwi fruit cultivars and can be used for determination of these compounds in any plants. The relative knowledge would contribute to the pharmaceutical development and clinical application of extracts of kiwi fruit extracts.

Keywords: antioxidant activity, fluorescence, infrared spectra, kiwi fruit, spectroscopic methods, total phenolics

Introduction

Consumption of fruits is important, because of their action in the prevention and treatment of various diseases.^[1–3] Most used kiwi fruits have several cultivars, which differ significantly.^[4] To distinguish and compare the cultivars, it is necessary to apply rapid analytical methods. There are a number of reports on the application of different analytical methods for the determination of bioactive compounds.^[5–7]

Human serum albumin (HSA) 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 obtain useful information on the properties of polyphenol–protein complex.^[8–10] Therefore the functional properties of new kiwi fruit cultivars will be studied by the interaction of ethanol polyphenol extracts with HSA, using three-dimensional (3D) and two-dimensional (2D) fluorescence (FL). Information on the combination of spectroscopic and fluorometric methods for the comparison of different kiwi fruit cultivars is limited.^[11,12] Therefore it was decided to investigate the bioactive compounds, antioxidants, and binding activities using spectroscopic methods and to compare with fluorometric and infrared (IR) results in six kiwi fruit cultivars. To the best of our knowledge, no results of such investigations were published.

Materials and Methods

Reagents and Materials

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Folin–Ciocalteu reagent (FCR), β -carotene, linoleic acid, and Tween-40 (polyoxyethylene sorbitan monopalmitate), $\text{FeCl}_3 \times 6\text{H}_2\text{O}$, $\text{CuCl}_2 \times 2\text{H}_2\text{O}$; and 2,9-dimethyl-1,

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10-phenanthroline (neocuproine), rutin, and HSA were purchased from Sigma Chemical Co., St Louis, MO, USA. 2, 4, 6-Tripyridyl-*s*-triazine (TPTZ) was from Fluka Chemie, Buchs, Switzerland. All reagents were of analytical grade.

Extraction and Samples Preparation

Kiwi fruits of six cultivars were harvested at the optimal stage in orchard, located in Haenam county (longitude 126° 15" and latitude 34° 18"), Jeonnam province, Korea, 2013. All cultivars, except "Hort 16A," are bred in Korea. "Hort 16A" is a New Zealand gold kiwi fruit and was purchased from a farmer settled in Jeju Island. "SKK 12" is a green kiwi fruit cultivar 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 homogenized 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 hr (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 analyzed.

The lyophilized samples of kiwi fruit cultivars were extracted with ethanol at room temperature and hot water during 3 hr. The extracts were filtered in a Buchner funnel. After removal of the solvents in a rotary evaporator at a temperature below 40°C, the aqueous solution was freeze-dried. The organic fractions were dried.

Determination of Total Phenolics

The polyphenols were determined by the Folin–Ciocalteu method by measuring at 750 nm with a spectrophotometer (Hewlett-Packard, model 8452A, Rockville, USA). The results were expressed as mg of gallic acid equivalents (GAE) per g DW.^[13]

Determination of Total Flavonoids

Flavonoids, extracted with 5% NaNO₂, 10% AlCl₃ × 6H₂O, and 1 M NaOH, were measured at 510 nm. As mentioned previously, (+)-catechin served as a standard for flavonoids and flavanols, and the results were expressed as catechin equivalents (CE).

Determination of Total Chlorophylls and Carotenoids

Total chlorophylls, chlorophylls *a* and *b*, and total carotenoids were extracted with 100% acetone and determined spectrophotometrically at the absorbances (nm) of 661.6, 644.8, and 470, respectively.^[14]

IR Spectra

Total phenols in the investigated kiwifruit extracts were studied by IR spectroscopy. A Nicolet iS 10 FT-IR Spectrometer (Thermo Scientific Instruments LLC, Madison, WI, USA), with the smart iTRTM ATR (attenuated total reflectance) accessory was used to record IR spectra.^[11,12,15]

Determination of Total Antioxidant Capacity

Ferric-reducing/antioxidant power (FRAP) assay measures the ability of the antioxidants in the investigated samples to reduce ferric-tripyridyltriazine (Fe³⁺ TPTZ) to a ferrous form (Fe²⁺). FRAP reagent (2.5 mL of a 10 mmol ferric-tripyridyltriazine solution in 40 mmol HCl plus 2.5 mL of 20 mmol FeCl₃ × H₂O 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.^[16]

Cupric reducing antioxidant capacity (CUPRAC): This assay is based on utilizing the copper (II)-neocuproine [Cu (II)-Nc] reagent as the chromogenic oxidizing agent. To the mixture of 1 mL of copper (II)-neocuproine and NH₄Ac buffer solution, acidified and nonacidified methanol extracts of berries (or standard) solution (*x*, in mL) and H₂O [(1.1 – *x*) mL] were added to make the final volume of 4.1 mL. The absorbance at 450 nm was recorded against a reagent blank.^[17,18]

Antioxidant assay, using β-carotene linoleate model system (β-carotene): β-carotene (0.2 mg) in 0.2 mL of chloroform, linoleic acid (20 mg), and Tween-40 (polyoxyethylene sorbitan monopalmitate) (200 mg) were mixed.^[19] Chloroform was removed at 40°C under vacuum, and the resulting mixture was diluted with 10 mL of water and mixed well. To this emulsion was added 40 mL of oxygenated water. Four milliliter aliquots of the emulsion were pipetted into different test tubes containing 0.2 mL of fruit extracts (50 and 100 ppm) and butylated hydroxyanisole (BHA; 25 and 50 ppm) in ethanol. BHA was used for comparative purposes. A control containing 0.2 mL of ethanol and 4 mL of the above emulsion was prepared. The tubes were placed at 50°C in a water bath, and the absorbance at 470 nm was taken at zero time (*t* = 0). Measurement of absorbance was continued until the color of β-carotene disappeared in the control tubes (*t* = 180 min) at an interval of 15 min. A mixture prepared as above without β-carotene served as blank. The antioxidant activity (AA) of the extracts was evaluated in terms of bleaching of the β-carotene using the following formula: AA = 100 [1 – (*A*₀ – *A*_{*t*})/(*A*₀ – *A*_{*t*})], where *A*₀ and *A*₀[°] are the absorbance values measured at zero time of incubation for the test sample and the control, respectively, and *A*_{*t*} and *A*_{*t*}[°] are the absorbance values measured in the test sample and the control, respectively, after incubation for 180 min.

Fluorometric Measurements

Fluorometric measurements were to evaluate the bioactive compounds of kiwi fruit extracts. 2D-FL 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. 2D-FL was taken at emission wavelengths from 310 to 500 nm and at an 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. Rutin was used as standard.^[20] 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 rutin in the proportions of HSA:extract = 1:1.^[21,22]

Statistical Analyses

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

Results and Discussion

Total Phenolics, Flavonoids, Chlorophylls, Carotenoids, and AA

The results of the determination of the main bioactive compounds of kiwi fruit cultivars in ethanol and water extracts are shown in Table 1.

Polyphenols in water extracts (Table 1) were the highest in “SKK-12” and the lowest in “Hayward” (16.3 ± 1.1 and 5.3 ± 0.5 mg GAE/g dry weight, DW). Flavonoids for “SKK-12” and “Hayward” differ, but not always significantly. Our present results slightly differ from the previous data, but the two season’s collections showed similar relationship between the same cultivars. Relatively high content of bioactive compounds and antioxidant properties of kiwi fruit determined by the advanced analytical methods justify its use as a source of valuable antioxidants.^[11,12] The bioactive compounds in kiwi fruit as an indication of quality after extraction using different solvents were studied in recent publications.^[11]

The values of β -carotene activities ($27.6 \pm 2.3\%$ and $8.3 \pm 0.9\%$) and FRAP [$(\mu\text{M TE/g DW})$ 24.6 ± 2.2 and 7.1 ± 0.7] were the highest in “SKK 12.” The lowest results were estimated in “Hayward” (Table 1). The highest amount of total chlorophyll and carotenes was in “Bidan” and “SKK 12” (Table 2). All kiwi fruit cultivars showed a high level of correlation between the contents of phenolic compounds and their antioxidant values. Our recent results differed from the ones showed by^[23] where the antioxidant

Table 1. The bioactive compounds and antioxidant activities in six kiwi fruit cultivars in ethanol and water extracts^{1,2,3}

Extracts of kiwi fruit cultivars	Indices				
	TPC mg GAE	FLA mg CE	FRAP μMTE	CUPRAC μMTE	β -carotene %
Hayward EtOH	4.5 ± 0.4^a	1.2 ± 0.1^b	6.1 ± 0.6^a	20.2 ± 2.1^a	5.3 ± 0.7^a
Daheung EtOH	4.2 ± 0.4^a	1.0 ± 0.1^{ab}	5.4 ± 0.5^a	19.4 ± 1.9^a	5.1 ± 0.5^a
Haenam EtOH	6.8 ± 0.6^{ab}	4.3 ± 0.4^d	10.2 ± 1.1^b	24.1 ± 2.3^{ab}	8.1 ± 0.9^{ab}
Bidan EtOH	11.5 ± 1.1^b	4.3 ± 0.4^d	18.4 ± 1.8^c	35.4 ± 3.2^c	20.2 ± 2.0^c
Hort16A EtOH	10.2 ± 1.1^b	1.2 ± 0.1^b	11.3 ± 1.1^b	32.1 ± 2.2^{bc}	17.0 ± 1.6^{bc}
SKK12 EtOH	14.5 ± 1.5^{bc}	2.4 ± 0.2^c	21.2 ± 1.7^{cd}	38.2 ± 3.9^{cd}	10.3 ± 1.2^b
Hayward H ₂ O	5.3 ± 0.5^a	0.6 ± 0.1^a	7.2 ± 0.7^{ab}	21.1 ± 2.1^a	8.3 ± 0.9^{ab}
Daheung H ₂ O	5.5 ± 0.5^a	0.6 ± 0.1^a	7.9 ± 0.7^{ab}	23.4 ± 1.9^{ab}	9.1 ± 0.9^b
Haenam H ₂ O	7.7 ± 0.7^{ab}	0.7 ± 0.1^a	11.3 ± 1.1^b	27.4 ± 2.1^b	13.8 ± 1.2^{bc}
Bidan H ₂ O	14.0 ± 1.3^{bc}	1.0 ± 0.1^{ab}	21.3 ± 1.8^{cd}	40.2 ± 3.2^{cd}	24.2 ± 2.2^{cd}
Hort16A H ₂ O	11.1 ± 1.1^b	1.4 ± 0.1^b	13.1 ± 1.3^{bc}	35.6 ± 2.8^c	22.1 ± 2.1^{cd}
SKK12 H ₂ O	16.3 ± 1.1^c	1.8 ± 0.1^{bc}	24.6 ± 2.2^d	43.3 ± 3.2^d	27.6 ± 2.3^d

¹Values are means \pm SD of five measurements;

²values in columns for every bioactive compound with the same solvent bearing different superscript letters are significantly different ($p < 0.05$);

³per g dry weight.

TPC, total polyphenol content FLAVON, flavonoids; CE, catechin equivalent; GAE, gallic acid equivalent; FRAP, ferric-reducing/antioxidant power; CUPRAC, cupric-reducing antioxidant capacity.

Table 2. Contents of chlorophylls and total carotenes in kiwi fruit cultivars (per g dry weight)^{1,2}

Kiwi fruit cultivars	Chlorophyll <i>a</i> μg	Chlorophyll <i>b</i> μg	Total chlorophylls μg	Total carotenes μg
Bidan	101 ± 15^d	39.1 ± 2.5^c	140 ± 11.8^d	4.7 ± 0.3^b
Hayward	21 ± 1.9^c	10.1 ± 1.9^b	31.1 ± 2.9^c	1.5 ± 0.1^a
Haenam	6.1 ± 0.5^{ab}	0.7 ± 0.1^a	6.8 ± 0.5^a	3.5 ± 0.2^{ab}
Daheung	4.4 ± 0.3^a	0.35 ± 0.1^a	4.8 ± 0.3^a	2.1 ± 0.1^a
Hort 16A	7.5 ± 0.7^b	8.7 ± 0.7^b	16.2 ± 1.4^b	4.9 ± 0.3^b
SKK 12	100 ± 7.7^d	31.1 ± 2.7^c	131.1 ± 9.5^d	7.5 ± 0.5^c

¹Values are means \pm SD of five measurements;

²values in columns for every bioactive compound bearing different superscript letters are significantly different ($p < 0.05$).

capacities were determined by ABTS, DPPH, and CUPRAC. Our results are in full correspondence with^[24] where a strong correlation was found between total polyphenol contents (TPC) and AA in hardy kiwi fruits. Phenolics affect the AA of hardy kiwi fruits.^[24]

The 2D-FL of ethanol extracts of kiwi fruit cultivars differ by the wavelength of the main peak and its fluorescence intensity (FI). All main FL peaks were located between λ_{em} from 329 to 338 nm with FI from 292 to 109. According to the value of FI in the main peak the ethanolic extracts kiwi

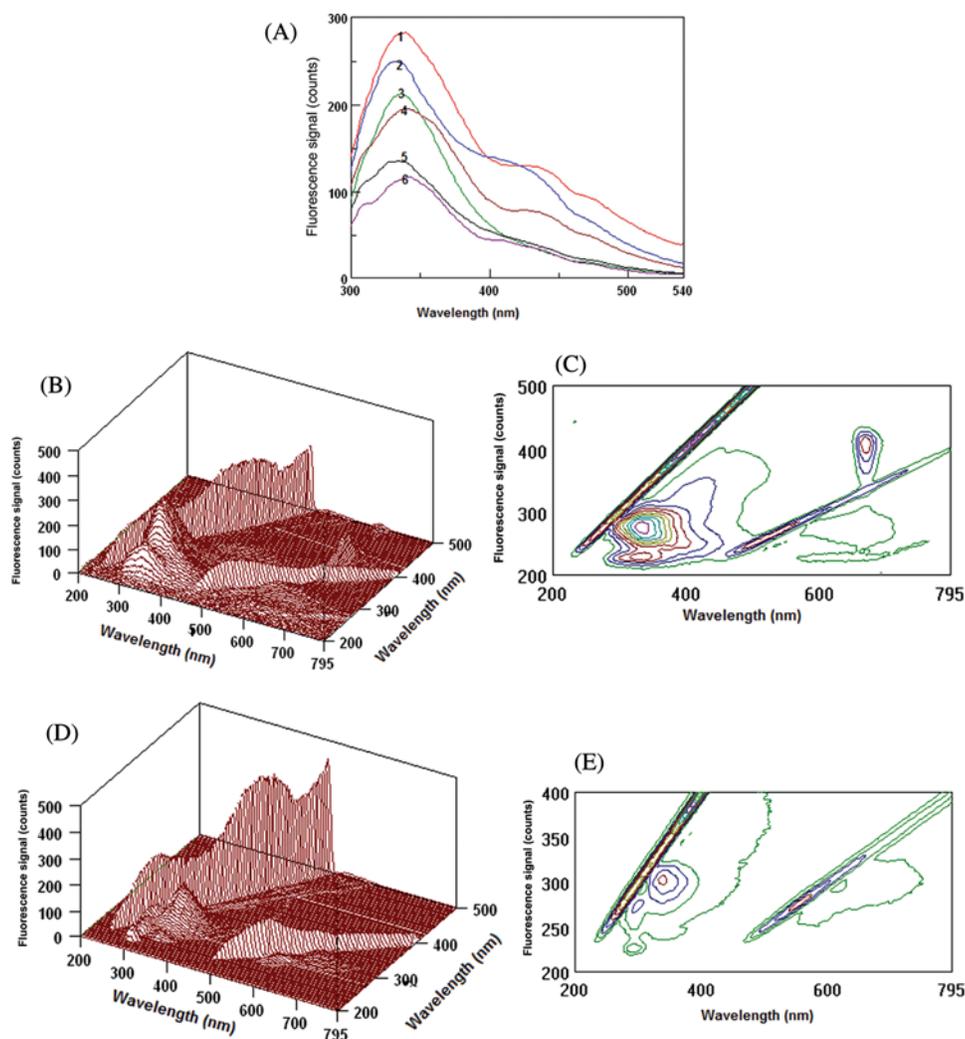


Fig. 1. Two-dimensional fluorescence spectra (A), with fluorescence intensity (FI) of ethanol extracts of the following kiwi fruit cultivars: “SKK 12” (first line from the top with FI of 308.2), “Hort16A” (second line from the top with FI = 252), rutin (third line, FI = 157.5), “Haenam” (fourth line, FI = 138.8), “Hayward” (fifth line, FI = 95.5), “Daehung” (sixth line, FI = 89.6). (B) 3D-FL spectra of “Haenam” ethanol extract. (C) Cross-section view from 3D-FL spectrum of ethanol extract of “Haenam.” (D) 3D-FL spectra of rutin ethanol extract. (E) Cross-section view of rutin ethanol extract. Fluorescence intensities are on y-axis and emission wavelengths are on x-axis. In all spectra the following conditions were used: $\lambda_{em}/\lambda_{ex}$ = 310/295; rutin (2.0×10^{-5} mol/L); ethanol extracts in concentration of $50 \mu\text{g}/\text{mL}$. 2D-FL spectra illustrate the interaction between human serum albumin (HSA), ethanol extracts of kiwi fruit cultivars, and rutin. (F) The change in the FI as a result of binding affinity of HSA with ethanol extracts: HSA [line 1, first line from the top with FI of 958]; HSA + “Daehung” (second line from the top with FI = 911), HSA + “Hayward” (third line, FI = 888.9), HSA + rutin (fourth line, FI = 863.3), HSA + “Haenam” (fifth line, FI = 852.5), HSA + “Hort16A” (sixth line, FI = 827.8), HSA + “Bidan” (seventh line, FI = 791.8), HSA + “SKK12” (eighth line, FI = 771.2), (G) 2D-FL spectra illustrate the interaction between HSA, rutin, and ethanol extracts of kiwi fruit cultivars. The change in FI as a result of binding affinity of HSA with ethanol extracts and rutin: HSA [first line from the top with FI of 958]; HSA + rutin (second line, FI = 863.3), HSA + “Daehung” (third line from the top with FI = 827.8), HSA + “Hayward” (fourth line, FI = 742.5), HSA + “Haenam” (fifth line, FI = 727.8), HSA + “Hort16A” (sixth line, FI = 714.8), HSA + “Bidan” (seventh line, FI = 683.6), HSA + “SKK12” (eighth line, FI = 679.6). In all reactions the following conditions were used: HSA (2.0×10^{-6} mol/L); rutin (1.7×10^{-6} mol/L); ethanol extracts in concentration of $50 \mu\text{g}/\text{mL}$. The binding was for 1 hr at 25°C . Fluorescence intensities are on y-axis and emission wavelengths are on x-axis.

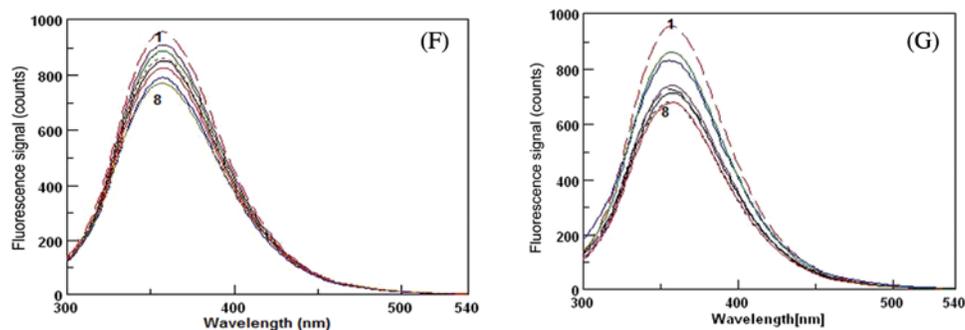


Fig. 1. Continued.

fruit cultivars were as follows: “SKK 12” ($\lambda_{em} = 331$ nm and FI = 291.7), followed by “Bidan” ($\lambda_{em} = 337$ nm and FI = 253.4), “Hort 16A” ($\lambda_{em} = 329$ nm and FI = 226.7), “Haenam” ($\lambda_{em} = 337$ nm and FI = 190.1), “Hayward” ($\lambda_{em} = 332$ nm and FI = 150.3), and the lowest “Daheung” ($\lambda_{em} = 338$ nm and FI = 108.5). There were various small peaks between wavelengths 614 and 669 nm and FIs varied from 22.1 to 188.3. All main FL peaks for water extracts were located between λ_{em} from 336 to 651 nm, with FI from 400 to 26.4. According to the value of FI in the main peaks the water extracts kiwi fruit cultivars were as follows: “SKK 12” ($\lambda_{em} = 340$ nm and FI = 404.1), followed by “Bidan” ($\lambda_{em} = 336$ nm and FI = 236.6), “Hort 16A” ($\lambda_{em} = 340$ nm and FI = 194.9), “Haenam” ($\lambda_{em} = 338$ nm and FI = 129.9), “Daheung” ($\lambda_{em} = 338$ nm and FI = 121.9), and the lowest in “Hayward” ($\lambda_{em} = 339$ nm and FI = 113.4). There were various small peaks between wavelengths 433 and 623 nm and FIs varied from 19.8 to 112.1. It was the best correlation between the obtained results of polyphenols using the spectroscopic method with the data of FI measurements in different extracts. The relationship between the FIs starting from the top line (line 1) and the determined polyphenols (“SKK 12” with FI = 308.2, Fig. 1A and polyphenols = 14.5, 14.5, Table 1) to the lowest line 9 “Daheung” (FI = 89.6, Fig. 1A, line 6, and polyphenol = 4.2, Table 1) was about 3.4 in both cases. The intensities of ethanol extract of “Haenam” and rutin (Fig. 1B, with similar λ_{em} of 341 nm of the main peak) can be compared and correlated. The binding properties of the kiwi fruit samples compared with the pure flavonoids such as rutin are shown in 2D-FL, Fig. 1F and G. One of the main peaks for HSA was found at $\lambda_{ex/em}$ of 220/357 nm (Fig. 1F, G, line 1). The interaction of HSA and the ethanol extracts of kiwi fruit cultivars (Fig. 1F) showed a slight change in the position of the main peak at the wavelength of 357 nm and a decrease in 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 958.5 (Fig. 1F, G, the upper line is HSA)]. The reaction with the kiwi fruit extracts and rutin decreased the FI of HSA (Fig. 1F, the fourth line). The following decrease in the FI (%) occurred during the interaction of ethanol extracts with HSA/ethanolic extracts and rutin with HSA: HSA + “Daheung” = 4.9%/13.6%; HSA + “Hayward” = 7.3%/22.5%; HSA +

“Haenam” = 11.1%/24.1%; HSA + “Hort16A” = 13.6%/25.4%; HSA + “Bidan” = 17.4%/28.7%; HSA + “SKK12” = 19.5%/29.1%; HSA + rutin = 9.9%. These results were in direct relationship with the antioxidant properties of the extracts. The synergism of bioactive compounds is shown when rutin was added to the mixture of HSA and extracts of kiwi fruit. Our results are similar to other reports, where the binding of aucubin to bovine serum albumin has been studied by FL.^[8,9] The experimental results showed that the FL quenching mechanism between BSA and three amide compounds of phenolic acids was mainly static quenching. The results indicated that the hydrophobic force was the dominant intermolecular force in stabilizing the complex, and the probable quenching mechanism of the bovine serum albumin–phenolic interaction was initiated by complex formation.^[10,22] Our very recent results showed that FL is significantly quenched, because of the conformation of proteins, phenolic acids, and flavonoids.^[11] The strong binding properties of phenolics show that they may be effective in the prevention of atherosclerosis under physiological conditions. 3D-FL can be used as an additional tool for the characterization of the polyphenol extracts of kiwi fruit cultivars and their binding properties. The IR spectra of ethanol and water kiwi fruit extracts were compared between them and also with standards in the range of common peaks (Fig. 2). IR spectrum of polyphenol shows main peaks at 3377, 1586, 1488, 1454, 1384 (small shoulder), 1209, 1162 (small peak), 892, 818, 745, and 687 cm^{-1} . To assign the IR peaks of polyphenols, the IR spectrum of phenolic extracts is used. Its IR peaks in water (Fig. 2A) appear at 3337, 1375, 1174, 812, 803, 788, and 718 cm^{-1} . Its IR peaks in ethanol (Fig. 2B) appear at 3328, 1590, 1401, 1340, 1235, and 816 cm^{-1} . The peak pattern of poly(rutin) was similar to that of the rutin monomer, although all the peaks of the polymer became broader. In the spectrum of the methanol-soluble fraction of poly(rutin), a broad peak centered at 3300 cm^{-1} due to the vibration of O–H linkage of phenolic and hydroxyl groups and peaks at ca. 1570 cm^{-1} ascribed to the C–C vibration of aromatic group were observed.^[25,26] Figure 2C shows the FT-IR spectra of rutin, where the most important peaks had a small shift (3335 cm^{-1}), 1652 and 1598 cm^{-1} in comparison with other studies.^[26] A shift in the difference between the standard and the investigated samples can be explained by the

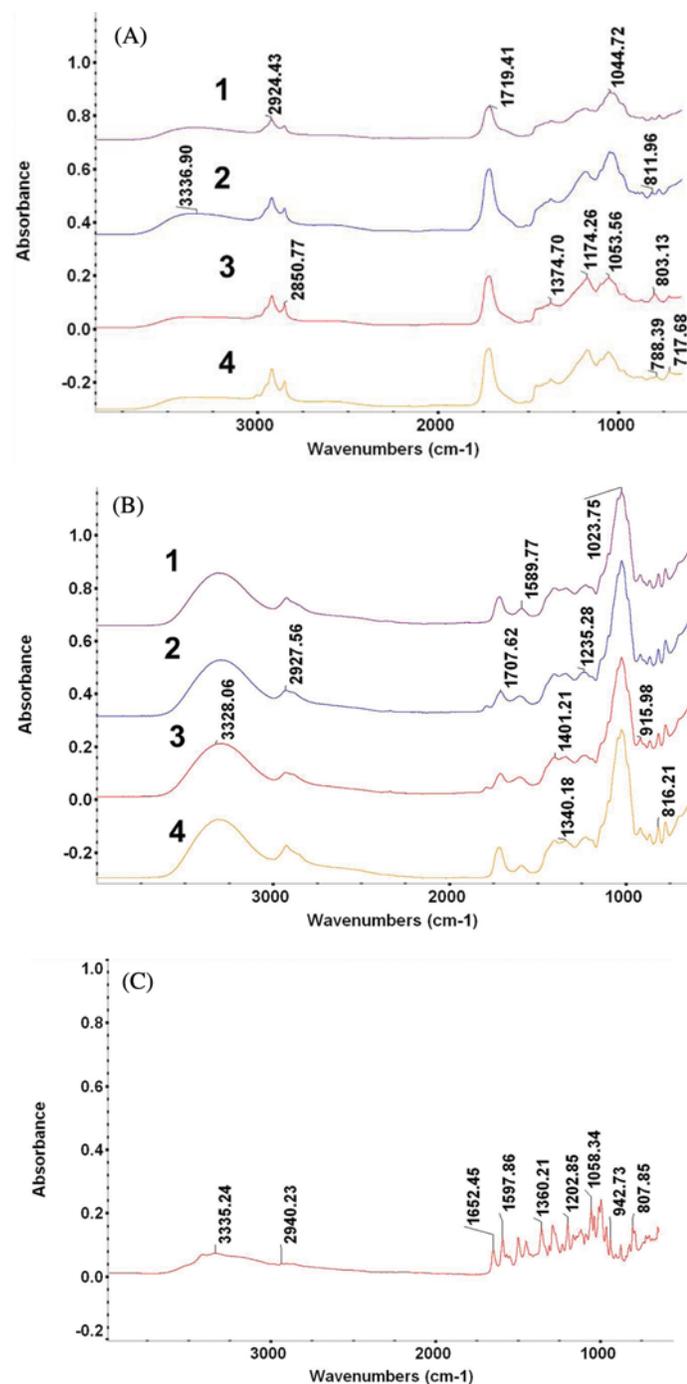


Fig. 2. Infrared spectra of water (A) and ethanol (B) extracts of four kiwi fruit cultivars. From the top lines of the following kiwi fruit cultivars are “SSK 12,” “Hort16A,” “Haenam,” “Hayward”; (C) rutin. A Nicolet iS 10 FT-IR Spectrometer with the smart iTRTM ATR (attenuated total reflectance) accessory was used to record IR spectra. Comparison and matching were carried out on the basis of wavenumbers peaks of rutin as a standard and between the two different extracts of four kiwi fruit cultivars in the region from 4000 to 650 cm^{-1} .

extraction procedures of the total phenols. The matching (%) of the peaks in the region from 4000 to 650 cm^{-1} (Fig. 2A) showed the highest matching (99%) between “SKK12” and

“Hayward” of phenols extracted with water. Rutin in the kiwi fruit water extracts showed high matching of 86–89%. Slightly different data were obtained in the ethanol extracts. These matching results for the first time showed that IR spectra can be used for a rapid estimation of extracted bioactive compounds. In our previous study the IR spectra data showed that the main bands in the kiwi fruit samples slightly shifted.^[11,12] Similar to the fluorometric methods, IR spectroscopy^[7,25] can also be used as an additional indication of similarity and differences between the cultivars.

Conclusions

The applied spectrophotometric methods showed that the bioactive compounds and their antioxidant activities of the studied kiwi fruit cultivars in ethanol and water extracts were significantly different ($p < 0.05$). According to the used tests it was significantly minimal ($p < 0.05$) in ethanol, and the highest was in the extract with water. The obtained results of polyphenols and antioxidant activities in kiwi fruit cultivars correlated with the FL results. The used FL and IR spectra were compared with the spectroscopic data and can be proposed as a rapid estimation of kiwi fruit analysis.

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