FOOD COMPOSITION AND ADDITIVES

Analytical Determination of Bioactive Compounds as an Indication of Fruit Quality

YONG SEO PARK
Mokpo National University, Department of Horticultural Science, Muan, Jeonnam, South Korea
BUK-GU HEO
Naju Foundation of Natural Dyeing Cultural Institute, Naju, South Korea
KYUNG-SIK HAM, SEONG-GOOK KANG, and YANG-KYUN PARK
Mokpo National University, Department of Food Engineering, Muan, Jeonnam, South Korea
ALINA NEMIROVSKI, ZEEV TASHMA, and SHELA GORINSTEIN[1]
The Hebrew University, Hadassah Medical School, The Institute for Drug Research, School of Pharmacy, Jerusalem 91120, Israel
HANNA LEONTOWICZ and MARIA LEONTOWICZ
Warsaw University of Life Sciences (SGGW), Faculty of Veterinary Medicine, Department of Physiological Sciences, Warsaw, Poland

The aim of this investigation was to determine the bioactive compounds in kiwifruit as an indication of quality after extraction using methanol and ethyl acetate. Using FTIR and three-dimensional fluorescence spectroscopy and electrospray ionization/MS, the contents of polyphenols, flavonoids, flavanols, and tannins, and the level of the antioxidant activity by 2, 2-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt, 1, 1-diphenyl-2-picrylhydrazyl, ferric-reducing/antioxidant power, and cupric reducing antioxidant capacity assays were determined and compared. It was found that the methanol extracts of kiwifruit showed significantly higher amounts of bioactive compounds and antioxidant activities than the ethyl acetate extracts. The cultivar Bidan, in comparison with the classic Hayward, showed significantly higher bioactivity. For the first time, Bidan organic kiwifruit was analyzed for its antioxidant activities and compared with the widely consumed Hayward organic based on its bioactive compounds and fluorescence properties. Relatively high content of bioactive compounds and positive antioxidant and antiproliferative properties of kiwifruit determined by the advanced analytical methods justify its use as a source of valuable antioxidants. The methods used are applicable for bioactivity determination, in general, for any food products.

Consumption of fruit and vegetables has the potential to reduce nontransmissible diseases, such as cardiovascular diseases and cancer, which are major public health concerns (1–3). Many epidemiologic studies have shown that a diet rich in apples can reduce cardiovascular events (myocardial infarction and stroke) and some type of cancers (1). Increased consumption of vegetables, particularly cruciferous ones, and fruits promote cardiovascular health and overall longevity (2). Among the widely consumed fruits is kiwifruit, which is popular in the United States and Europe (4, 5). Kiwifruits have many cultivars; the best-known is Hayward. Bidan is less spread and consumed than Hayward. Both belong to the *Actinidia deliciosa* species that is known for good taste (6–8). However, the good taste of this fruit is not the only positive property: Duttaroy and Jøorgensen, in a clinical trial of human volunteers, showed that kiwifruit is effective in the prevention of coronary atherosclerosis (9). It was found that consumption of two or three kiwifruits/day lowers the blood triglyceride level by 15% and reduces platelet aggregation response by 18% compared with controls ($P < 0.05$). There is growing interest concerning organically versus conventionally grown fruits and vegetables. The differences in the bioactivity between kiwifruit cultivars grown in conventional and organic conditions have been less studied (10). Therefore, it was decided to compare their bioactivity using two different solvents (methanol and ethyl acetate). Not all investigators used complementary assays for determination of the antioxidant activity (11). In order to obtain reliable data about the antioxidant activity of the studied samples, four generally accepted assays were used: 2, 2-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt (ABTS; 12); 1, 1-diphenyl-2-picrylhydrazyl (DPPH; 13); ferric-reducing/antioxidant power (FRAP; 13); and cupric reducing antioxidant capacity (CUPRAC; 14). As far as we know, results of such investigations have not been published.

Received March 26, 2012. Accepted by SG April 30, 2012.
[1] Corresponding author's e-mail: gorin@cc.huji.ac.il
DOI: 10.5740/jaoacint.12-130

Experimental

Chemicals

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; catechin; quercetin; Tris, tris (hydroxymethy1) aminomethane; bovine serum albumin, ABTS, DPPH, Folin-Ciocalteu reagent; lanthanum (III) chloride heptahydrate, $FeCl_3 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$, 2,9-dimethyl-1,10-phenanthroline (neocuproine); and bovine serum albumin (BSA) were purchased from Sigma Chemical Co., St. Louis, MO. 2,4,6-Tripyridyl-*s*-triazine (TPTZ) was purchased from Fluka Chemie (Buchs, Switzerland). All reagents were of analytical grade. Deionized and distilled water (Bio-Lab Ltd, Jerusalem, Israel) was used throughout.

Sample Preparation

Samples of kiwifruit cultivars Hayward and Bidan at their commercial maturity stage were grown in conventional and organic conditions in an orchard in Heanam County, Jeonnam Province, Korea, 2009. The samples were cleaned with tap water and dried. For the investigation five replicates of five fruits each were used. Their edible parts were prepared manually without using steel knives. The peeled fruits were weighed, chopped, and homogenized under liquid nitrogen in a high-speed blender (Hamilton Beach Silex professional model, Memphis, TN) for 1 min. A weighed portion (50–100 g) was then lyophilized for 48 h (VirTis Model 10–324, Gardiner, NY), and the dry weight (dw) was determined. The samples were ground to pass through a 0.5 mm sieve (Cole-Parmer, El-Hamma Instruments Kibbutz Mevo Hamma Ramat Hagolan, Israel) and stored at –80°C until the bioactive substances were analyzed. The bioactive compounds and the antioxidant activity (AA) were determined in methanol and ethyl acetate extracts of conventional and organic grown Hayward and Bidan cultivars: CHmet, OHmet, CHetlylac, OHethylac, CBmet, OBmet, CBetlylac, and OBethylac.

Determination of Bioactive Compounds and AA

The extracts from kiwifruit were prepared in the same way for all tests (bioactive compounds, antioxidant, and anticancer assays). The extracts were phenols from methanol and ethyl acetate solvents (concentration 25 mg/mL) prepared at room temperature and extracted twice during 3 h. The polyphenols were determined by the Folin-Ciocalteu method, with absorbance measurement at 750 nm with a spectrophotometer (Hewlett-Packard, Model 8452A, Rockville, MD). The results were expressed as mg of gallic acid equivalents (GAE)/g dw. Total flavonoid content was determined by an aluminum chloride colorimetric method with some modifications. Briefly, 0.25 mL kiwifruit sample extract was diluted with 1.25 mL distilled water. Then 75 µL 5 g/100 g $NaNO_2$ solution was added to the mixture. After 6 min, 150 µL 10 g/100 g $AlCl_3 \cdot H_2O$ solution was added, and the mixture was allowed to stand for another 5 min. Half of a milliliter of 1 M NaOH was added, and the total was made up to 2.5 mL with distilled water. The solution was mixed well. The absorbance was measured immediately against the blank at 510 nm in comparison with the standards prepared similarly with known (+)-catechin concentrations. The results are expressed as milligrams of catechin equivalents. 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 kiwifruit extract. As mentioned previously, (+)-catechin served as a standard for flavonoids and flavanols, and the results were expressed as catechin equivalents (15, 16). Total ascorbic acid was determined by CUPRAC assay. The water extract was prepared from 100 mg sample and 5 mL water, then mixed, stirred for 24 h, and centrifuged. The extract (1 mL) was mixed with 2 mL 3.0×10^{-3} M lanthanum(III) chloride heptahydrate. Ethyl acetate was used for extraction in order to avoid the interference of flavonoids. For determination of ascorbic acid by CUPRAC assay, the aqueous phase was examined. One milliliter of Cu (II)-neocuproine (Nc) in ammonium acetate-containing medium at pH 7 was mixed with 1 mL of the obtained extract, and the absorbance of the formed bis (Nc)-copper (I) chelate was measured at 450 nm (17).

Determination of AA, µmol Trolox Equivalent (TE)/g dw

The AA was determined by four complementary assays: (*1*) $ABTS^{\cdot+}$ method for the screening of antioxidant activity is reported as a decolorization assay applicable to both lipophilic and hydrophilic antioxidants, including flavonoids, hydroxycinnamates, carotenoids, and plasma antioxidants. The performed radical monocation ABTS is generated by oxidation of ABTS with potassium persulfate and is reduced in the presence of hydrogen-donating 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 AA. $ABTS^{\cdot+}$ radical cation was generated by the interaction of ABTS (7 mmol/L) and $K_2S_2O_8$ (2.45 mmol/L). This solution was diluted with water until the absorbance in the samples reached 0.7 at 734 nm. (*2*) FRAP assay measures the ability of the antioxidants in the investigated samples to reduce ferric-tripiridyltriazine (Fe^{3+}-TPTZ) to the ferrous form (Fe^{2+}). FRAP reagent (2.5 mL 10 mmol ferric-tripiridyltriazine solution in 40 mmol HCl plus 2.5 mL 20 mmol $FeCl_3 \cdot H_2O$ and 25 mL 0.3 M acetate buffer, pH 3.6; 900 µL) was mixed with 90 µL distilled water and 30 µL kiwifruit samples as the appropriate reagent blank. The absorbance was measured at 595 nm. (*3*) CUPRAC: this assay is based on utilizing Cu(II)-Nc reagent as the chromogenic oxidizing agent. To a mixture of 1 mL copper (II)-Nc ammonium acetate buffer solution, acidified and nonacidified water extracts (or standard) solution (x, in mL), and H_2O [(1.1-x) mL] were added to make a final volume of 4.1 mL. The absorbance at 450 nm was recorded against a reagent blank. (*4*) Scavenging free radical potentials were tested in a solution of DPPH. The degree of decoloration of the solution indicates the scavenging efficiency of the added substance. In its radical form, DPPH has an absorption band at 515 nm that 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 (12–14).

Fluorometric Measurements

Fluorescence spectra for all kiwifruit extracts in methanol at a concentration of 0.01 mg/mL were recorded on a Model FP-6500 Jasco spectrofluorometer, serial No. N261332 (Tokyo, Japan), equipped with 1.0 cm quartz cells and a thermostat bath. The widths of the excitation and emission slits were set at 10.0 and 5.0 nm, respectively. The three-dimensional fluorescence (3D-F1) spectra were collected with subsequent scanning of emission spectra from 250 to 500 nm at 1.0 nm increments by varying the excitation wavelength from 250 to 450 nm in 10 nm increments. The scanning speed was set at 1000 nm/min for all measurements, which were performed with the emission mode and intensity up to 550.

Table 1. Contents of bioactive compounds and the AA (μmol TE/g dw) in methanol extracts of two kiwifruit cultivars: Hayward conventional (CH) and organic (OH); and Bidan conventional (CB) and organic (OB)

Samples[1]	CH	OH	CB	OB
Polyph, mg GAE/g	8.87 ± 0.5[a, 2, 3]	12.42 ± 0.6[b]	24.76 ± 1.3[c]	34.85 4.3[d]
Tannins, mg CE/g	2.86 ± 0.1[a]	4.21 ± 0.6[b]	2.23 ± 0.2[a]	3.68 ± 0.7[c]
Flavonoids, mg CE/g	9.02 ± 0.9[a]	12.63 ± 1.7[b]	5.27 ± 0.6[c]	8.69 ± 0.9[a]
Flavanols, μg CE/g	618.65 ± 25.1[a]	866.11 ± 27.1[b]	251.13 ± 11.1[c]	414.36 ± 13.2[d]
ABTS	21.23 ± 3.5[a]	29.72 ± 1.2[b]	107.48 ± 10.9[c]	147.34 ± 14.2[d]
DPPH	8.14 ± 1.2[a]	11.45 ± 1.3[b]	98.56 ± 8.4[c]	132.62 ± 11.6[d]
FRAP	10.23 ± 7.1[a]	14.67 ± 0.9[b]	92.13 ± 4.5[c]	142.76 ± 7.7[d]
CUPRAC	22.15 ± 2.2[a]	31.07 ± 3.2[b]	118.89 ± 11.1[c]	166.22 ± 11.8[d]

[1] Abbreviations: Polyph = polyphenols; CE = catechin equivalent; GAE = gallic acid equivalent; CGE = cyanidin-3-glucoside equivalent; ABTS = 2, 2-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammoniumsalt; CUPRAC = cupric reducing antioxidant capacity; FRAP = ferric-reducing/anti-oxidant power; DPPH = 1, 1-diphenyl-2-picrylhydrazyl method.

[2] Values are means ± SD of five measurements.

[3] Values in rows [a, b, c, d] for every bioactive compound with the same solvent bearing different superscript letters are significantly different ($P < 0.05$) per g dry weight.

FTIR Spectroscopy Studies

The presence of polyphenols in the investigated kiwifruit samples was studied by FTIR spectroscopy. A Nicolet iS 10 FT-IR Spectrometer (Thermo Scientific Instruments LLC, Madison, WI) with a smart iTR™ attenuated total reflectance accessory was used to record IR spectra.

Extraction of Phenolic Compounds for MS

For proanthocyanidin and flavonoid identification, 1 g of kiwifruit powder was extracted three times with 25 mL of acetone–water–acetic acid (70 + 29.5 + 0.5, v/v/v) for 45, 45, and 30 min, respectively, using an Orbi-Shaker™ orbital shaker (Benchmark Scientific, Inc., Edison, NJ) at 300 rpm. The mixture was centrifuged, the supernatant collected, and the acetone evaporated under reduced pressure to yield a slurry (8 mL). To obtain the proanthocyanidins, the slurry was liquid–liquid extracted with ethyl acetate, and the ethyl acetate fraction was analyzed using electrospray ionization (ESI)-MS. For flavonoid identification and quantification, the slurry was diluted four times with methanol and filtered through a Sartorius Minisart (Sartorius Group, Göttingen, Germany) 45 μm porosity and analyzed using ESI-MS (16).

MS Analysis

A TSQ Quantum Access Max mass spectrometer (Thermo Fisher Scientific, Basel, Switzerland) was used. Analytes were ionized by ESI in the negative mode. Vaporizer temperature was kept at 100°C. 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, 30 AU; capillary temperature, 200°C; and skimmer offset, 0 V.

MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] Assay

The MTT assay measures antiproliferative activity. Antiproliferative activity of methanol extracts of the studied kiwifruits on human cancer cell lines (Calu-6 for human pulmonary carcinoma and SNU-601 for human gastric carcinoma) were measured using the MTT assay. The cell lines were purchased from Korean Cell Line Bank (Cancer Research Institute, Seoul, Korea) for the MTT assay. Cells were grown in Roswell Park Memorial Institute (RPMI) 1640 medium at 37°C under 5% CO_2 in a humidified incubator. Serum (10 g/100 g) and antibiotics (1 g/100 g) were added to the RPMI-1640 medium. Cells were trypsinized and then centrifuged to harvest. Cells were harvested, counted (3×10^4 cells/mL), transferred into a 96-well plate, and incubated for 24 h prior to the addition of test compounds. Serial dilutions of test samples were prepared by dissolving compounds in methanol followed by dilution with RPMI-1640 medium to give final concentration of 10, 30, 100, 300, and 1000 μg/mL. Stock solutions of test samples were prepared for cell lines of 90 μL medium and 10 μL samples and incubated for 72 h. MTT solution (at 5 mg/mL) was dissolved in 1 mL phosphate buffer solution, and 10 μL was added to each of the 96 wells. The wells were wrapped with aluminum foil and incubated at 37°C for 4 h. The solution in each well containing media, unbound MTT, and dead cells was removed by suction and 150 μL dimethylsulfoxide was added to each well. The plates were then shaken, and optical density was recorded using a micro plate reader (FLUOstar galaxy; BMG Labtechnologies, Offenburg, Germany) at 540 nm. Distilled water was used as a positive control and methanol as a solvent control. Controls and samples were assayed in duplicate for each concentration and replicated three times for each cell line. The cytotoxicity was obtained by comparing the absorbance between the samples and the control.

Statistical Analyses

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

Table 2. Contents of bioactive compounds and the AA (μmol TE/g dw) in ethyl acetate extract of two kiwifruit cultivars: Hayward conventional (CH) and organic (OH); and Bidan conventional (CB) and organic (OB)

Samples	CH	OH	CB	OB
Polyph, mg GAE/g	6.33 ± 0.6[a, 1, 2]	8.28 ± 0.7[b]	16.54 ± 1.8[c]	23.28 ± 2.3[d]
Tannins, mg CE/g	2.04 ± 0.1[a]	2.86 ± 0.6[b]	1.49 ± 0. 1[c]	2.45 ± 0.6[b]
Asc acid, mg/100g FW[4]	20.47 ± 1.3[a]	26.36 ± 1.5[b]	99.26 ± 7.4[c]	142.45 ± 16.4[d]
Flavonoids, mg CE/g	6.22 ± 0.7[a]	8.42 ± 0.8[b]	3.63 ± 0.4[c]	5.57 ± 0.6[a]
Flavanols, μg CE/g	412.43 ± 21.2[a]	577.45 ± 24.2[b]	167.56 ± 9.1[c]	276.24 ± 9.2[d]
ABTS	13.79 ± 2.6[a]	19.35 ± 1.4[b]	70.72 ± 8.9[c]	98.45 ± 11.2[d]
DPPH	5.43 ± 0.9[a]	7.63 ± 1.1[b]	65.71 ± 4.4[c]	94.73 ± 8.6[d]
FRAP	6.82 ± 0.9[a]	10.12 ± 0.9[b]	61.42 ± 3.5[c]	95.17 ± 7.7[d]
CUPRAC	14.76 ± 3.2[a]	20.71 ± 2.2[b]	79.26 ± 9.1[c]	110.82 ± 10.8[d]

[1] Values are means ± SD of five measurements.

[2] Values in rows [a, b, c, d] for every bioactive compound with the same solvent bearing different superscript letters are significantly different ($P < 0.05$) per g dry weight.

[3] Abbreviations: Polyph = polyphenols; CE = catechin equivalent; GAE = gallic acid equivalent; CGE = cyanidin-3-glucoside equivalent; ABTS, 2 = 2-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammoniumsalt; CUPRAC = cupric reducing antioxidant capacity; FRAP = ferric-reducing/anti-oxidant power; DPPH = 1, 1-diphenyl-2-picrylhydrazyl method.

Results and Discussion

Polyphenols, Flavonoids, Flavanols, Tannins, and Ascorbic Acid

The results of the determination of the contents of these bioactive compounds in samples of conventional and organic grown kiwifruits extracted by two different solvents are shown in Tables 1 and 2. As can be seen, the contents of polyphenols, flavonoids, flavanols, and tannins were significantly higher in methanol extracts of both organically grown cultivars ($P < 0.05$) than in ethyl acetate extracts. In Bidan cultivar, all the studied properties were significantly higher than in Hayward.

Antioxidant Activity

The results of the determination of the level of AA (μmol micromole trolox equivalent/g) of the conventionally and organically grown kiwifruits by two different extracts are shown in Tables 1 and 2. The same relationship as with bioactive compounds was obtained with the values of the AA: it was significantly higher in Bidan than Hayward, and higher in organic fruits than in conventional. The order of AA was identical with all assays (ABTS, DPPH, FRAP, and CUPRAC) for the tested cultivars: CH < OH < CB < OB.

Fluorometric Data

Fluorometric data measured the quenching properties of the kiwifruit samples in comparison with the pure flavonoids such as quercetin, catechin, and others. 3D-FL spectra (Figure 1) illustrated the elliptical shape of the contours. The x-axis represents the emission spectra from 250 to 500 nm, while the y-axis is the excitation spectra from 250 to 450 nm, for conventional (B) and organic (D) kiwifruit. The results show that the 3D-FL contour maps of conventional in comparison with organic fruit were similar. One main peak can easily be observed at the approximate location of λex/λem 275/305 nm, and the other at λex/λem 350/430 nm. Conventional kiwifruit extract showed the following fluorescence intensities (FIs): at λex/λem 270/275 nm 174.19, and at λex/λem 275/310 nm 96.67; for organic kiwifruit, at λex/λem 275/330 nm FI was 183.99 and at λex/λem 275/310 nm FI was 112.49.

FTIR Spectra

The FTIR spectra of the methanol fraction of organic kiwifruit (Figure 2A, OHmet line 1), of the methanol fraction of conventional kiwifruit (Figure 2A, CHmet, line 2), and of gallic acid (Figure 2A, GA, line 3) were compared between the fruits and separately with gallic acid in the range of common peaks. In the range of peaks (1040–1800 cm^{-1}), the match

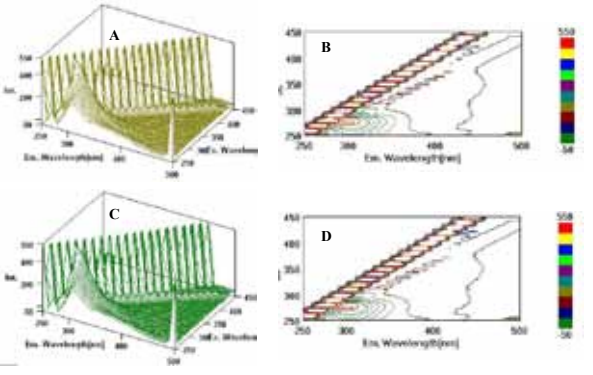

Figure 1. (A), (C), 3D-FI spectra of methanol extracts of conventional and organic kiwifruits (0.25 mg/mL); (B), (D), elliptical shapes of the contours of methanol extracts of conventional and organic kiwifruits (0.25 mg/mL); the 3D-FI spectroscopy was run at emission mode and fluorescence intensity up to 550, emission wavelengths from 250 to 500 nm, and excitation wavelengths from 250 to 450 nm; scanning speed was 1000 nm/min; (B), (D), emission wavelength on x-axis and fluorescence intensity on y-axis; Em., emission wavelength on x-axis and Ex., excitation wavelength on y-axis.

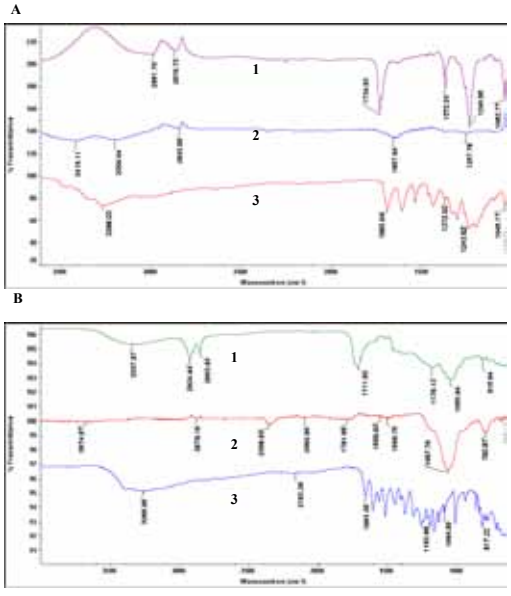

Figure 2. Infrared study of FTIR spectra of (A) methanol extracts of organic kiwifruit (line 1); conventional kiwifruit (line 2); gallic acid (line 3). (B) Ethyl acetate extracts of organic kiwifruit (line 1); conventional kiwifruit (line 2); and quercetin (line 3).

between two samples of kiwifruits (CHmet:OHmet) was about 24%, and in the range 2800–3600 cm^{-1} was about 54%. Lower matches were found in two ranges of the peaks between CHmet and GA (12 and 8%), and in comparison with the OHmet (17 and 30%). Slightly different data were obtained for matching in the two ranges of the peaks between CHmet and quercetin (4 and 19%), and in comparison with the OHmet (6 and 34%). In comparison with catechin (CAT), the following numbers were obtained: CHmet: CAT = 20% and CHmet: CAT = 30% for two regions for conventional kiwifruit and for organic kiwifruit, 3 and 15%, respectively. As can be seen, the FTIR spectra showed the highest match with the main three standards from 2800 to 3600 cm^{-1}, ranging from 54 to 8%. The three standards were used for comparison (gallic acid, quercetin, and CAT) because of their relatively high AA. Lower matching was in the range 1040–1800 cm^{-1}, from 24 to 3%. The two peaks that appeared around 3415 and 3200 cm^{-1} (broad phenolic OH band; Figure 2A, CHmet, line 2) are not present in organic fruit. Probably the peak at 3200 cm^{-1} shifted to 2992 cm^{-1}. The peaks at 2844 and 2871 cm^{-1} (C–H bond of saturated carbons) were present in the two investigated kiwifruits. One main peak appeared at 1735 cm^{-1} in OHmet, C=O band, and a small one in CHmet (1658 cm^{-1}), a small shoulder due to the overlapping of the dominant –CO stretching. The other bands were similar in the two samples (1257 and 1241 cm^{-1}). The observation and comparison of the peaks showed that the organic kiwifruits had better matching than the conventional with gallic acid and quercetin (QU).The two ethyl acetate extracts were compared with quercetin (Figure 2B, OHethylac, line 1) and conventional kiwifruit (Figure 2B, CHethylac, line 2) and QU (Figure 2A, line 3) were compared between themselves and separately with QU in the range of common peaks. In the range of peaks, 650–1800 cm^{-1}, the matching between two samples of kiwifruits (CHethylac:OHethylac) was about 18%, at 790–1600 cm^{-1}, 43%, at 2000–3000 cm^{-1}, 20%. Lower matching was in the three ranges of the peaks between CHethylac and QU (2, 2, and 4%) and in comparison with the OHethylac (7, 11, and 14%; Figure 2B). Slightly different data were obtained in the matching in three ranges of the peaks between CHethylac and GA (8, 7, and 21%) and in comparison with the OHethylac (7, 14, and 21%). In comparison, with CAT, the following numbers were obtained: CHethylac: CAT = 5%, CHethylac: CAT = 7% and CHethylac: CAT = 4% for three regions for conventional kiwifruit, and for organic kiwifruit, 4, 8, and 20%, respectively. Ethyl acetate extracts had the highest matching with the main three standards from 2000 to 3000 cm^{-1}, ranging from 20 to 2%. The peaks that were in methanol extract in organic fruits appeared in ethyl acetate extract (in the range of 3500–3000 cm^{-1} with some shifting and changes in the range of 1500–800 cm^{-1}). The same relationship was found also in the conventional sample, where additional big peaks appeared in the range from 1500 to 794 cm^{-1}.

Mass Spectra

The spectra show that in methanol extract of conventional kiwifruit (Figure 3A) and organic kiwifruit (Figure 3B), 15 peaks were found. The main peaks for conventional and organic kiwifruits in *m/z* units were the following: 4-hydroxybenzoic acid at 133, with relative abundance (RA, %) of 11 and 19; gallic acid at 173, with RA of 3; 3-caffeoyl quinic acid at 191, with RA of 100; methyl syringate at 215 with RA of 13; sinapic acid at 225, with RA of 2; phloretin at 277, with RA 5 and 7%; chlorogenic acid at 353, with RA of 48 and 42; hydroxy-FA-hexoside at 371, with RA of 17 and 15; and pelargonidin-3-malonylglucoside at 515 with RA of 10 and 8. The spectra showed that in ethyl acetate extract of conventional kiwifruit (Figure 4A) and organic kiwifruit (Figure 4B), 10 peaks were found. The main peaks for conventional and organic kiwifruits in *m/z* units were the following: 4-hydroxybenzoic acid at 133, with RA of 26 and 38; 3-caffeoyl quinic acid at 191, with RA of 100; apigenin at 265, with RA of 16 only in organic kiwifruit; caffeic acid at 281, with RA of 12 only in conventional kiwifruit; caftaric acid at 311, with RA of 10 and 13; at 339, with RA of 12 for organic kiwifruit; chlorogenic acid at 353, with RA of 5 and 12; and at 391, with RA of 23 and 4.

Inhibition of Proliferation

It was observed that the percentage of proliferativity of the methanol extracts of conventional and organic kiwifruit samples on two cell lines Calu-6 for human pulmonary carcinoma and human gastric carcinoma (Figure 5) were different. The proliferativity (%) for concentrations of 1000 µg/mL (Figure 5A and B) for organic Hayward kiwifruit on Calu-6 was 87.21%, and on SNU-601 was 89.04%, showing higher antiproliferative activity in comparison with a conventional sample for Calu-6 (90.83%) and SNU-601 (93.56%). The proliferativity (%) for concentrations of 1000 µg/mL (Figure 5C and D) for organic Bidan kiwifruit on Calu-6 was 79.11%, and on SNU-601 was 80.23%, showing higher antiproliferative activity in comparison with a conventional sample for Calu-6 (82.54%) and SNU-601 (84.54%). Our investigation shows that AA of the studied samples was highly correlated with their antiproliferative activity.

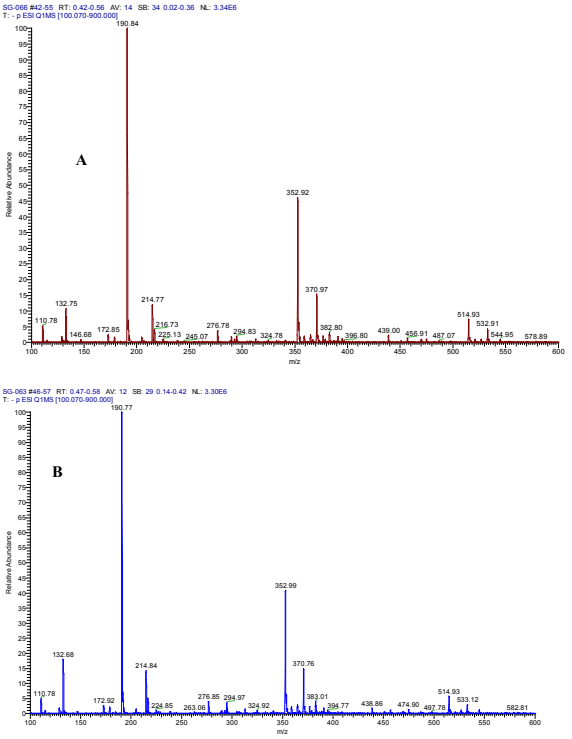

Figure 3. ESI-MS spectra of methanol fractions of (A) conventional kiwifruit and (B) organic kiwifruit in the negative ion mode.

It is well known that consumption of fruits and vegetables with high contents of bioactive compounds and high AA prevents and treats even the most dangerous diseases (1, 18–20). Dauchet et al. (3) revealed that consumption of fruit and vegetables is associated with a reduced rate of coronary heart disease (CHD) in observational cohorts. They assessed the strength of this association in a meta-analysis. Nine studies were eligible for inclusion in the meta-analysis that consisted of 91 379 men, 129 701 women, and 5007 CHD events. The risk of CHD was decreased by 4% [Relative risks (95% confidence interval; CI): 0.96 (0.93–0.99), $P = 0.0027$] for each additional portion/day of fruit and vegetable intake and by 7% [0.93(0.89–0.96), $P < 0.0001$] for fruit intake. The meta-analysis of cohort studies shows that fruit and vegetable consumption is inversely associated with the risk of CHD. Therefore, investigators recommend consuming only such fruits (21, 22). An interest was shown by the scientific community concerning organically versus conventionally grown fruits and vegetables (23–26). In the present investigation, samples of conventionally and organically grown kiwifruit cultivars Hayward and Bidan were studied with the aim of finding which was best for human consumption. It must be underlined once again that these samples were at the same stage of ripening and grown in the same geographic and climatic conditions. Therefore, no doubt, the determined data were reliable as it was shown in other reports (27, 28). These data were similar to that published in our previous investigation (29). The results of the present investigation show that kiwifruit cultivars contain high quantities of bioactive compounds. We found that Hayward and Bidan methanol extracts of the organic grown cultivars contain significantly higher amounts of the studied bioactive compounds. Other authors found high amount of phenolics (mg GAE/g dw) in Hayward kiwifruit patterns: 2.19 (30), 2.94 (31), and 3.5 (32), but relatively lower than our results. The same patterns were reported for other fruits (33). It was reported (33) that organic food is associated by the general public with improved nutritional properties. The effect of organic and conventional cultivation methods on dry matter, protein, minerals, and total phenolic content of eggplants was different. Organically produced eggplants had higher mean contents of total phenolics (49.8 versus 38.2 mg/100 g). The results of our last investigation support the data of the above-cited authors (34). The AA of all samples was higher in methanol extract than in ethyl acetate, probably showing that hydrophilic (polar) antioxidants were in the majority in the tested extracts. Other authors reported high AA in kiwifruit (30, 31, 35, 36). Fisk et al. (35) reported that the AA ranged from 1.6 to 2.3 ascorbic acid equivalents/g fresh weight with no significant difference between treatments. The obtained data in this investigation, using FTIR, and 3D-FL spectroscopy and ESI-MS showed that the composition of polyphenols, flavonoids, flavanols, and tannins was similar to other recently reported data (37). The obtained results of the used analytical methods can be applied to any food product as was shown in other reports (38–41).

Conclusions

The AA of the studied methanol and ethyl acetate extracted samples is significantly different ($P < 0.05$); according to the used tests AA was significantly lower ($P < 0.05$) in ethyl acetate and higher in methanol extract. Bidan cultivar showed higher bioactivity than Hayward. Significantly higher

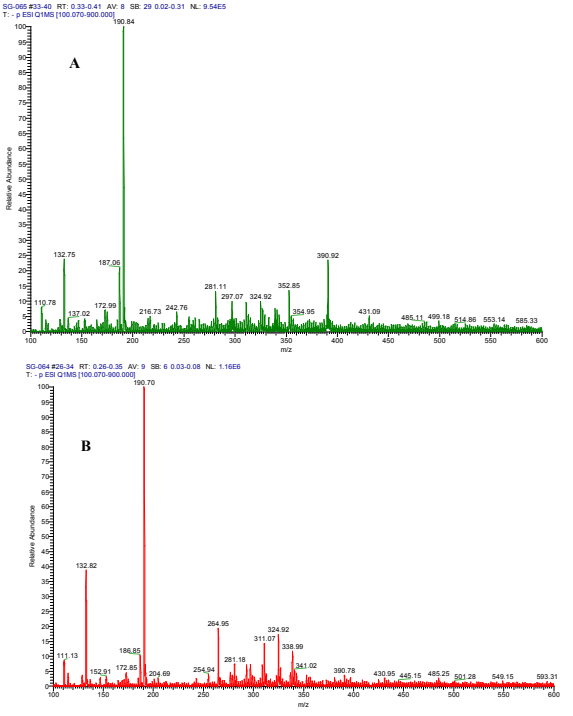

Figure 4. ESI-MS spectra of ethyl acetate fractions of (A) conventional kiwifruit and (B) organic kiwifruit in the negative ion mode.

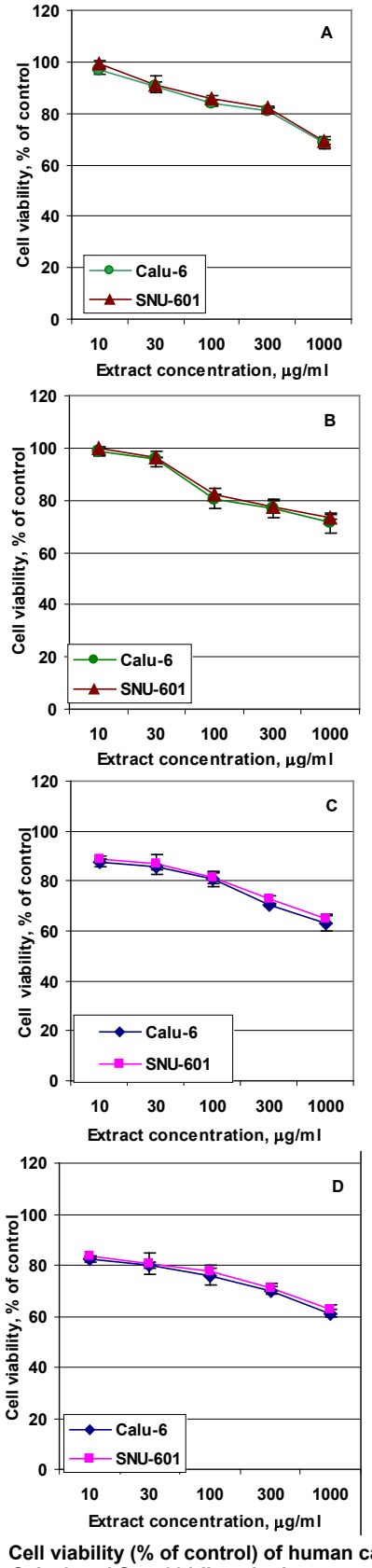

Figure 5. Cell viability (% of control) of human cancer cells of the Calu-6 and Snu-601 lines in the presence of methanol extracts: (A) conventional Hayward kiwifruit; (B) organic Hayward kiwifruit; (C) conventional Bidan kiwifruit; and (D) organic Bidan kiwifruit. Each point represents the mean ± SD (*n* = 6).

bioactivity was found in organic kiwifruits. The correlations between the polyphenolic compounds, and the antioxidant and antiproliferative activities were relatively high (R^2 from 0.82 to 0.99). Positive antioxidant and antiproliferative properties of kiwifruit justify the use of this fruit as a source of valuable antioxidants. The applied analytical methods can be widely used in any of the investigated food product as a rapid estimation of its quality, especially FTIR spectroscopy.

Acknowledgments

This research was partly supported by the Rural Development Administration (RDA), Korea. The authors are thankful to Elena Katrich (The Institute for Drug Research, School of Pharmacy, The Hebrew University, Hadassah Medical School, Jerusalem, Israel) for her technical assistance in determination of AA and fluorescence in the investigated plants.

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

References

(1) Palomo, G.I., Antonio Yuri, S.J., Moore-Carrasco, R., Quilodrán, P.A., & Neira, E.A. (2010) *Rev. Chilena Nutr*. **37**, 377–385

(2) Zhang, X., Shu, X.-O., Xiang, Y.B., Yang, G., Li, H., Gao, J., Cai, H., Gao, Y.T., & Zheng, W. (2011) *Am. J. Clin. Nutr*. **94**, 240–246. http://dx.doi.org/10.3945/ajcn.110.009340

(3) Dauchet, L., Amouyel, P., Hercberg, S., & Dallongeville, J. (2006) *J. Nutr*. **136**, 2588–2593

(4) Atkinson, R.G., & MacRae, E.A. (2007) *Biotechnol. Agric. Forest* **60**, 329–346. http://dx.doi.org/10.1007/978-3-540-49161-3-13

(5) Celik, A., Ercisli, S., & Turgut, N. (2007) *Int. J. Food Sci. Nutr*. **58**, 411–418. http://dx.doi.org/10.1080/09637480701252518

(6) Tavarini, S., Degl'Innocenti, E., Remorini, D., Massai, R., & Guidi, L. (2008) *Food Chem*. **107**, 282–288. http://dx.doi.org/10.1016/j.foodchem.2007.08.015

(7) Mohammadi, A., Rafiee, S., Keyhani, A., & Emam-Djomeh, Z. (2009) *Pakistan J. Nutr.* **8**, 78–82

(8) Du, G., Li, M., Ma, F., & Liang, D.(2009) *Food Chem*. **113**, 557–562. http://dx.doi.org/10.1016/j.foodchem.2008.08.025

(9) Duttaroy, A.K., & Jøorgensen, A. (2004) *Platelets* **15**, 287–292. http://dx.doi.org/10.1080/09537100410001710290

(10) Park, Y.S., Jung, S.T., Kang, S.G., Heo, B.G., Arancibia-Avila, P., Toledo, F., Drzewiecki, J., Namiesnik, J., & Gorinstein, S. (2008) *Food Chem*. **107**, 640–648. http://dx.doi.org/10.1016/j.foodchem.2007.08.070

(11) Fiorentino, A., D'Abrosca, B., Pacifico, S., Mastellone, S., Scognamiglio, M., & Monaco, P. (2009) *J. Agric. Food Chem*. **57**, 4148–4155. http://dx.doi.org/10.1021/jf900210z

(12) Ozgen, M., Reese, R.N., Tulio Jr, A.Z. , Scheerens, J.C., & Miller, A.R. (2006) *J. Agric. Food Chem*. **54**, 1151–1157. http://dx.doi.org/10.1021/jf051960d

(13) Singh, R.P., Chidamdara, M., & Jayaprakasha, G.K. (2002) *J. Agric. Food Chem*. **50**, 81–86. http://dx.doi.org/10.1021/jf010865b

(14) Apak, R., Guclu, K., Ozyurek, M., & Karademir, S.E. (2004) *J. Agric. Food Chem.* **52**, 7970–7981. http://dx.doi.org/10.1021/jf048741x

(15) Haruenkit, R., Poovarodom, S., Leontowicz, H., Leontowicz, M., Sajewicz, M., Kowalska, T., Delgado-Licon, E., Rocha-Guzman, N.E., Gallegos-Infante, J.-A., Trakhtenberg, S., &

Gorinstein, S. (2007) *J. Agric. Food Chem.* **55**, 5842–5849. http://dx.doi.org/10.1021/jf070475a

(16) Isabelle, M., Lee, B.L., Ong, C.N., Liu, X., & Huang, D. (2008) *J. Agric. Food Chem*. **56**, 9410–9416. http://dx.doi.org/10.1021/jf801527a

(17) Ozyurek, M., Guçlu, K., Bektasoglu, B., & Apak, R. (2007) *Anal. Chim. Acta* **588**, 88–95. http://dx.doi.org/10.1016/j.aca.2007.01.078

(18) Pierce, J.P., Stefanick, M.L., Flatt, S.W., Natarajan, L., Sternfeld, B., Madlensky, L., Al-Delaimy, W.K., Thomson, C.A., Kealey, S., Hajek, R., Parker, B.A., Newman, V.A., Cann, B., & Rock, C.L. (2007) *J. Clin. Oncol.* **25**, 2345–2351

(19) Agudo, A., Cabrera, L., Amiano, P., Ardanaz, E., Barricarte, A., Berenguer, T., Chirlaque, M.D., Dorronsoro, M., Jakszyn, P., Larrañaga, N., Martinez, C., Navarro, C., Quirós, J.R., Sanchez, M.J., Tormo, M.J., & González, C.A. (2007) *Am. J. Clin. Nutr*. **85**, 1634–1642

(20) Radhika, G., Sudha, V., Sathya, R.M., Ganesan, A., & Mohan, V. (2008) *Br. J. Nutr.* **99**, 398–405. http://dx.doi.org/10.1017/S0007114507803965

(21) 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) *Free Rad. Res.* **36**, 217–233. http://dx.doi.org/10.1080/10715760290006484

(22) Sun, J., Chu, Y.F., Wu, X.Z., & Liu, R.H. (2002) *J. Agric. Food Chem*. **50**, 7449–7454. http://dx.doi.org/10.1021/jf0207530

(23) Wang, S.Y., Chen, C.T., Sciarappa, W., Wang, C.Y., & Camp, M.J. (2008) *J. Agric. Food Chem*. **56**, 5788–5794. http://dx.doi.org/10.1021/jf703775r

(24) Juroszek, P., Lumpkin, H.M., Yang, R.Y., Ledesma, D.R., & Ma, C.H. (2009) *J. Agric. Food Chem.* **57**, 1188–1194. http://dx.doi.org/10.1021/jf801992s

(25) Kim, G.D., Lee,Y.S., Cho, J.Y., Lee, Y.H., Choi, K.J., Lee, Y., Han, T.H., Lee, S.H., Park, K.H., & Moon, J.H. (2010) *J. Agric. Food Chem*. **58**, 12300–12306. http://dx.doi.org/10.1021/jf1028448

(26) Lester, G.E., & Saftner, R.A. (2011) *J. Agric. Food Chem.* **59**, 401–406. http://dx.doi.org/10.1021/jf202385x

(27) Afshar-Mohammadian, M., & Rahimi-Koldeh, J. (2010) *Aust. J. Crop Sci*. **4**, 49–54

(28) Castaldo, D., Lo Voi, A.,Trifiro, A., & Gherardi, S. (1992) *J. Agric. Food Chem*. **40**, 594–598. http://dx.doi.org/10.1021/jf00016a013

(29) 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) *Food Chem. Toxicol.* **47**, 1884–1891. http://dx.doi.org/10.1016/j.fct.2009.04.047

(30) Tavarini, S., Degl'Innocenti, E., Remorini, D., Massai, R., & Guidi, L. (2008) *Food Chem*. **107**, 282–288. http://dx.doi.org/10.1016/j.foodchem.2007.08.015

(31) Jeong, C.H., Lee, W.J., Bae, S.H., & Choi, S.G. (2007) *Han'guk Sikp'um Yongyang Kwahak Hoechi* **36**, 859–865

(32) Amodio, M.L., Colelli, G., Hasey, J.K., & Kader, A.A. (2007) *J. Sci. Food Agric*. **87**, 1228–1236. http://dx.doi.org/10.1002/jsfa.2820

(33) Raigon, M.D., Rodriguez-Burruezo, A., & Prohens, J. (2010) *J. Agric. Food Chem*. **58**, 6833–6840. http://dx.doi.org/10.1021/jf904438n

(34) Park, Y.S., Leontowicz, H., Leontowicz, M., Namiesnik, J., Suhaj, M., Cvikrova, M., Mariticova, O., Weisz, M., & Gorinstein, S. (2011) *J. Food Comp. Anal*. **24**, 963–970. http://dx.doi.org/10.1016/j.jfca.2010.08.010

(35) Fisk, C.L., McDaniel, M.R., Strik, B.C., & Zhao, Y. (2006) *J. Food Sci*. **71**, S204-S210. http://dx.doi.org/10.1111/j.1365-2621.2006.tb15642.x

(36) Latocha, P., Krupa, T., Wołosiak, R., Worobiej, E., & Wilczak, J. (2010) *Int. J. Food Sci. Nutr*. **61**, 381–394. http://dx.doi.org/10.3109/09637480903517788

(37) Sarbu, C., Nascu-Briciu, R.D., Kot-Wasik, A., Gorinstein, S., Wasik, A., & Namiesnik, J. (2012) *Food Chem.* **130**, 994–1002. http://dx.doi.org/10.1016/j.foodchem.2011.07.120

(38) Simal-Gandara, J., Sarria-Vidal, M., & Rijk, R. (2000) *J. AOAC Int*. **83**, 311–319

(39) Krueger, D.A. (2012) *J. AOAC Int*. **95**, 163–168. http://dx.doi.org/10.5740/jaoacint.11-178

(40) Brown, P.N., & Shipley, P.R. (2011) *J. AOAC Int*. **94**, 459–466

(41) Krzyzanowska, J., Janda, B., Pecio, L., Stochmal, A., Oleszek, W., Czubacka, A., Przybys, M., & Doroszewska, T. (2011) *J. AOAC Int*. **94**, 43–50