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Organic and Conventional Kiwifruit, Myths versus Reality: Antioxidant, Antiproliferative, and Health Effects

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ABSTRACT: Comparison between organic and conventional kiwifruit cultivars 'Hayward' and 'Bidan', which was done by four radical scavenging assays, ESI-MS, and DSC measurements, showed significant differences between the cultivars. Such results were not estimated in kiwifruit growing under organic and conventional conditions. The extraction of bioactive compounds was done by two different methods: sequential extraction with ethyl acetate followed by methanol and maceration with methanol and ethyl acetate. The highest yield of polyphenols was found in the new cultivar 'Bidan' in comparison with the classic 'Hayward', by direct extraction with methanol. This is the first investigation of 'Bidan' kiwifruit cultivar, grown under organic conditions and compared with 'Hayward' organic. High contents of bioactive compounds and antioxidant and antiproliferative properties of the two kiwifruit cultivars justify their use as sources of valuable antioxidants. It is necessary to continue this study as a long-term experiment to eliminate the influence of seasonality.

KEYWORDS: *conventional and organic kiwifruit, bioactives, antioxidativity*

INTRODUCTION

Coronary artery disease is the most important adult health problem in the world. In this connection the antioxidant properties of kiwifruit have received attention in regard to their possible health-promoting effects.[1,2] There is a great variety of kiwifruit, which belongs to the genus *Actinidia* (*A. melanandra* and *A. arguta* var. *purpurea*) with different colors and compositions[3] and *Actinidia deliciosa*.[4−8] The kiwifruit cultivar *A. deliciosa* (A. Chev.) C.F. Liang and A.R. Ferguson var. *deliciosa* 'Hayward' is a commercially important cultivar, which has a relatively long storage life.[5] 'Bidan' is less consumed and belongs to the *A. deliciosa* species.[9]

Nowadays, there is interest in the possible differences between organically and conventionally grown fruits and vegetables.[10−12] Organic foods are thought to have higher antioxidant capacity, because this form of agricultural management could induce synthesis of secondary compounds such as polyphenols.[12] Some differences were shown between organic and conventionally produced sweet oranges of Valencia variety.[10] The agronomic environments in which tomatoes are cultivated potentially affect the levels of antioxidants and other metabolites in commercial products.[11] Whether or not all foods marketed to consumers as organic meet specified standards for the use of that descriptor, or are nutritionally different from conventional foods, is uncertain.[12−14]

The comparison between organic and conventional fruits is important, taking into consideration that, as mentioned above, few papers have studied this aspect. Therefore, we decided to compare the bioactive compounds of organic and conventional kiwifruit cultivars using different extraction procedures and recent analytical methods.[15−19] To obtain reliable data, four generally accepted antioxidant assays were used: ABTS, DPPH, FRAP, and CUPRAC. Additionally, ESI-MS and DSC measurements[20−22] were made, and antiproliferative properties were investigated.[23]

As far as we know no results of such investigations have been published.

MATERIALS AND METHODS

Chemicals. 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), tris(hydroxymethyl)aminomethane (Tris), 2,2-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin−Ciocalteu reagent (FCR), lanthanum(III) chloride heptahydrate, $FeCl_3 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$, and 2,9-dimethyl-1,10-phenanthroline (neocuproine) were purchased from Sigma Chemical Co., St. Louis, MO, USA. 2,4,6-Tripyridyl-*s*-triazine (TPTZ) was purchased from Fluka Chemie, Buchs, Switzerland. All reagents were of analytical grade. Deionized and distilled water was used throughout.

Samples. The samples of kiwifruit cultivars 'Hayward' and 'Bidan' at their maturity stage, according to the acidity, firmness, and soluble solid content, were grown in conventional and organic conditions in an

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orchard in Heanam County, Jeonnam province, Korea, 2011 (Figure 1). Chemical fertilizers, pesticides, and fungicides were regularly used in the kiwifruit orchard for growing conventional fruits. Under organic growing conditions, no chemical fertilizers were applied during cultivation; only organic fertilizers such as manure or humus were used. 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) 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 −80 °C until the bioactive substances were analyzed.

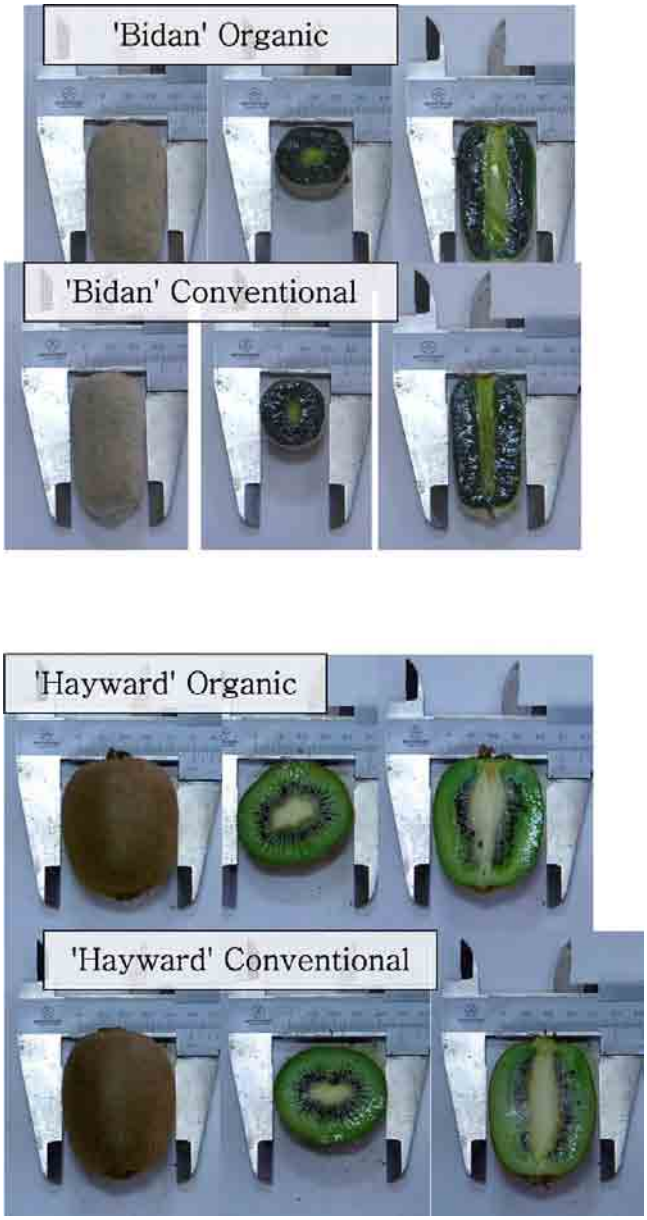

Figure 1. Two kiwifruit cultivars grown in Korea: 'Bidan' organic and conventional; 'Hayward' organic and conventional.

Extraction of Phenolic Compounds for Antioxidant Activities, Mass Spectrometry (MS), and Differential Scanning Calorimetry (DSC). The extracts from kiwifruit were prepared in the same way for all tests (bioactive compounds; antioxidant and anticancer assays). To find the best extraction of polyphenols two different methods were used: sequential extraction with ethyl acetate (and then with methanol) and maceration separately with methanol and ethyl acetate. The sequential extraction was done in the following way. For proanthocyanidin and flavonoid identifications, 1 g of the kiwifruit powder was extracted three times with 25 mL of acetone/water/acetic acid (AWA; 70:29.5:0.5) for 45, 45, and 30 min, respectively, using an orbital platform shaker at 300 rpm. The mixture was centrifuged, the supernatant was collected, and the acetone was 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 ESI-MS. For flavonoid identification and quantification, the slurry was diluted four times with methanol and filtered through a Sartorius Minisart 45 μm porosity and analyzed using ESI-MS.[19] Kiwifruit samples were extracted directly with methanol or ethyl acetate (concentration = 25 mg/mL) at room temperature twice for 3 h during maceration.

Determination of Bioactive Compounds and Antioxidant Activity. The polyphenols were determined by Folin−Ciocalteu method[24] with measurement at 750 nm with a spectrophotometer (Hewlett-Packard, model 8452A, Rockville, MD, USA). The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight (DW).

Total flavonoid content was determined by an aluminum chloride colorimetric method with some modifications.[25] The absorbance was measured immediately against the blank at 510 nm in comparison with the standards. The total flavanols amount was estimated using the *p*-dimethylaminocinnamaldehyde (DMACA) method, and then the absorbance at 640 nm was read. (+)-Catechin served as a standard for flavonoids and flavanols, and the results were expressed as catechin equivalents (CE).[15,25] The limit of concentration of catechin standard curves for flavonoids was from 10 to 100 μg/mL and for flavanols was from 0.6 to 25 μg/mL.

Determination of Antioxidant Activity (AA) [Micromoles of Trolox Equivalents (TE) per Gram of DW]. The AA was determined by four complementary assays: (1) The 2,2-azinobis(3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{\bullet+}$) 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.[17] The $ABTS^{\bullet+}$ 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) The ferric reducing/antioxidant power (FRAP) assay measures the ability of the antioxidants in the investigated samples to reduce ferric tripiridyltriazine (Fe^{3+}-TPTZ) to a ferrous form (Fe^{2+}). FRAP reagent (2.5 mL of a 10 mmol ferric tripiridyltriazine solution in 40 mmol of HCl plus 2.5 mL of 20 mmol of $FeCl_3 \cdot H_2O$ 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 kiwifruit samples as the appropriate reagent blank. The absorbance was measured at 595 nm.[18] (3) The cupric reducing antioxidant capacity (CUPRAC) 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 NH_4Ac buffer solution were added acidified and nonacidified water extracts (or standard) solution (x, in mL) and H_2O [$(1.1 - x)$ mL] to make the final volume of 4.1 mL. The absorbance at 450 nm was recorded against a reagent blank.[16] (4) Scavenging free radical potentials were tested in a 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 compound. DPPH solution (3.9 mL, 25 mg/L) in methanol was mixed with the sample extracts (0.1 mL), and then the reaction progress was monitored at 515 nm until the absorbance was stable.[17]

MS Analysis. A TSQ Quantum Access Max (Thermo Fisher Scientific, Basel, Switzerland) mass spectrometer was used. Analytes were ionized by electrospray ionoization (ESI) in negative mode. The 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; skimmer offset, 0 V.

Table 1. Bioactive Compounds (per Gram Dry Weight) in Fractions Obtained by Sequential Extraction with EtOAc and MeOH and in Extracts by Maceration with EtOAc and MeOH of Conventionally and Organically Grown Kiwifruit Cultivars[a]

	indices			
extracts and fractions of kiwifruit cultivars[b]	POL, mg GAE	FLAVAN, μg CE	TAN, mg CE	FLA, mg CE
KHaC, EtOAc extract	1.10 ± 0.1 e	25.47 ± 2.3 c	2.69 ± 0.3 d	8.48 ± 0.9 b
KHaO, EtOAc extract	1.27 ± 0.2 e	21.23 ± 2.5 cd	2.72 ± 0.3 d	10.80 ± 1.1 b
KBiC, EtOAc extract	2.26 ± 0.2 d	93.41 ± 8.5 b	8.00 ± 0.9 b	67.39 ± 4.6 a
KBiO, EtOAc extract	3.80 ± 0.4 d	97.66 ± 9.3 b	10.80 ± 1.2 b	66.38 ± 4.7 a
KHaC, EtOAc fraction	0.73 ± 0.1 f	4.63 ± 0.3 e	0.18 ± 0.1 f	1.64 ± 0.3 cd
KHaO, EtOAc fraction	0.75 ± 0.2 f	3.84 ± 0.5 e	0.22 ± 0.3 f	1.31 ± 0.6 d
KBiC, EtOAc fraction	0.83 ± 0.2 f	16.98 ± 1.5 d	0.42 ± 0.3 e	8.54 ± 0.7 b
KBiO, EtOAc fraction	0.89 ± 0.4 f	16.49 ± 1.3 d	0.38 ± 0.1 e	8.72 ± 0.7 b
KHaC, MeOH extract	7.08 ± 0.8 c	142.67 ± 9.1 a	4.48 ± 0.8 c	1.87 ± 0.4 c
KHaO, MeOH extract	7.98 ± 0.7 c	127.00 ± 7.1 ab	5.12 ± 0.9 c	1.48 ± 0.2 cd
KBiC, MeOH extract	64.40 ± 5.6 a	28.03 ± 2.8 c	19.07 ± 1.8 a	2.13 ± 0.2 c
KBiO, MeOH extract	60.86 ± 4.8 a	29.72 ± 2.7 c	17.87 ± 1.7 a	2.05 ± 0.3 c
KHaC, MeOH fraction	1.96 ± 0.2 e	31.85 ± 3.9 c	0.26 ± 0.1 f	0.03 ± 0.01 e
KHaO, MeOH fraction	1.81 ± 0.3 e	43.06 ± 4.3 bc	0.31 ± 0.1 f	0.03 ± 0.01 e
KBiC, MeOH fraction	18.02 ± 1.6 b	4.46 ± 0.7 e	1.92 ± 0.3 de	0.04 ± 0.03 e
KBiO, MeOH fraction	18.87 ± 1.7 b	4.35 ± 0.5 e	1.52 ± 0.4 de	0.02 ± 0.01 e

[a]Values are the mean ± SD of five measurements. Values in columns for every bioactive compound with different letters are significantly different ($P < 0.05$). [b]Abbreviations: KHaC, kiwifruit 'Hayward' conventional; KHaO, kiwifruit 'Hayward' organic; KBiC, kiwifruit 'Bidan' conventional; KBiO, kiwifruit 'Bidan' organic; POL, polyphenols; CE, catechin equivalent; GAE, gallic acid equivalent; FLAVAN, flavanols, TAN, tannins, FLA, flavonoids. Two extraction methods were used: sequential separation with EtOAc (EtOAc fraction), followed by MeOH (MeOH fraction) and maceration by direct extraction with MeOH (MeOH extract) and EtOAc (EtOAc extract).

DSC Measurements. The extent of the stability of extracted polyphenol fractions in different kiwifruit samples was estimated on a differential scanning calorimeter (DSC1, STAR System, Mettler Toledo). Portions (4−6 mg) of lyophilized kiwifruit samples were transferred into preweighed aluminum pans and sealed. An empty pan was used as the reference. A number of unique temperatures such as onset (T_i), left limit, right limit, and T peak (T_p, °C) and endothermal enthalpy changes (ΔH, J/g) were computed from the thermograms. The equipment was previously calibrated with standard reference of indium.[20−22]

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide (MTT) Assay. The antiproliferative activity of methanol extracts of the studied kiwifruit samples on human cancer cell lines (Calu-6 for human pulmonary carcinoma and SNU-601 for human gastric carcinoma) was measured using the MTT assay. The cell lines were purchased from Korean Cell Line Bank (KCLB) for MTT assay. Cells were grown in 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 concentrations at 10, 30, 100, 300, and 1000 μg/mL. Stock solutions of test samples were prepared for cell lines of 90 μL of medium and 10 μL of samples and incubated for 72 h. MTT solution at 5 mg/mL was dissolved in 1 mL of phosphate buffer solution (PBS), and 10 μL of it 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 of DMSO was added to each well. The plates were then shaken, and the optical density was recorded using a microplate reader at 540 nm. The cytotoxicity was obtained by comparing the absorbance between the samples and the control.[23]

Statistical Analyses. To verify the statistical significance, means ± SD of five independent measurements were calculated. Differences between groups were tested by two-way ANOVA. In the assessment of the antioxidant activity, Spearman correlation coefficients (R) were used. Linear regressions were also calculated. P values of <0.05 were considered to be significant.

RESULTS

The use of analytical and antioxidant assays and ESI-MS and DSC measurements for bioactive determination could characterize and distinguish the two kiwifruit cultivars.

Polyphenols, Flavonoids, Flavanols, Tannins, and Antioxidant Activity. The bioactive compounds and antioxidant activities in samples of conventionally and organically grown kiwifruits by two different extractions (maceration and sequential extraction with the same solvents methanol and ethyl acetate) are shown in Tables 1 and 2. The contents of polyphenols, flavonoids, and tannins and levels of antioxidant activities in ethyl acetate extracts (Table 1) and ethyl acetate fractions (Tables 1 and 2) in KHaC, KHaO, KBiC, and KBiO did not differ significantly. The same relationship was found in methanol extracts and in methanol fractions in the studied samples. In 'Bidan' cultivar all of the studied indices were significantly higher than in 'Hayward'. In methanol extracts and fractions of both organically and conventionally grown cultivars ($P < 0.05$) the amounts of studied compounds were higher than in the ethyl acetate extract and its fractions.

Mass Spectra. The spectra show that in methanol (Figure 2A) and ethyl acetate fractions obtained by sequential fractionation (Figure 2A, inset) of conventional kiwifruit 'Hayward' were found six main m/z peaks. In methanol (Figure 2B) and ethyl acetate fractions (Figure 2B, inset) of organic kiwifruit 'Hayward' were found approximately the same number of m/z peaks. The main peaks for conventional and organic 'Hayward' kiwifruit, using two different extraction procedures, were the following: 4-hydroxybenzoic acid at 133 with relative abundance (RA, %) of HaCMeOH, HaOMeOH, HaCEtOAc, and HaOEtOAc of 11, 18, 25 and 40, respectively; at 191 with RA of 100% for all samples; and chlorogenic acid at 353 with RA of

Table 2. Antioxidant Activities (Micromoles of Trolox Equivalents per Gram Dry Weight) Obtained in Fractions by Sequential Extraction with EtOAc and MeOH and Extraction with Maceration by EtOAc and MeOH of Conventionally and Organically Grown Kiwifruit Cultivars[a]

	indices			
fractions and extracts of kiwifruit cultivars[b]	FRAP	DPPH	CUPRAC	ABTS
KHaC, EtOAc extract	0.71 ± 0.1 f	1.25 ± 0.4 f	6.23 ± 0.8 f	2.07 ± 0.6 df
KHaO, EtOAc extract	0.61 ± 0.1 f	1.32 ± 0.5 f	7.95 ± 0.9 ef	2.05 ± 0.4 df
KBiC, EtOAc extract	2.83 ± 0.3 d	11.38 ± 1.4 d	13.61 ± 1.2 e	4.69 ± 0.3 e
KBiO, EtOAc extract	3.00 ± 0.4 d	11.38 ± 1.4 d	18.62 ± 1.8 de	7.65 ± 0.8 de
KHaC, EtOAc fraction	0.67 ± 0.1 f	0.57 ± 0.1 g	2.53 ± 0.4 g	0.41 ± 0.1 g
KHaO, EtOAc fraction	0.79 ± 0.1 f	0.78 ± 0.2 g	2.69 ± 0.3 g	0.38 ± 0.1 g
KBiC, EtOAc fraction	0.68 ± 0.2 f	0.79 ± 0.2 g	2.71 ± 0.2 g	0.72 ± 0.3 f
KBiO, EtOAc fraction	0.75 ± 0.3 f	0.75 ± 0.1 g	3.18 ± 0.8 fg	0.84 ± 0.3 f
KHaC, MeOH extract	11.00 ± 1.2 c	9.38 ± 0.9 de	28.44 ± 2.9 d	17.14 ± 1.8 d
KHaO, MeOH extract	11.24 ± 1.1 c	12.08 ± 1.1 d	36.70 ± 3.6 c	16.08 ± 1.6 d
KBiC, MeOH extract	94.08 ± 8.7 a	86.21 ± 8.7 a	180.28 ± 12.5 a	81.87 ± 7.6 a
KBiO, MeOH extract	82.04 ± 8.2 b	76.39 ± 7.6 b	158.19 ± 10.2 b	70.67 ± 6.7 b
KHaC, MeOH fraction	2.03 ± 0.3 e	3.36 ± 0.4 e	3.71 ± 0.6 fg	5.36 ± 0.8 e
KHaO, MeOH fraction	1.86 ± 0.2 e	3.76 ± 0.3 e	3.75 ± 0.5 fg	4.54 ± 0.6 e
KBiC, MeOH fraction	27.79 ± 2.9 c	21.46 ± 2.7 c	31.09 ± 3.3 d	31.34 ± 3.5 c
KBiO, MeOH fraction	31.22 ± 3.2 c	26.69 ± 2.6 c	32.35 ± 3.1 d	30.25 ± 3.1 c

[a]Values are the mean ± SD of five measurements. Values in columns for every antioxidant activity bearing different letters are significantly different ($P < 0.05$). [b]Abbreviations: ABTS, 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; CUPRAC, cupric reducing antioxidant capacity; FRAP, ferric reducing/antioxidant power; DPPH, 1,1-diphenyl-2-picrylhydrazyl method; KHaC, kiwifruit 'Hayward' conventional; KHaO, kiwifruit 'Hayward' organic; KBiC, kiwifruit 'Bidan' conventional; KBiO, kiwifruit 'Bidan' organic. Two extraction methods were used: sequential separation with EtOAc (EtOAc fraction), followed by MeOH (MeOH fraction), and maceration by direct extraction with MeOH (MeOH extract) and EtOAc (EtOAc extract).

44, 41, and 12% for HaCMeOH, HaOMeOH, and HaCEtOAc, respectively. Equal m/z peaks appeared for HaOEtOAc at 338 (11%); hydroxy-FA-hexoside at 371 with RA of 15% was seen in HaCMeOH and HaOMeOH. The spectrum shows that in the methanol fraction of conventional kiwifruit 'Bidan' (Figure 3A) and organic kiwifruit (Figure 3B) were found the same number of m/z peaks.

The insets of the ethyl acetate fraction of both conventional and organic 'Bidan' showed higher numbers of peaks than the methanol ones. The main peaks for conventional and organic 'Bidan' kiwifruit were the following: 4-hydroxybenzoic acid at 133 with **RA, %,** of BiCMeOH, BiOMeOH, BiCEtOAc, and BiOEtOAc being 11, 10, 19 and 18, respectively; at 191 with RA of 100% for all samples; at 311 with RA of 12 and 10%; at 391 with RA of 21 and 30%, for both MeOH fractions of 'Bidan' conventional and organic, respectively. In general, no significant changes were found in organic and conventional kiwifruit samples.

DSC Measurements. The DSC profiles of the kiwifruit samples fractionated and extracted with two solvents, methanol and ethyl acetate, from two different cultivars are shown in Figure 4. It is apparent that the three ethyl acetate treated fractions showed multiple peaks in DSC profiles (Figure 4Ab,Ac,Ad), whereas the other samples from both methanol and ethyl acetate extracts and methanol fraction showed only a single peak (Figure 4B,C). The polyphenolic mixture with high molecular mass, fractionated with ethyl acetate, showed for most samples two or three peaks: for KHaC (Figure 4Ab, two temperature peaks of T_p = 132.45 °C with T_i = 129.71 °C and T_p = 140 °C); for KBiO (Figure 4Ac, two temperature peaks of T_p = 114.57 °C with T_i = 99.71 °C and T_p = 137 °C); for KBiC (Figure 4Ad, three temperature peaks of T_p = 112.83 °C with T_i = 99.14 °C, T_p = 123 °C, and T_p = 140 °C). Only the ethyl acetate fraction of KHaO (Figure 4Aa) showed one peak at 135.32 °C with T_i = 125.20 °C. In most of the DSC profiles only one peak appeared and showed a unique population of molecules with a defined thermal stability. The multiple peaks indicate that there is more than one fraction of phenolics in terms of their thermal transitional properties. These results are in full correspondence with others.[20−22] The DSC profile of the KBiC (Figure 4Ad) sample has three peaks with T_p, where the first and third peaks are similar to that of KBiO (Figure 4Ac). The other two peaks occurred at slightly higher temperatures for two extracts, KHaO and KHaC (Figure 4Aa,Ab). The samples of kiwifruit 'Hayward' showed narrow profiles. In contrast, the ethyl acetate fractions showed broad profiles. The kiwifruit 'Hayward' and 'Bidan' methanol extracts showed only one temperature peak in all samples with similar values for KHaO and KHaC of 125.16 °C (T_i = 116.34 °C) and 125.57 °C (T_i = 105.70 °C) (Figure 4Ba,Bb). The methanol fractions of organic and conventional 'Bidan' (Figure 4Bc,Bd) exhibited also only one peak (Table 3) with similar values of T_p = 105.61 °C with T_i = 87.69 °C and T_p = 103.50 °C with T_i = 89.47 °C. The methanol extracts of four samples showed only one peak (Figure 4C). The T_p for 'Hayward' conventional was higher by about 5 °C than for organic (Figure 4Ca,Cb; and T_i = 91.31 and 85.85 °C, respectively). The T_p for 'Bidan' conventional was nearly equal to that for 'Bidan' organic (difference of about 1.1 °C, T_i = 84.52 and 81.98 °C for Figure 4Cc,Cd, respectively). The width of DSC peaks is an indication of the sharpness of the thermal transition; therefore, a broader peak is an indication of a broader distribution of phenolic molecules having different thermal stabilities. The broadest peaks were found in the ethyl acetate fraction of 'Bidan' conventional (Figure 4Ad) and in the methanol fraction of 'Hayward' conventional (Figure 4Bb).

Inhibition of Poliferation. It was observed that the percentages of proliferation of the methanol extracts of conventional and organic kiwifruit samples on two cell lines Calu-6 for human pulmonary carcinoma human gastric

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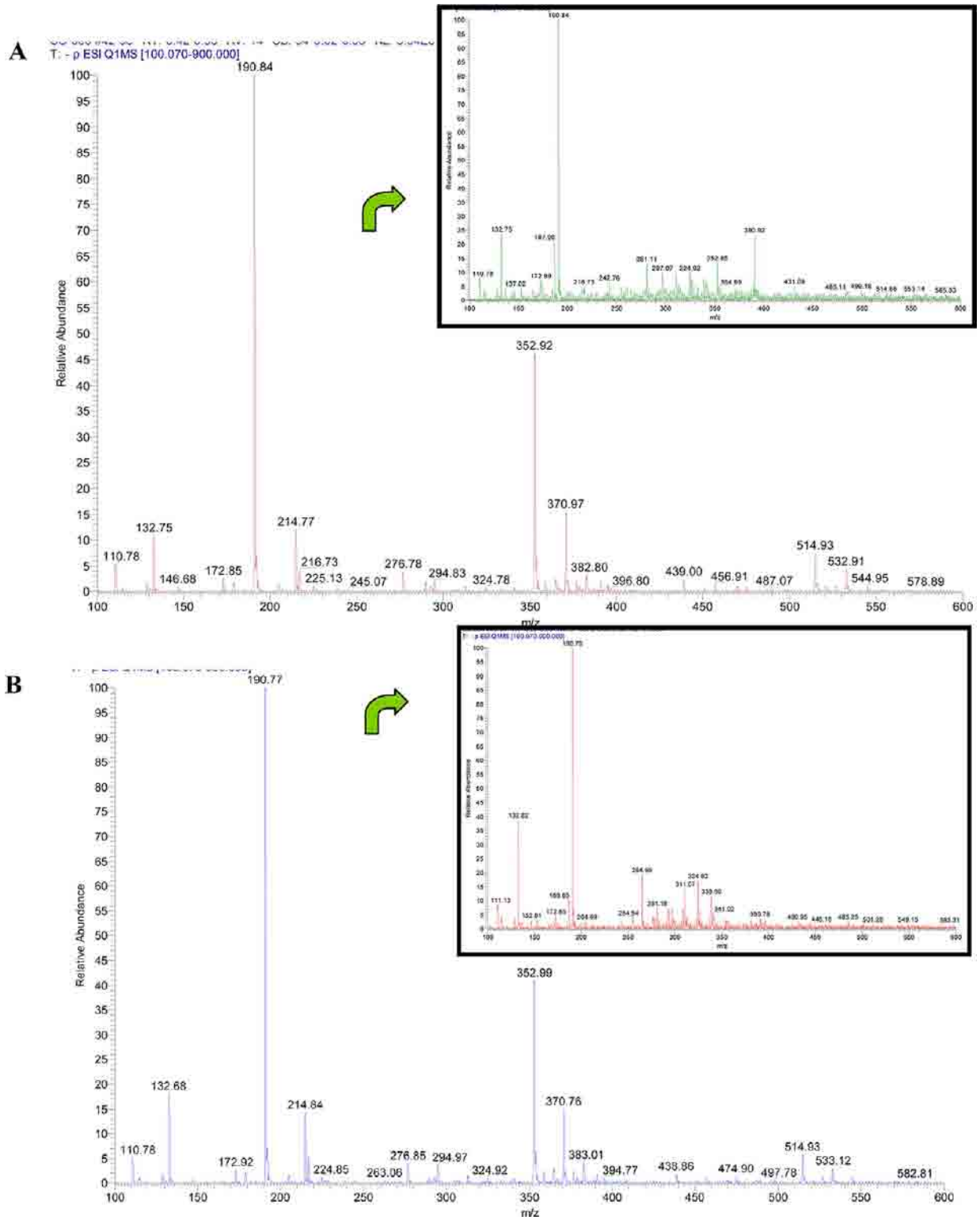

Figure 2. ESI-MS spectra of (A) methanol fraction of kiwifruit 'Hayward' conventional (inset, ethyl acetate fraction of kiwifruit 'Hayward' conventional) and (B) methanol fraction of kiwifruit 'Hayward' organic (inset, ethyl acetate fraction of kiwifruit 'Hayward' organic in negative ion mode).

carcinoma were different. The proliferation (%) for concentrations of 1000 μg/mL (Figure 5) for organic 'Hayward' kiwifruit on Calu-6 was 54.54% and that on SNU-601 was 60.08%, showing higher antiproliferation activity in comparison with conventional sample for Calu-6 (56.23%) and SNU-601 (61.56%). The proliferation (%) for concentrations of 1000 μg/mL (Figure 5) for organic 'Bidan' kiwifruit on Calu-6 was 48.84% and that on SNU-601 was 53.36%, showing lower antiproliferation activity in comparison with conventional sample for Calu-6 (47.25%) and SNU-601 (52.28%). Our investigation shows that the antioxidant activity of the studied samples highly correlated with their antiproliferation activity. Significantly different values between the organic and conventional samples were not found.

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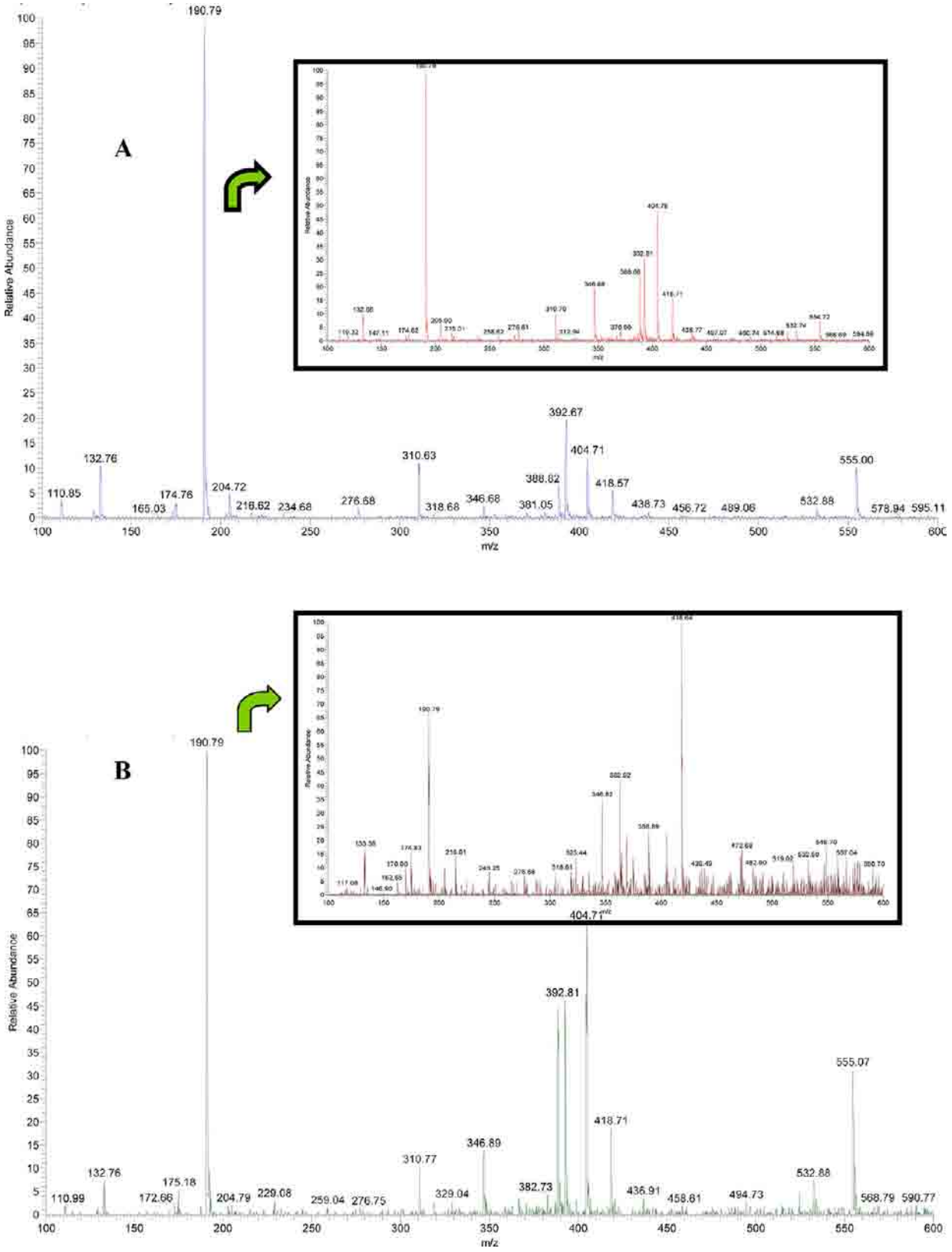

Figure 3. ESI-MS spectra of (A) methanol fraction of kiwifruit 'Bidan' conventional (inset, ethyl acetate fraction of kiwifruit 'Bidan' conventional) and (B) methanol fraction of kiwifruit 'Bidan' organic (inset, ethyl acetate fraction of kiwifruit 'Bidan' organic in negative ion mode).

DISCUSSION

As mentioned in the Introduction an interest has been shown by the scientific community concerning organically versus conventionally grown fruits and vegetables.[10−14] Many scientists believe that organically grown produce is healthier (i.e., has a higher content of health-promoting bioactivities) than conventionally grown produce of the same type.[14] The obtained and cited data are highly variable. The numbers of papers that describe a health

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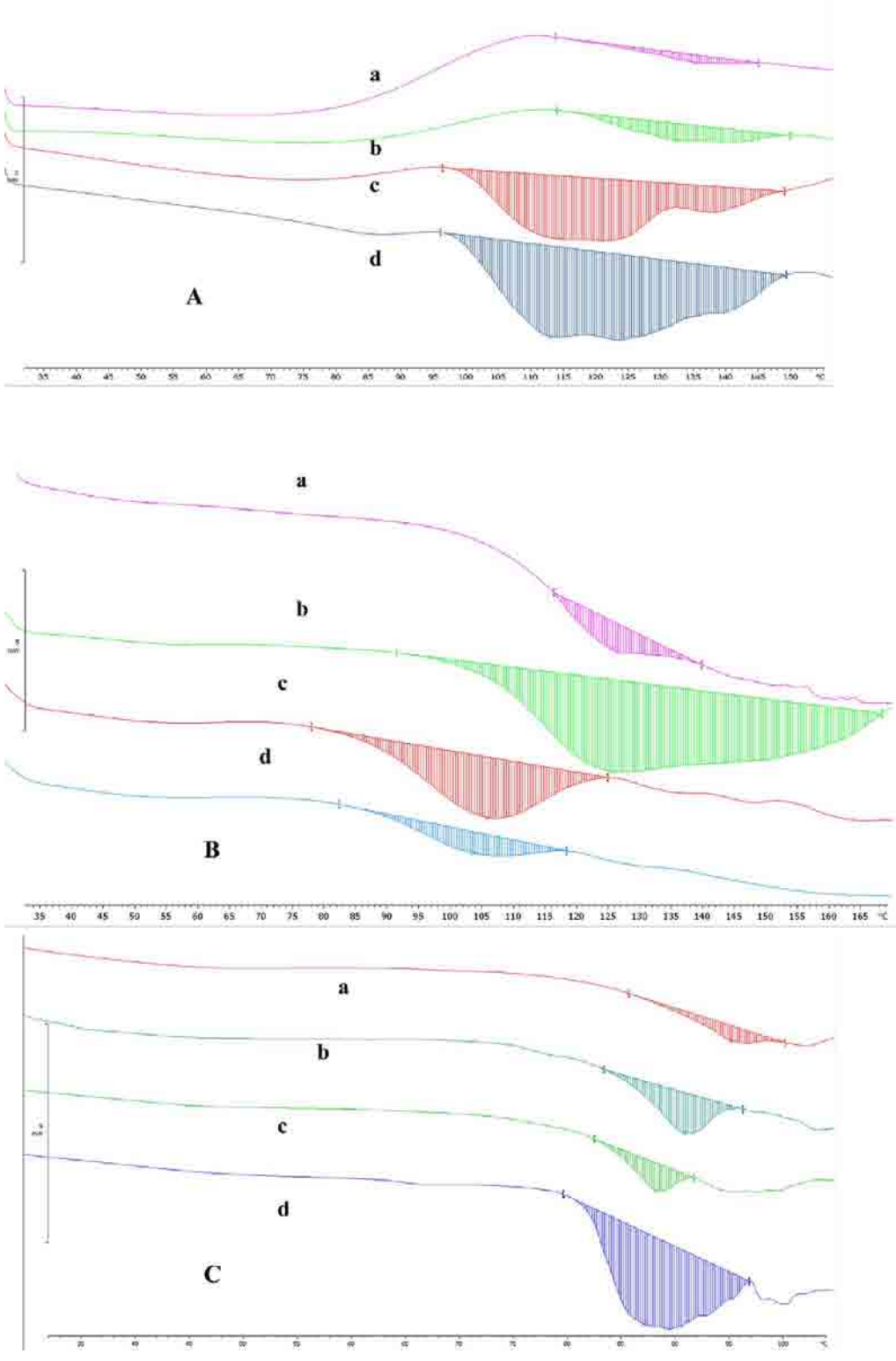

Figure 4. DSC curves of (A) ethyl acetate fractions of kiwifruit samples [KHaO (a), KHaC (b), KBiO (c), KBiC (d)]; (B) methanol fractions [KHaO (a), KHaC (b), KBiO (c), KBiC (d)]; and (C) methanol extracts [KHaC (a), KHaO (b), KBiC (c), KBiO (d)]. The pans were heated in the calorimeter at 10 °C min over the range of 25−200 °C. Abbreviations: KHaC, kiwifruit 'Hayward' conventional; KHaO, kiwifruit 'Hayward' organic; KBiC, kiwifruit 'Bidan' conventional; KBiO, kiwifruit 'Bidan' organic.

benefit of organic food[11] are about equal to the number that show no difference in benefit or composition between organic and conventional food. It is still difficult to choose the control of variables: ripeness or maturity of the plants, irrigation, mineral content of the soil, weather conditions, and other indices.[14]

There have been some studies showing a higher level of bioactive compounds in organic fruits compared with conventional fruits, but not all studies have been consistent in this respect. Therefore, the main purpose of our research was to compare two different kiwifruit cultivars grown under organic and conventional

Table 3. Thermodynamic Properties of Ethyl Acetate and Methanol Fractions and Methanol Extracts of Conventionally and Organically Grown Kiwifruit Cultivars[a]

kiwifruit fractions and extracts[b]	*T* peaks, °C	−(H fusion), J/g
KHaC, EtOAc fraction	132.5/140 ± 7.4/12.6	5.2 ± 0.9
KHaO, EtOAc fraction	135.3 ± 9.1	1.8 ± 0.1
KBiC, EtOAc fraction	112.8/123/140 ± 9.9/7.9	50.6 ± 5.3
KBiO, EtOAc fraction	114.6/137 ± 4.8/11.3	28.4 ± 2.5
KHaC, MeOH fraction	125.6 ± 12.1	94.3 ± 8.3
KHaO, MeOH fraction	125.2 ± 10.3	6.7 ± 3.8
KBiC, MeOH fraction	103.5 ± 8.1	10.0 ± 6.6
KBiO, MeOH fraction	105.6 ± 8.1	33.3 ± 3.3
KHaC, MeOH extract	95.2 ± 6.8	1.8 ± 0.4
KHaO, MeOH extract	90.8 ± 8.7	4.0 ± 0.5
KBiC, MeOH extract	88.2 ± 8.3	2.6 ± 0.4
KBiO, MeOH extract	87.1 ± 7.7	13.6 ± 1.4

[a]Values are the mean ± SD of five measurements. [b]Abbreviations: KHaC, kiwifruit 'Hayward' conventional; KHaO, kiwifruit 'Hayward' organic; KBiC, kiwifruit 'Bidan' conventional; KBiO, kiwifruit 'Bidan' organic. Two extraction methods were used: sequential separation with EtOAc (EtOAc fraction), followed by MeOH (MeOH fraction), and maceration by direct extraction with MeOH (MeOH extract).

technologies. At the time of the harvest the fruits were at the same maturity according to the main indices ('Bidan', 25 g each fruit, acid level = 0.5%, firmness = 18 N, soluble solid compounds (SSC) = 7%; 'Hayward', 90 g each fruit, acid level = 0.29%, firmness = 42 N, SSC = 7.8%). Color did not change during maturation, so we did not analyze flesh color in 'Bidan' and 'Hayward'. This analysis in the case of gold kiwifruit is important, but it is not the main index for the studied cultivars. Cultivars and culture systems are known to affect the rate of maturation in many fruits. As was mentioned in the description of the organically grown investigated samples no spraying of pesticides and fungicides in the orchard was performed. In this case the possibility of pathogen infection was higher in organic than in conventional kiwifruit. To protect against pathogen infection, kiwifruit increases the amount of phenolic compounds produced as defense compounds. The higher bioactivity in organic kiwifruit is closely related to the content of phenolic compounds.

In this experiment five fruits from five trees from one orchard were collected. There is only one orchard in Jeonnam province, Korea, where kiwifruit is grown under an organic system. Therefore, it was not possible to collect the kiwifruit samples from other orchards. The analyzed data of bioactive compounds would have significance among orchards due to different growing conditions and cultural practices (different shapes of the trees, manure content, irrigation, and other factors). It was only possible to compare the fruits by the year of harvesting. There was no rainfall during the summer season in 2011. Long sunshine duration and low relative humidity could reduce the rate of disease occurrence during the growing season. As mentioned, the rate of disease occurrence can affect the synthesis of phenolic compound as a defense mechanism.

Epidemiological studies and laboratory experiments have shown that fruit and vegetable consumption has protective effects against coronary artery disease (CAD),[1,2] and it does not seem to be important if the fruits were grown under different conditions. Regular consumption of kiwifruit exerts beneficial effects on the antioxidative status and the risk factors for CAD in hyperlipidemic subjects.[2] The antioxidant capacity of kiwifruit was discussed in the context of biologically relevant in vitro assays for predicting antioxidant activity. The ability of kiwifruit to protect cells from dying after exposure to an oxidative insult by hydrogen peroxide (cytoprotection) was studied as well[23] and is in agreement with our data. Some recent results are included, where extracts from 20 kiwifruit genotypes were compared for their cellular antioxidant activity and cytoprotection, using human gut-derived epithelial cell lines.[1] Our results can be compared with others,[8] where the effects of polyphenol-rich methanolic extracts from 13 fruit species on the proliferation of MCF-7 human mammary cancer cells were studied. The inhibitory effects of the fruit extracts on cancer cell proliferation were measured by the MTT assay as was done in this study. Kiwifruit showed the average inhibitory activity compared to the other fruits tested such as Chiku and dragon fruit, as well as the amount of polyphenols. The amount of polyphenols in DMSO kiwifruit extract was 50 mg GAE/100 g DW and of flavonoids, 7 mg quercetin equivalent (QE)/100 g DW. The percent of cell viability of kiwifruit extract at 600 μg/mL fruit extract was about 74. Thus, fruit phytochemicals can inhibit cell proliferation. Anthocyanins of most *Actinidia* species are usually conjugated

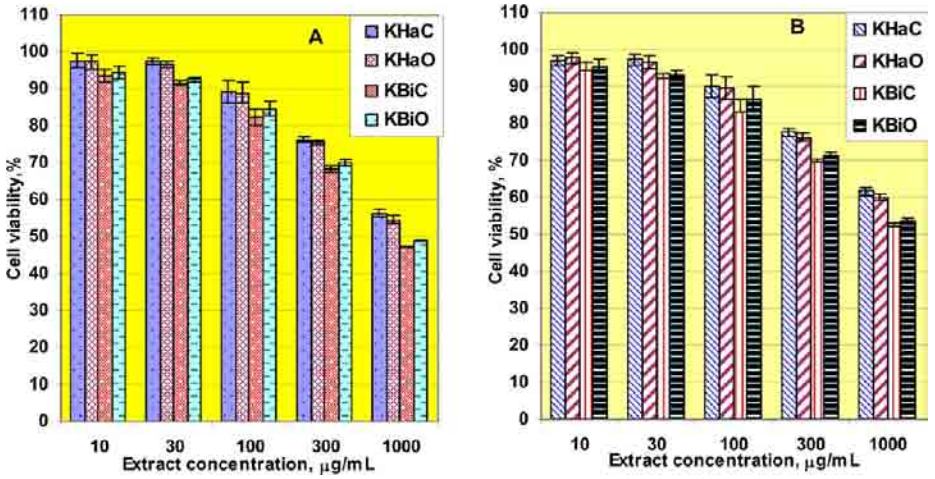

Figure 5. Cell viability (% of control) of human cancer cells of the Calu-6 (A) and Snu-601 (B) lines of methanol kiwifruit extracts. Each bar represents the mean ± SD (n = 6). Abbreviations: KHaC, conventional 'Hayward'; KHaO, organic 'Hayward'; KBiC, conventional 'Bidan'; KBiO, organic 'Bidan'.

with either xylosylgalactose or galactose, whereas *A. deliciosa* anthocyanins are conjugated with glucose and galactose.[3]

Our results can be compared with others, where the ascorbic acid was about 16.47 mg/100 mL; DPPH, 10 mM TE/mL; and ABTS, 1.2 mM TE/mL.[5] The antioxidant potency and total phenolic and flavonoid contents of kiwifruit *A. deliciosa* in vitro by analyzing the radical-scavenging activity of lyophilized water extract from kiwifruit (LEK) for ABTS and DPPH have similar values.[6] The kiwifruit showed the following values of antioxidants activities for FRAP and DPPH of 1620 and 735 mg TE/100 g DW, respectively, and total phenolic acids of 550 mg GAE/100 g DW. Polyphenol content and antioxidant capacity varied among organic and conventional vegetables with no prevalence from either agricultural type. The organic practices result in different effect patterns according to the plant species analyzed, with fruits being more susceptible to the induction of polyphenol synthesis, and the greatest accumulation of polyphenols in external plant tissues. Our results are in correspondence with other studies,[11] where the organic agriculture results in food products with similar or slightly higher polyphenol content and antioxidant capacity. Our results were different compared with Stracke et al.,[13] who found that the polyphenol and antioxidant contents of Golden Delicious apples were significantly higher in the organic apples, and this effect was consistent over a multiyear period. Organic cultivation was found to provide tomatoes and tomato-derived products with a significantly higher content of antioxidant microconstituents, whereas glutamylphenylalanine and *N*-malonyltryptophan were detected only in conventional ketchups.[11] The antioxidant capacity of conventional grape skin extracts of Riesling *Vitis vinifera* L.[26] was significantly higher, according to the higher contents in catechin, epicatechin, and procyanidin B. Pesticide loads did not affect the antimutagenic or antimicrobial properties of the extracts. The levels of total phenolics in sweet peppers grown organically and conventionally as well depend on soil and climate conditions in a greenhouse under the same soil and climate conditions,[27,28] and increasing availability of plant-available nitrogen reduces the accumulation of defense-related secondary metabolites and vitamin C, whereas the contents of secondary metabolites such as carotenes that are not involved in defense against diseases and pests may increase.[25] The data obtained in this investigation showed that the composition of polyphenols, flavonoids, flavanols, and tannins was similar to other recently reported data.[29−32] Also, other authors found amounts of phenolics (mg GAE/g DW) in 'Hayward' kiwifruit similar to our data: 2.19,[30] 2.94,[31] and 3.5.[32]

Our results obtained from DSC measurements support the previous data on polyphenols and antioxidant activities and can be compared with others,[20] where extracted raw suberin showed two endothermic peaks at approximately 45 and 59 °C, suggesting that the native raw suberin is a mixture of at least two different types of compounds. In another study the thermodynamic properties of chitosan have been developed for the controlled release of polyphenolic antioxidants such as catechin.[21] DSC data obtained from the fractionated and extracted polyphenols support the results from two extracted procedures: in the case of ethyl acetate fractions two and three resolved endothermic peaks were detected, suggesting that these samples are a mixture of at least two different types of substances such as polyphenols and lipids. In the case of methanol fractions and extracts phenolics, sugars, and acids were resolved in a single peak. The highest temperature peak was observed in the ethyl acetate fraction of KBiC and the lowest in the methanol fraction of KBiO. Ethyl acetate and methanol fractions and methanol extracts of the organic and conventional kiwifruit 'Hayward' cultivar showed endothermic peaks at the same temperatures with 3- and 14-fold more heat, which was required to soften the conventional sample relative to the organic sample. In the case of methanol extract 2-fold more heat was required for the organic extract. 'Bidan' organic and conventional showed the same temperatures with the same numbers of peaks for ethyl acetate fractions and their extracts. The enthalpy of transition was different as for 'Hayward' (1.8-fold more heat required for conventional fruit). The difference was in methanol fractions and methanol extracts of conventional 'Bidan', where 3.3- and 5.2-fold more heat were required for organic 'Bidan'. Our results are in agreement with others,[20] that the difference in thermal behaviors of the phenolic components clearly points toward the success of the extraction and fractionation procedures because in the case of the phenolic extracts with methanol only one clear band was detected.

Our obtained results are in agreement with others[14] that more studies of this type are needed to underpin a better understanding of how production methods influence the phytonutrient content of food crops. More information would be required to understand isotopic variations and fractionation effects between vegetables and soil over time as the technique does not discriminate organic from conventional regimens.[33]

In conclusion, the antioxidant activity of the studied methanol and ethyl acetate extracted samples is significantly different between cultivars ($P < 0.05$): it was significantly minimal ($P < 0.05$) in ethyl acetate, and the highest was in methanol extract. 'Bidan' cultivar showed higher bioactivity than 'Hayward'. Such differences were not found between the organic and conventional fruits in the recent harvest. The correlations between the polyphenol compounds, thermodynamic properties, and antioxidant and antiproliferative activities were relatively high. The use of analytical and antioxidant assays and ESI-MS and DSC measurements for bioactive determination could characterize and distinguish the two kiwifruit cultivars. The antioxidant and antiproliferative properties of kiwifruit justify its use as a source of valuable antioxidants. It would be necessary to continue this study as a long-term experiment to eliminate the influence of seasonality.

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Notes

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