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The comparative characteristics of snake and kiwi fruits

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ABSTRACT

In the time of globalization many of the tropical fruits can be find at the markets of Europe and North America. Most customers are not familiar with the nutritional and proliferative values of these fruits. Therefore, a less known snake fruit was compared with better known kiwi fruit, using fluorometry, FT-IR spectroscopy, several radical scavenging and proliferative assays and statistical evaluation.

It was found similarity between snake fruit (cultivar Sumalee) and kiwi fruit (cultivar Hayward) in the contents of polyphenols (8.15–7.91, mg GAE g^{-1} DW), antioxidant values by DPPH (11.28–10.24, μMTE g^{-1} DW), and antiproliferative activities on both human cancer cell lines (Calu-6 for human pulmonary carcinoma, and SMU-601 for human gastric carcinoma, 90.5–87.6 and 89.3–87.1%, cell survival, respectively).

In conclusion, snake fruit cultivar Sumalee is comparable with kiwi fruit cultivar Hayward. Two fruits can be used as supplements to the normal diet. Consumption of a combination of both fruits could be recommended in order to receive the best results.

1. Introduction

Nowadays a variety of tropical fruits are common ingredients of different diets in Europe and North America (Kondo et al., 2005; Luximon-Ramma et al., 2003; Murcia et al., 2001; Yuka et al., 2003). The high nutritional value of the subtropical and tropical fruits led to significant increase in their consumption. However, most customers are not familiar with the nutritional values of these fruits. Therefore, in this investigation a less known and less investigated snake fruit (*Salacca edulis* Reinw, cultivar Sumalee) was compared with the better known kiwi fruit (*Actinidia chinensis*, cultivar Hayward) and the main nutritional components and the antioxidant and proliferative potentials of both fruits were determined and compared.

In order to receive reliable results it was decided to use snake and kiwi fruits of the same ripeness and to determine their antioxidant potentials by four different assays: 1. FRAP, ferric-reducing/antioxidant power assay (Ozgen et al., 2006). 2. ABTS−+, 2,2-azino-bis (3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt assay (Ozgen et al., 2006); 3. DPPH, 1,1-diphenyl-2-picrylhydrazyl method (Ozgen et al., 2006); and 4. CUPRAC, cupric reducing antioxidant capacity assay (Apak et al., 2004). The role of ascorbic acid in the total antioxidant potentials (AP) of fruits is controversial. Some authors claim that the AP of fruits might be attributed mainly to the content of phenols (Wang et al., 1996; Rapisarda et al., 1999), and the contribution of ascorbic acid to the total AP is less than 15% (Wang et al., 1996). On the other hand, there are investigators who claim that ascorbic acid plays a major role in the total AP (Vinson et al., 2002). It was decided to determine the content of ascorbic acid in the studied samples and its contribution to AP.

As far as we know there are no published results of such investigations.

2. Materials and methods

2.1. Chemicals

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent (FCR), lanthanum (III) chloride heptahydrate,

Abbreviations: Kiwi OHE, kiwi fruit organic Hayward ethylene treated; Kiwi CHE, kiwi fruit conventional Hayward ethylene treated.

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$FeCl_3 \times 6H_2O$, $CuCl_2 \times 2H_2O$, 2,9-dimethyl-1, 10-phenanthroline (neocuproine), butylated hydroxyanisole (BHA), were purchased from Sigma Chemical Co., St. Louis, MO, USA. 2,4,6-Tripyridyl-*s*-triazine (TPTZ) was purchased from Fluka Chemie, Buchs, Switzerland. All reagents were of analytical grade. Deionized and distilled water was used throughout.

2.2. Samples and preparation

Snake fruit (*S. edulis* Reinw) is originated from Southeast Asia. The fruit is egg like in shape and the skin of the mature fruit is brown. The fruit has pineapple-, pear-, and banana-like aroma. The weight is up to 70 g at the last maturation stage. The fruit contains three pieces of seeds covered with white flesh. Most of this fruit is freshly consumed and some are processed into fruit juice, canned fruit or jam.

The kiwi fruit (*A. chinensis*) is native to the Yangtze River valley of northern China and Zhejiang Province on the coast of eastern China. When sliced, the fruit yields an attractive emerald green flesh with rows of small, dark, edible seeds, and a light cream colored center. Its flavor is similar to a blend between strawberry and pineapple.

Hayward cultivars of kiwi fruits (conventional and organic) at their commercial maturity stage were harvested in the orchard (Heanam County, Jeonnam province, Korea, 2008).

The kiwi fruit samples [organic 'Hayward' ethylene treated (OHE) and conventional 'Hayward' ethylene treated (CHE)] were treated with 100 ppm of ethylene for 24 h at 20° C in a growth chamber (Percival Scientific Inc., Perry, IA, USA). The samples were put into an 18 l glass jar and ventilated with humidified flow of ethylene at 300 ml min^{-1}. Then the ethylene-treated kiwi fruits were ripened at 20° C in the same growth chamber for 10 days (Park et al., 2008).

Two cultivars of snake fruits Noen Wong (old variety, 2006) and Sumalee (new variety, 2008) were sampled from orchard (Muang district, Chantaburi province, Thailand).

All fruits were cleaned with tap water and dried, using five replicates of five fruits each. The edible parts of the above-mentioned fruits were prepared for this investigation 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 −20° C until the bioactive substances were analyzed.

2.3. Determination of bioactive compounds, antioxidant potentials, basic nutritional compounds, minerals and trace elements

The bioactivity in snake and kiwi fruits such as antioxidant potentials, dietary fibers, proteins, fats, carbohydrates, minerals and trace elements determined by a Perkin–Elmer 5100 ZL atomic absorption spectrometer [Perkin–Elmer Ltd., Beaconsfield, Buckinghamshire, England, using the flame and flameless method] were determined as previously described (Leontowicz et al., 2006, 2007; Haruenkit et al., 2007; Gorinstein et al., 2009).

2.4. Determination of the contents of the main bioactive compounds, Fourier transform infrared (FT-IR) spectra of polyphenols and fluorometry

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

Fluorescence measurements were done using a model FP-6500, Jasco Spectrofluorometer, serial N261332, Japan. Fluorescence emission spectra for all fruit samples at a concentration of 0.25 mg/ml were taken at emission wavelength (nm) of 330, and recorded from wavelength of 265 to a wavelength of 310 nm, at emission wavelengths of 685 nm from 300 to 750 nm; and at excitation of 350 nm from 370 to 650 nm. Standards of quercetin of 0.01 mM in methanol were used.

Phenols were extracted from lyophilized fruits with methanol (concentration 25 mg/ml) at room temperature twice during 3 h and then were determined by Folin-Ciocalteu method with measurement at 750 nm with spectrophotometer (Hewlett–Packard, model 8452A, Rockvile, USA). The results were expressed as mg of gallic acid equivalents (GAE) per g DW. Phenols were extracted as well in the same concentration with ethanol and water.

Flavonoids, extracted with 5% $NaNO_2$, 10% $AlCl_3 \times 6H_2O$ and 1 M NaOH, were measured at 510 nm. The total flavanols were estimated using the *p*-dimethylaminocinnamaldehyde (DMACA) method, and then the absorbance at 640 nm was read. The extracts of condensed tannins (procyanidins) with 4% methanol vanillin solution were measured at 500 nm. (+)-Catechin served as a standard for flavonoids, flavanols, and tannins, and the results were expressed as catechin equivalents (CE). Total ascorbic acid was determined by CUPRAC assay (Ozyurek et al., 2007). Water extract was prepared from 100 mg of lyophilized sample and 5 ml of water. This extract (1 ml) was mixed with 2 ml of 3.0×10^{-3} M of lanthanum (III) chloride heptahydrate. Ethylacetate (EtAc) was used for extraction of flavonoids in order to avoid the interference. One millilitre of Cu(II)–neocuproine (Nc), in ammonium acetate-containing medium at pH 7, added to 1 ml of the obtained extract. The absorbance of the formed bis (Nc)-copper(I) chelate was measured at 450 nm.

2.5. Determination of the antioxidant potentials

The following antioxidant assays were used: (1) ferric-reducing/antioxidant power (FRAP) assay measures the ability of the antioxidants contained in the samples to reduce ferric tripyridyltriazine (Fe^{3+}-TPTZ) to a ferrous form (Fe^{2+}) which absorbs light at 593 nm. The antioxidant activity was determined at constant concentration and also with different concentrations of the fruits from 5 to 25 mg/ml; (2) 2,2-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{\cdot+}$) was generated by the interaction of ABTS (mmol/l) and $K_2S_2O_8$ (2.45 mmol/l). This solution was diluted with methanol until the absorbance reached 0.7 at 734 nm; (3) 1-diphenyl-2-picrylhydrazyl method (DPPH) solution (3.9 ml, 25 mg/l) in methanol was mixed with the samples extracts (0.1 ml). The reaction progress was monitored at 515 nm until the absorbance was stable; (4). Cupric reducing antioxidant capacity (CUPRAC) is based on utilizing the copper(II)–neocuproine [Cu(II)–Nc] reagent as the chromogenic oxidizing agent. To the mixture of 1 ml of Cu(II), Nc, and NH_4Ac buffer solution, extract of durian sample (or standard) solution (x ml) and H_2O [$(1.1 - x)$ ml] were added to make the final volume of 4.1 ml. The absorbance at 450 nm was recorded against a reagent blank.

2.6. Chemometrical processing

Samples with different portion of fruit extracts (5, 10, 15, 20 and 25 mg/ml) were analyzed by FRAP antioxidant activity assay. The data set consisted of a 150×3 matrix in which rows represented the different extracts of fruit species and columns the three FRAP variables (several readings of absorbance A1–A3 and Trolox equivalent values). Basic chemometric characterization of the investigated fruit extracts according to their ability to reduce the Fe^{3+} ions was carried out by descriptive (normal probability, box/whisker and dot plots) and multivariate (principal component, factor and discriminant analysis) statistics. All statistical procedures were realized by statistical programme Unistat® (Unistat, London, United Kingdom) and basic descriptive statistics by Microsoft® Office Excel 2003.

2.7. Determination of the antiproliferative activity

Anticancer activity of methanol extracts of the studied plants on human cancer cell lines (Calu-6 for human pulmonary carcinoma and SNU-601 for human gastric carcinoma) were measured using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The cell lines (suspended) 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. Cells cultured were harvested, counted (3×10^4 cells/ml), and transferred into a 96-well plate, and incubated for 24 h prior to the addition of fruit ethanol, methanol and water extracts. Serial dilutions of the extracts were prepared by dissolving compounds in dimethyl sulfoxide (DMSO) followed by dilution with RPMI-1640 medium to give final concentration at 125, 250, 500, 1000, and 2000 μg ml^{-1}.

To give final concentration of extracts from 125 to 2000 μg ml^{-1}, solutions were prepared for cell lines at 90 μl and samples (plant extracts) at 10 μl, and incubated for 72 h. MTT solution at 5 mg/ml^{-1} 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 were removed by suction, and then 150 μl of DMSO was added to each well. Final concentration of DMSO was 10%. The plates were then shaken and optical density was recorded using a microplate reader at 540 nm. Distilled water was used as positive control and DMSO as solvent control. The effect of the fruit extract on the proliferation of cancer and normal cells was expressed as cell viability: percent viability = OD of fruit extract of treated sample/OD of none treated sample) × 100, where OD is an optical density (Boivin et al., 2008; Chon et al., 2009). Three different extracts were used (methanol, ethanol and water).

2.8. Statistical methods

The results of the investigation are mean ± SD of five measurements. Where it was appropriate, differences between groups were tested by two-way analysis of variance (ANOVA). The *P*-values of < 0.05 were considered significant.

3. Results

3.1. Nutrients in soil, minerals and trace elements and basic nutritional compounds

The soil where the investigated fruits were grown was a sandy loam with the following data: pH 5.4, EC 296 μS cm^{-1}, organic

matter 3.7%, available *P* (Bray II) 1400 mg kg^{-1}, exchangeable (NH_4OAc) K, Ca and Mg 105, 1773 and 75.2 mg kg^{-1}, respectively, extractable (DTPA) Fe, Mn, Cu and Zn 104, 14.9, 15.8 and 7.2 mg kg^{-1}, respectively.

The main minerals and trace elements (Table 1) were in the acceptable range determined of these fruits. As can be seen, the contents of the studied minerals are higher in kiwi fruit, particular in the samples of organic (OHE), but only the following minerals such as for P, K, Ca, Mn and B the significance is shown ($P < 0.05$). The content of the studied minerals are higher in Kiwi OHE than in Kiwi CHE, but significantly only for Fe and S ($P < 0.05$). The potassium/magnesium ratio for snake fruit was from 18.68 to 21.10 and for kiwi fruit: 20.16–22.41; and the magnesium/calcium ratio for snake fruit was from 2.76 to 1.98 and for kiwi fruit −0.57 to −0.50.

As can be seen, total dietary fibers, crude protein, lipids, carbohydrates and water-soluble pectin were significantly higher in kiwi than in snake fruits (Table 2, $P < 0.05$), and all studied indices, excluding WSP, were significantly higher in Kiwi OHE than in Kiwi CHE ($P < 0.05$).

3.2. Bioactive compounds

The wavelengths numbers of FT-IR spectra for catechin at 827, 1039, 1115, 1143, 1286, 1478, 1511 and 1610 cm^{-1} were assigned to C–H alkenes, –C–O alcohols, C–OH alcohols, –OH aromatic, C–O alcohols, C–H alkanes, C=C aromatic ring, and C=C alkenes. Gallic acid showed the following wavenumbers (cm^{-1}) of 866, 1026, 1238, 1450, 1542 and 1618. Snake fruit sample (line C, Fig. 1) in the region of polyphenols showed slightly different bands than the standards. The three lines [C– for snake fruit, A and B – for organic (Kiwi OHE) and for conventional (Kiwi CHE) kiwi fruits] showed the following peaks in the region of polyphenols: 772.4, 818.7, 867.9, 922.9, 1056.1, 1105.4, 1137.2 and 1238.6 cm^{-1}. Between 1337 and 1455.7 cm^{-1} were shown five peaks. For snake fruit additional band (nm) was at 992.4, at 772.4 and 818.7; and at 1238.6 and 1745.3 the peaks were small or not detected.

Fluorimetric measurements showed (Fig. 2A–E) the following peaks (nm) with the absorbance units (AU): A, snake fruit with emission of 685 nm and the spectra (290–400 nm) with a peak of 343 nm and AU of 240.142; conventional (Kiwi CHE) and organic (Kiwi OHE) kiwi fruits at 344 nm and AU of 249.11. The organic at the same wavelength as conventional was with slightly higher AU of 259.99. Catechin of 0.001 mM as a standard for flavonoids was measured at all the following conditions and showed the same peak as the fruit extracts with at 343.5 nm and with a higher absorbance of 666.83. At excitation wavelength of 350 nm (Fig. 2C) the following peaks appeared: the first peak for kiwi fruit organic, conventional and snake fruit was the same at 391 nm, with slightly different AU of 78.49, 75.06 and 60.44 (position C). A shift appeared in the second peak: organic at 427 nm with 26.13; conventional at 430 and with 68.31 and snake fruit at 437 with 53.96. Catechin showed one peak at 390.5 with AU of 35.09. At emission of 330 nm (Fig. 2E) the recording was for snake fruit, Kiwi CHE and Kiwi OHE with the shift of the main peak and was detected at 280.5 with 255.66; 281 with 251.60; and 285 with 249.52. The second peak was measured exactly at the same wavelength of 300 nm with 149.29, 127.15 and 118.49 for snake fruit, Kiwi CHE and Kiwi OHE, respectively. Catechin showed one peak at 282 with AU of 700.39. At emission of 450 nm for Kiwi OHE and Kiwi CHE peak at 345 with 62.03 and for snake fruit at 340.5 with 55.92 and at 285 with 25.81 (Fig. 2D) with catechin at 286 with 9.72. Fig. 2B at emission of 740 nm shows the following data: for Kiwi OHE and Kiwi CHE one peak exactly at 372 with 739.5 and 739.2, the second peak was at 739.5 nm and 739.2 with 46.94 and 43.14. Catechin showed two peaks at 371.5 and 739.5 with 590.86 and 311.90.

Phenolic compounds in snake and kiwi fruit extracts were in the range from 8.15 ± 0.81 to 5.62 ± 0.65 mg GAE/g DW and flavonoids from 3.33 ± 0.31 to 1.68 ± 0.38 mg CE/g DW, respectively (Table 3).

3.3. Antioxidant potentials

The antioxidant potentials by ABTS, DPPH, and CUPRAC assays in snake and kiwi fruits were in the range from 13.81 to 20.99; 8.42 to 11.28; and from 16.41 to 27.42 μM TE/g, respectively (Table 3). As can be seen, among the studied indices CUPRAC, ABTS, flavonoids (FL), flavanols (FLAV) and tannins were significant higher in methanol extract of snake fruit Sumalee that in snake fruit Noen Wong and kiwi fruit ($P < 0.05$). The studied indices of CUPRAC, FL and FLAV were significantly higher in Kiwi OHE than in Kiwi CHE ($P < 0.05$).

Three different extracts were compared on the basis of polyphenol and DPPH values (Table 4). The studied indices are higher in snake fruit Sumalee that in snake fruit Noen Wong. However, the differences were significant only in the contents of polyphenols in water and polyphenols and DPPH values in methanol extracts ($P < 0.05$). The contents of the studied indices were higher in Kiwi OHE than in Kiwi CHE. However, the differences were not significant ($P > 0.05$) in the cases of DPPH in ethanol extract. Most studied indices were higher in snake extracts, but not significant, excluding DPPH in water ($P < 0.05$).

3.3.1. Descriptive statistics

The pattern recognition techniques were used in order to evaluate the possibility of differentiating the fruit extracts according to

Table 1
Mineral content (mg kg^{-1} DW) of snake and kiwi fruits cultivars.

Indices	Snake Sumalee	Snake Noen W	Kiwi OHE	Kiwi CHE
P	1161 ± 51^b	896 ± 45^a	2640 ± 111^c	2498 ± 111^c
K	$11{,}339 \pm 509^a$	$11{,}963 \pm 509^a$	$17{,}418 \pm 639^b$	$16{,}987 \pm 619^b$
Ca	220 ± 9^a	287 ± 12^a	1512 ± 15^b	1488 ± 15^b
Mg	607 ± 31^a	567 ± 25^a	864 ± 41^b	758 ± 37^b
Na	231 ± 11^a	220 ± 11^a	242 ± 12^a	227 ± 11^a
Fe	12.0 ± 0.5^a	12.9 ± 0.6^a	16.1 ± 0.8^b	11.2 ± 0.4^a
Mn	10.4 ± 0.3^a	27.8 ± 1.3^c	22.4 ± 1.2^b	19.7 ± 0.9^b
Cu	3.36 ± 0.2^c	1.48 ± 0.1^b	1.14 ± 0.1^a	0.99 ± 0.1^a
Zn	10.4 ± 0.3^a	11.4 ± 0.4^a	10.8 ± 0.3^a	10.4 ± 0.3^a
B	5.07 ± 0.2^a	3.23 ± 0.2^a	6.11 ± 0.3^b	5.73 ± 0.3^b
S	139 ± 5.2^a	176 ± 5.9^b	164 ± 5.7^b	143 ± 5.3^a

Values are mean ± SD of five measurements. Means in rows with different superscript letters are significantly different ($P < 0.05$).
Abbreviations: W, Wong; Kiwi OHE, kiwifruit organic Hayward ethylene treated; Kiwi CHE, kiwifruit conventional Hayward ethylene treated.

Table 2
Some nutritional indices (mg/g DW) in snake and kiwifruit cultivars.

Indices	Snake Sumalee	Snake Noen W	Kiwi OHE	Kiwi CHE
TDF	71.3 ± 0.4^b	69.8 ± 0.3^a	84.5 ± 0.4^b	80.9 ± 0.3^a
IDF	48.5 ± 0.5^c	49.7 ± 0.4^c	54.9 ± 0.3^b	56.6 ± 0.2^b
SDF	22.8 ± 0.1^a	20.1 ± 0.1^a	29.6 ± 0.2^b	24.3 ± 0.1^a
CP	33.17 ± 0.2^a	30.91 ± 0.2^a	47.85 ± 0.2^a	41.69 ± 0.2^a
CL	41.25 ± 0.4^c	39.18 ± 0.3^b	45.63 ± 0.4^c	43.15 ± 0.2^a
Carb	711.32 ± 0.6^b	684.15 ± 0.5^a	989.11 ± 0.6^b	876.18 ± 0.4^a
WSP	13.91 ± 0.6^b	10.12 ± 0.5^a	18.24 ± 0.6^b	16.49 ± 0.4^a

Values are mean ± SD of five measurements. Means in rows with different superscript letters are significantly different ($P < 0.05$).
Abbreviations: TDF, total dietary fiber; IDF, insoluble dietary fiber; SDF, soluble dietary fiber; CP, crude protein; CL, crude lipids; Carb, carbohydrates; WSP, water-soluble pectin; W, Wong; DW, dry weight; Kiwi OHE, kiwifruit organic Hayward ethylene treated; Kiwi CHE, kiwifruit conventional Hayward ethylene treated.

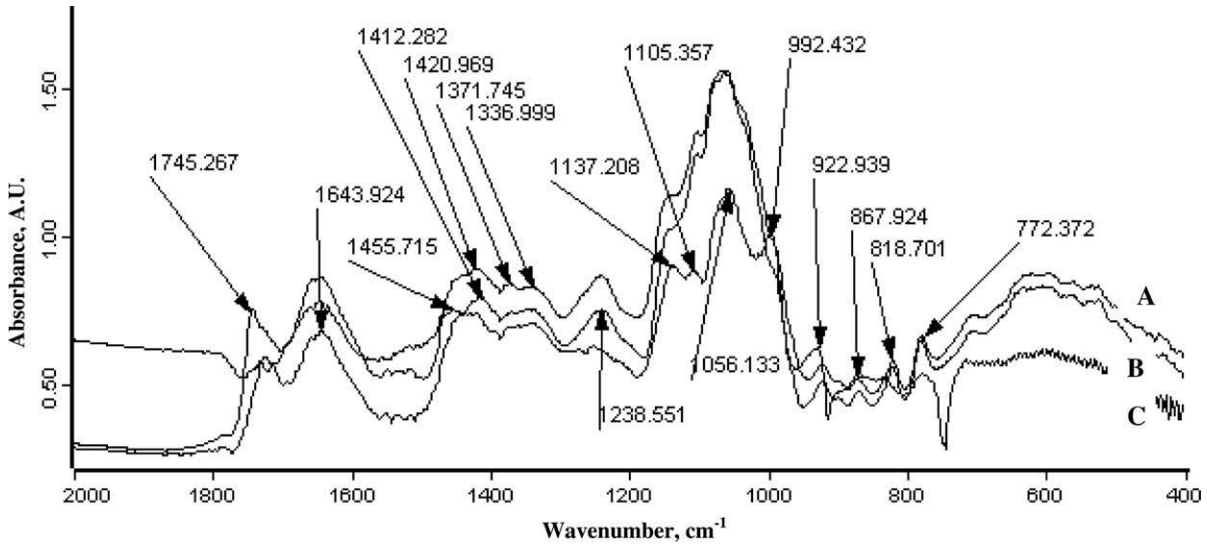

Fig. 1. FT-IR spectra of shake fruit (line C), organic (line A) and conventional (line B) kiwifruit.

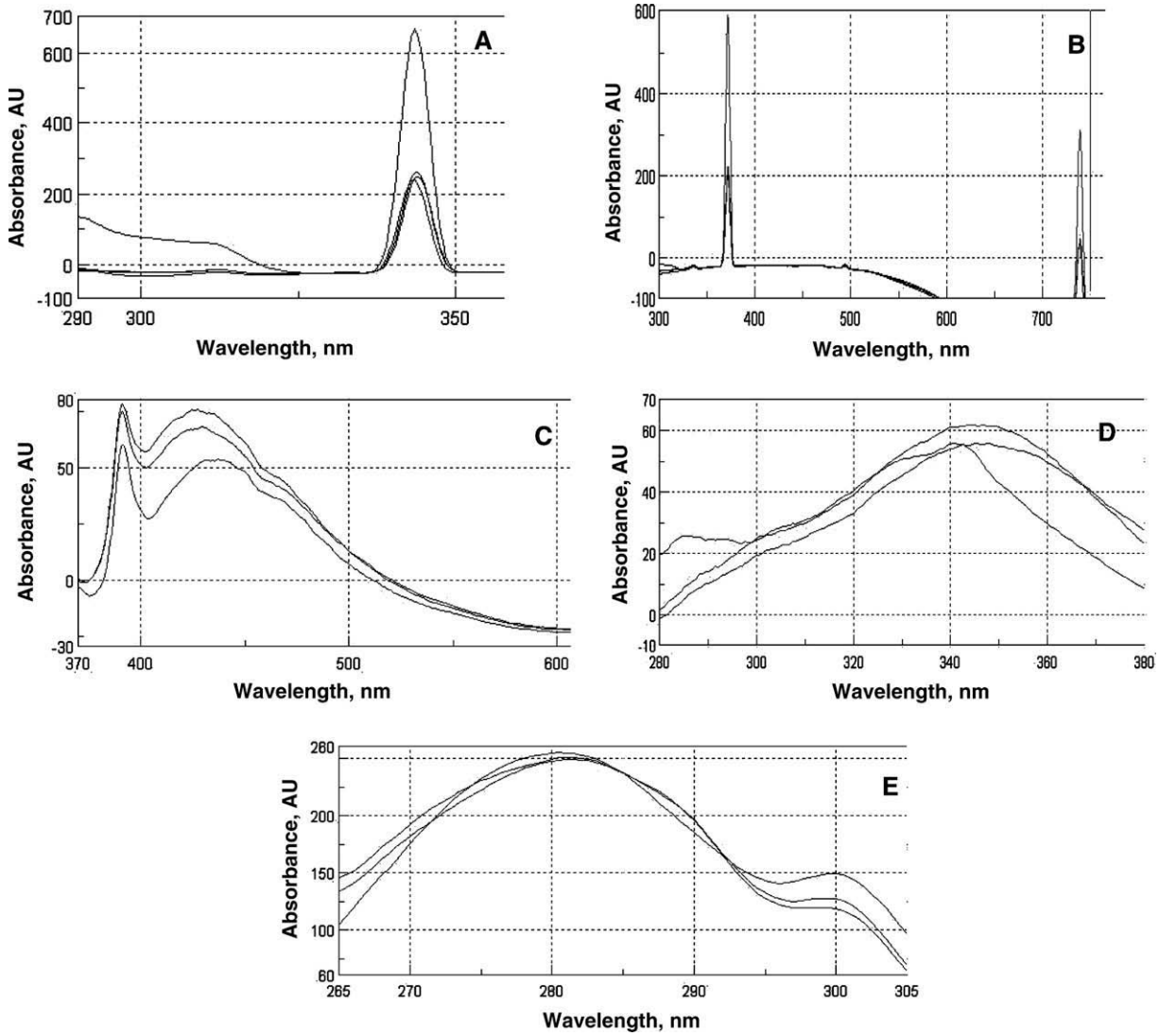

Fig. 2. Fluorimetric excitation spectra of fruit extracts (0.25 mg/ml) with: (A) emission wavelength at: 685 nm recorded over the frequency range from the excitation wavelength from 290 to a wavelength of 400 nm from upper to lower lines: catechin, organic and conventional kiwifruit, snake fruit; (B) emission at 740 nm and recording from 300 to 750 nm; (C) excitation at 350 nm and recording from 370 to 650 nm; (D) emission at 450 nm and recording from 280 to 380 nm; (E) emission at 330 nm and the recording from 265 to 305 nm.

Table 3
Bioactive compounds and antioxidant activity in methanol extracts of snake and kiwifruit cultivars.

Indices/g DW	Snake S	Snake NW	Kiwi OHE	Kiwi CHE
POL, mg GAE	8.15 ± 0.4[b]	7.08 ± 0.3[a]	7.91 ± 0.4[b]	5.62 ± 0.2[a]
CUPRAC, μMTE	27.42 ± 1.5[c]	21.15 ± 1.3[b]	23.04 ± 1.4[b]	16.41 ± 0.8[a]
ABTS, μMTE	20.99 ± 0.9[c]	17.41 ± 0.8[b]	14.36 ± 0.7[a]	13.81 ± 0.6[a]
DPPH, μMTE	11.28 ± 0.5[a]	9.40 ± 0.4[a]	10.24 ± 0.5[a]	8.42 ± 0.4[a]
FL, mg CE	3.33 ± 0.1[c]	2.91 ± 0.1[b]	2.84 ± 0.1[b]	1.68 ± 0.1[a]
FLAV, mg CE	0.38 ± 0.02[c]	0.31 ± 0.02[b]	0.29 ± 0.01[b]	0.21 ± 0.01[a]
Tannins, mg CE	6.48 ± 0.3[b]	2.38 ± 0.1[a]	2.60 ± 0.1[a]	2.30 ± 0.1[a]
AA, mg	13.98 ± 0.7[b]	12.03 ± 0.6[b]	6.56 ± 0.3[a]	5.93 ± 0.3[a]

Values are mean ± SD of five measurements. Means in rows with different superscript letters are significantly different ($P < 0.05$).
Abbreviations: POL, polyphenols; CUPRAC, cupric reducing antioxidant capacity; ABTS, 2,2-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt; DPPH, 1-diphenyl-2-picrylhydrazyl method; FL, flavonoids; FLAV, flavanols; AA, ascorbic acid; Snake S, Snake Sumalee; Snake NW, Snake Noen Wong; DW, dry weight; TE, trolox equivalent; CE, catechin equivalent; GAE, gallic acid equivalent; Kiwi OHE, kiwifruit organic Hayward ethylene treated; Kiwi CHE, kiwifruit conventional Hayward ethylene treated.

their ability to reduce the Fe^{3+} ions and with the aim to improve the reliability of the interpretation of the data by extracting the useful information from the experimental data. Principal component analysis (PCA) or factor analysis (FA) is widely used statistical methods for data evaluation. The analysis can graphically present inter sample and inter variable relationships and reduce dimensionality of the data (but retain most of the original variability in the data set) by linear combinations of original dependent variables to a smaller set of new uncorrelated variables (called principal components). This is in the case of factor analysis to a new set of variables (called factors) based on patterns of correlation among the original variables. Canonical discriminant analysis was performed to identify the FRAP variables, which contribute most to antioxidant activity differences and discrimination between examined fruits. Figs. 3 and 4 show box/whisker and dot plots of all FRAP values for each fruit species and fruit concentration in extracts with values of median, minimum, maximum and interquartile ranges. From the investigated fruits kiwi fruit organic (OHE) has the highest ability to reduce the ferric ions to a ferrous form. Principal component analysis (PCA) is a tool to get an overview of these data and to detect similarities among the samples, their groupings, trends, outliers and to evaluate correlations among variables and to reduce data dimensionality without loss of information. The results of PCA are depicted on a three-dimensional plot (Fig. 3), which is able to explain 99.9% of total variance. Thus the eight descriptors of FRAP ability were reduced to three principal components with only 0.1% loss of variance. The first component by itself condensed 74.1% and the second component represented almost 24.9% of the total information. According to the eigenvector values (not presented), the first component could be associated with the absorbance reading values, the second one with the Trolox values calculated for fruits' dry matter content and the third one – with the Trolox equivalents found in liquid phase of these three fruits.

Table 4
Polyphenols and DPPH values in water, ethanol and methanol extracts of snake and kiwifruit cultivars.

Indices/g DW	Snake Sumalee	Snake Noen W	Kiwi OHE	Kiwi CHE
POL W, mg GAE	7.87 ± 0.4[b]	6.98 ± 0.3[a]	7.70 ± 0.4[b]	6.18 ± 0.3[a]
DPPH W, μMTE	8.72 ± 0.5[c]	7.64 ± 0.4[c]	6.79 ± 0.3[b]	5.87 ± 0.2[a]
POL EtOH, mg GAE	3.59 ± 0.1[a]	3.14 ± 0.1[a]	4.98 ± 0.2[b]	3.64 ± 0.1[a]
DPPH EtOH, μMTE	5.40 ± 0.2[a]	4.94 ± 0.2[a]	5.95 ± 0.2[a]	4.62 ± 0.2[a]
POL MeOH, mg GAE	8.15 ± 0.4[c]	7.08 ± 0.3[b]	7.91 ± 0.4[c]	5.62 ± 0.2[a]
DPPH MeOH, μMTE	11.28 ± 0.6[b]	9.40 ± 0.5[a]	10.24 ± 0.6[b]	8.42 ± 0.4[a]

Values are mean ± SD of five measurements. Means in rows with different superscript letters are significantly different ($P < 0.05$).
Abbreviations: W, Wong; POL W, POL EtOH, POL MeOH, polyphenols in water, ethanol, methanol extracts; DPPH W, DPPH EtOH, DPPH MeOH, 1,1-diphenyl-2-picrylhydrazyl in water, ethanol, methanol extracts; polyphenols in ethanol extracts; DW, dry weight; GAE, gallic acid equivalent; Kiwi OHE, kiwifruit organic Hayward ethylene treated; Kiwi CHE, kiwifruit conventional Hayward ethylene treated; TE, trolox equivalent.

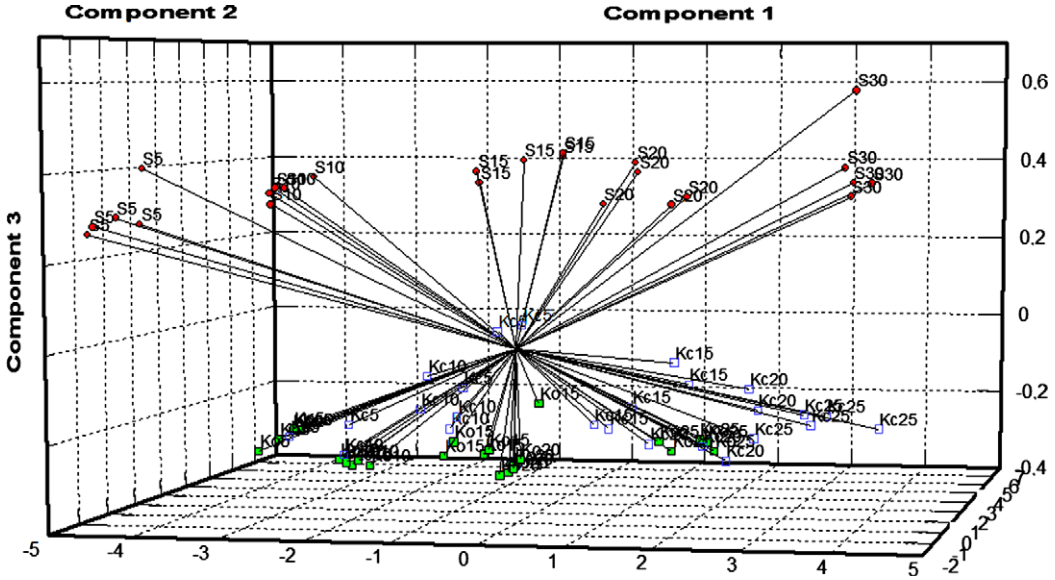

Fig. 3. Principal component analysis of FRAP parameters of snake fruit (S), Kiwi OHE (Ko) and kiwi CHE (Kc) extracts (5–25 mg/ml).

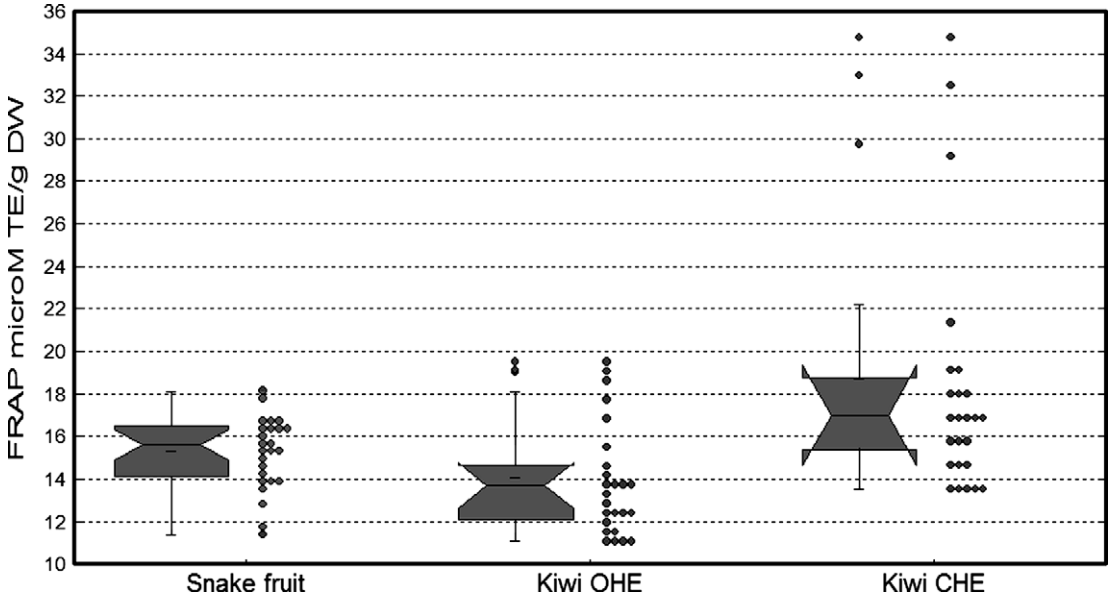

Fig. 4. Box and dot plots of the frequency distribution of the snake fruit, and two kiwifruit samples: conventional 'Hayward' (CHE) and organic 'Hayward' ethylene treated.

Box and dot plots in Fig. 3 show the frequency distribution of the snake fruit, and two kiwi fruit samples: conventional 'Hayward' ethylene treated (CHE) and organic 'Hayward' ethylene treated (OHE). The antioxidant activities (μMTE/g DW) were determined according to FRAP assay in the wide-ranking interval of fruit extract concentrations from 5 to 25 mg/ml. FRAP data vary widely and all box plots have approximately the same variation. The data dots are plotted against their actual values on a vertical scale. There is a degree of resemblance between these two kiwi fruits (CHE and OHE), but the largest similarities exist between snake and kiwi fruits. The medians of the kiwi fruits are roughly very near to the snake fruit. Table 5 shows that snake fruit and kiwi (OHE) are very similar in their FRAP ability examined in a quite range of fruit extract concentration tested from 5 to 25 mg/ml. Ferric-reducing ability of these two fruits is nearly identical evaluated according to values of mean, median, minimum, maximum and interquartile ranges.

3.4. Antiproliferative activity

Three extracts of snake fruit (EtOH, MeOH and water) were investigated on cell lines as having relatively high antioxidant activity (Fig. 5). It was observed that the antiproliferative activity of the following extracts on two cell lines [Calu-6 for human pulmonary carcinoma (Fig. 5A) and SNU-601 for human gastric carcinoma (Fig. 5B)] was different. The cell survival rate (%) for concentrations of 2000 μg ml^{-1} was for MeOH extract of snake fruit on Calu-6 of 90.50 ± 2.44, and on SNU-601 of 89.25 ± 2.26%, showing the highest antiproliferative activity in comparison with other extracts. The EtOH and water extracts slightly differ. Based on these results only MeOH extracts of snake fruit (Sumalee), as having higher antioxidant activity then that of the old variety Noen Wong were used for the comparison of kiwi fruit (Fig. 5C). The cell survival rate (%) at 2000 μg ml^{-1} for MeOH extract of Hayward conventional kiwi fruit (CHE-C) on Calu-6 was exactly the same as for the snake fruit (Sumalee) – 90.83 ± 5.90, and on SNU-601 – 87.06 ± 1.73% for MeOH extract of Hayward organic kiwi fruit (OHE-S), showing slightly higher antiproliferative activity in comparison with the snake fruit sample.

Table 5
Comparison of snake, kiwi (OHE) and kiwi (CHE) fruits according to the summary statistics of FRAP data (μM TE/g DW).

	Snake fruit	Kiwi (OHE)	Kiwi (CHE)
Valid cases	25	25	25
Mean	15.3316	14.0416	18.6728
Median	15.6300	13.6800	16.9900
Variance	2.9787	6.7010	31.7646
Standard deviation	1.7259	2.5886	5.6360
Standard error	0.3452	0.5177	1.1272
Coefficient of variation	0.1126	0.1844	0.3018
Minimum	11.3800	11.0600	13.5200
Maximum	18.1300	19.4900	34.7300
Range	6.7500	8.4300	21.2100
Lower quartile	14.1300	12.0700	15.4000
Upper quartile	16.4800	14.6800	18.7300
Interquartile range	2.3500	2.6100	3.3300

Abbreviations: Kiwi OHE, kiwifruit organic Hayward ethylene treated; Kiwi CHE, kiwifruit conventional Hayward ethylene treated.

4. Discussion

Tropical and subtropical fruits, such as mango, guava, papaya and kiwi are well known in countries of Europe and North America (Samadi-Maybodi and Shariat, 2003; Ajila et al., 2007; Ribeiro et al., 2008). However, snake fruit is less known and less investigated (Shui and Leong, 2005; Leontowicz et al., 2006, 2007).

In our previous investigations we have found (Leontowicz et al., 2006, 2007) that total polyphenols and antioxidant potentials of snake fruit were significantly higher than those of mangosteen ($P < 0.05$). A cholesterol-containing diet supplemented with the studied exotic fruits showed a positive affect on rat's plasma lipid levels, especially on fibrinogen fraction, and on the antioxidant activity. This was a comparison within the group of exotic fruits, which are not so spread for the customers. Therefore, it was decided to compare the nutritional value of this exotic fruit with well known kiwi fruit. In this study, we tried to discover whether the good taste of snake fruit justified its inclusion in different known disease preventive diets.

It was found that the contents of the studied minerals in both snake and kiwi fruits were high and comparable (Samadi-Maybodi and Shariat, 2003). Also results of other authors are similar to our data (Jeong et al., 2007). Castaldo et al. (1992) showed similar data

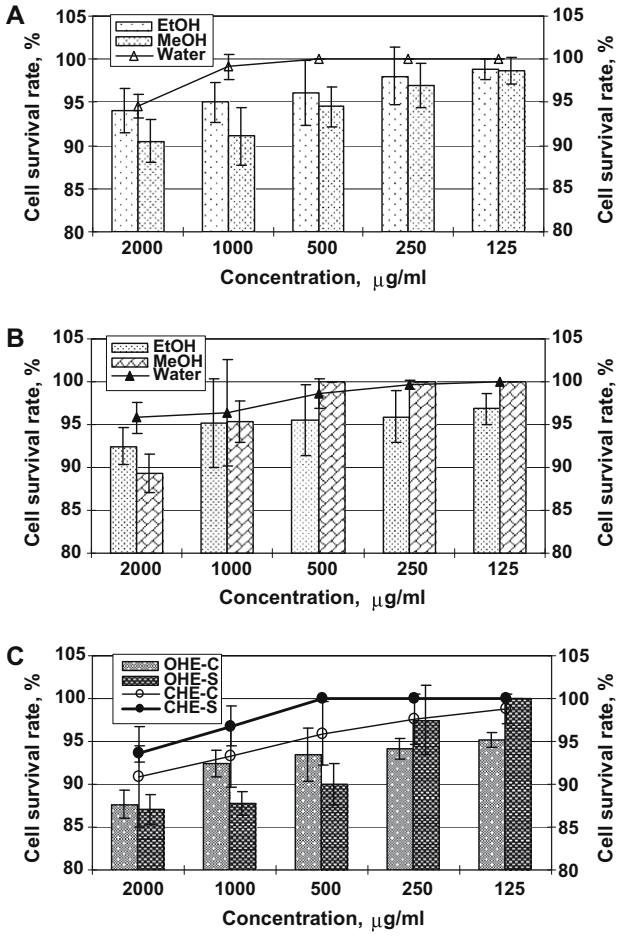

Fig. 5. Cell survival rate of (A) Calu-6 (for human pulmonary carcinoma) and (B) SNU-601 (for human gastric carcinoma) cells treated with the snake fruit extracts with ethanol, methanol and water. (C) Methanol extract of OHE-C and OHE-S (kiwifruit Hayward organic on Calu-6 and on SNU-601, respectively) and CHE-C and CHE-S, kiwifruit Hayward conventional on Calu-6 and SNU-601, respectively. Antiproliferative effects of the fruit cultivars were expressed as cell survival rate after exposure to treatment for 24 h.

for Na and K. Most of the minerals, determined in the present study, were lower than determined by others (Amodio et al., 2007). The obtained ratios of some important minerals show high mineral value.

The contents of the basic nutritional compounds (fibers, crude proteins, crude fats and crude carbohydrates) was higher in snake fruit, but not significantly ($P > 0.05$). Also other investigators confirmed our data (Lestari et al., 2003; Jeong et al., 2007). The above cited authors reported that in different snake fruit cultivars the content of total dietary fibers (mg/g DW) was the highest in 'Gading Jawa' of 93.5, followed by other cultivars and varied from 63.3 to 69.7. Total carbohydrates (989.11–684.15 mg/g DW) and the water-soluble pectin fraction of snake and kiwi fruits varied (10.12–18.24) and agreed with the cited data Lestari et al. (2003). The amount of crude protein (mg/g DW) in our investigation (57.85–30.91) corresponded with 83.75 (Jeong et al., 2007) and 38.5 (Castaldo et al., 1992), as well as lipids of 43.75 mg/g DW, and carbohydrates of 1022.5 mg/g DW (Jeong et al., 2007). The high values of the dietary fibers and carbohydrates reflected not only the high nutritional value, but also the sensory and textural quality compounds which are very important in two cultivars, but were higher in the new snake cultivar Sumalee. The results of our previous investigations (Leontowicz et al., 2006) and findings of others (Luximon-Ramma et al., 2003) showed that snake fruit was an excellent source of antioxidants. Also kiwi fruit contains high quantities of antioxidants. According to the cited authors the amount of phenolics (mg GAE/g DW) in Hayward kiwi fruit – 2.19 (Tavarini et al., 2008), 2.94 (Jeong et al., 2007) and 3.5 (Amodio et al. 2007). Our results were higher that in the cited references. The amount of ascorbic acid (mg/g DW) in these samples was higher than determined by others: for kiwi fruits 1.88–3.00 (Tavarini et al., 2008), 2.06 (Amodio et al. (2007), 6.67 (Castaldo et al., 1992), and the lowest value about 1.69 (Jeong et al., 2007).

The lipophilic fraction was low in the investigated fruits. Most of the antioxidants were in the hydrophilic fraction, which corresponds with reports (Ozgen et al., 2006).

Also others reported that snake fruit is a good source of natural antioxidants, but it was still not clear which compounds were responsible for its antioxidant property (Shui and Leong, 2005). The antioxidants in this fruit were identified to be chlorogenic acid, (−)-epicatechin, and singly linked proanthocyanidins that mainly existed as dimers through hexamers of catechin or epicatechin. In snake fruit chlorogenic acid was identified to be an antioxidant of the slow reaction type as it reacted with free radicals much more slowly than either (−)-epicatechin or proanthocyanidins (Shui and Leong, 2005).

As was shown in this investigation that the antioxidant potential of snake fruit was significantly higher than of the kiwi fruit ($P < 0.05$). Also other authors confirmed our data (Leong and Shui, 2002). The above-mentioned team investigated a group of fruits obtained in the Singapore markets. A total of 27 fruit pulps were tested for their general antioxidant capacity using two antioxidant tests. According to the results, ciku shows the highest antioxidant capacity, followed by strawberry, plum, star fruit, guava, seedless grape, snake fruit, mangosteen, avocado, orange, solo papaya, mango, kiwi fruit and others. We registered a good correlation between the content of phenolics and the antioxidant potential. Also others found that there were strong correlations between antioxidant activity as assessed by both DPPH and FRAP and the total phenolics (Luximon-Ramma et al., 2003; Yuka et al., 2003).

As was written above the role of ascorbic acid in the total AP of fruits is controversial: based on the different results of investigation and calculated correlations of the influence of ascorbic acid on overall antioxidant activity (Wang et al., 1996; Rapisarda et al., 1999; Vinson et al., 2002; Du et al., 2009). Therefore, it was decided to determine the content of ascorbic acid in the studied samples and its contribution to AP. We found that the content of the ascorbic acid was higher in kiwi than in snake fruit. The results of our investigation show that the contribution of ascorbic acid to the total antioxidant potential of the studied fruits is moderate – $R^2 = 0.56$.

With the aim to improve the reliability of the interpretation of the data by extracting the useful information from the experimental data, the pattern recognition techniques were used to evaluate and to compare some kiwi and snake fruits according to their FRAP ability. The highest reducing ability was observed for shake fruit, then for organic and conventional kiwi fruit extracts. Canonical discriminant analysis (CDA) found A2 and TE/L values as the most important variables for these fruits FRAP ability evaluation, more stepwise discriminant analysis selected both absorbance readings (A1 and A2) and TE values (TE/L and TE/g DW) as the most effective descriptors to enable differentiation and classification of estimated samples according to their ability to reduce Fe^{3+}. The actual percent of correct recognition of fruit extracts according to their FRAP values estimated by the CDA procedure was 83.3%.

Epidemiological studies have consistently linked abundant consumption of fruits and vegetables to a reduction of the risk of developing several types of cancer. The methanolic fraction of snake fruit showed the highest antioxidant activity in comparison with the ethanolic and water extracts. Therefore the methanol

fractions were selected for testing of the effect on cells for kiwi fruit. In most cases, however, the identification of specific fruits and vegetables that are responsible for these effects is still lacking, retarding the implementation of effective dietary-based chemopreventive approaches. Our previous investigations showed that the results of the antiproliferative effect of different lotus cultivars were not consistent with the findings of DPPH radical scavenging activity or total phenolic content (Park et al., 2009). This can be explained not only by relatively high antioxidant activity, but by the amount of flavonoids and other bioactive compounds. The results on cell proliferation can be explained as a synergistic effect of flavonoids, flavanols and ascorbic acid in the fruits. Our data correspond with others, that combinations of flavonoids, which are naturally present in whole fruits and vegetables, are more effective in cancer cell growth inhibition than the individual flavonoids. These fruits can be used as a potential source of high-value phytochemicals with nutraceutical and functional food additive applications.

In conclusion:

1. Snake and to a less degree kiwi fruit contains high comparable quantities of basic nutritional compounds, antioxidants and exercises high level of antioxidant and proliferative activities.
2. Both fruits show high level of correlation between the contents of phenolic compounds and the antioxidant potential.
3. The contribution of ascorbic acid to the total antioxidant potential is moderate.
4. Snake and the kiwi fruit could be a valuable addition to known disease preventing diets.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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