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Bioactivity and nutritional properties of hardy kiwi fruit *Actinidia arguta* in comparison with *Actinidia deliciosa* 'Hayward' and *Actinidia eriantha* 'Bidan'

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ABSTRACT

The aim of this research is to identify and compare the bioactive compounds, antioxidant capacities and binding potentials to human protein in different varieties of hardy kiwi (*Actinidia (A.) arguta*), 'Hayward' (*Actinidia deliciosa*) and less – known 'Bidan' (*Actinidia eriantha*). Polyphenols, flavonoids, flavanols, tannins, vitamin C, lutein, zeaxanthin and dietary fibers were significantly higher in cultivar 'M1' among the *A. arguta* than in 'Hayward'. The binding properties of studied kiwi fruits were determined by interaction of polyphenols with human serum albumin (HSA). An internal standard FTIR technique allowed the quantitative comparison of specific IR absorption bands (Amides I, II, III) of different kiwi fruit samples after interaction with HSA. It was shown that the antioxidant and binding capacities and FTIR quantitative estimations of *A. arguta* fruits were significantly higher than in 'Hayward', but lower than the 'Bidan'. In MS spectra were found some slight differences in *A. arguta* kiwis in comparison with 'Hayward' and 'Bidan'. Two *A. arguta* cultivars were similar to 'Bidan'. The interaction of polyphenols with HSA, evaluated by fluorometry/FTIR, made it possible to compare the bioactivity of different cultivars and families. In conclusion, for the first time fruits *A. arguta*, cultivated in Poland, were compared with widely consumed kiwi fruits, using advanced analytical methods. The high bioactivity and nutritional value of *A. arguta* fruits from Polish ecological plantation enables us to recommend them for marketing and consumption.

1. Introduction

Fruits contain significant levels of biologically active substances that have physiological and biochemical benefits and are important for human health. Exotic fruits, especially kiwi fruit, avocado, mango and persimmon, have high nutritional and bioactive properties due to their composition (Gorinstein and Leontowicz et al., 2011; Gorinstein and Poovarodom et al., 2011; Park et al., 2008). The most popular species of *Actinidia (A.)* kiwi fruit are *Actinidia deliciosa*, *Actinidia chinensis* and *Actinidia eriantha*, but other species such as *Actinidia arguta*, *Actinidia kolomikta* and *Actinidia purpurea* can grow also in cooler regions due to their frost resistance (Chesoniene, Daubaras, & Viskelis, 2004). As it is known, kiwi fruit possess antioxidant properties, which are influenced by their biologically active substances. A high correlation between the content of total polyphenols and vitamin C on the one hand and antioxidant activity on the other was found (Du, Li, Ma, & Liang, 2009; Park et al., 2011). According to Park et al. (2011) the overall bioactivity of four cultivars of *A. deliciosa and A. eriantha* families was established in the following order: 'Bidan' > 'Haenam' > 'Deheung' = 'Hayward'. Cultivar 'Bidan' (relatively new one) was recommended for consumption. Increasing consumption of exotic fruits in Poland, including kiwi fruits (*A. deliciosa* and *A. eriantha*), which are imported from different countries, led us to study the *A. arguta* (hardy or mini kiwi) grown at organic farms. Hardy kiwis possess a rich chemical composition, which depends on the geographic and climatic conditions and on the method of growing fruits and cultivars. Fruits contain a high level of vitamin C [up to 185 mg/100 g fresh weight (FW)], which only slightly declines during cold

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storage. Dietary fiber (2–3%) and proteolytic enzyme actinidin, which is similar to papain, was also determined (Yamanaka, Oota, Fukuda, & Nishiyama, 2004). Hardy kiwis can be considered as the richest source of lutein among available fruits. Kim, Beppu, and Kataoka (2009) found that the concentration of phenolics in the skin of *A. arguta* was 15 times higher than in the pulp. Therefore, the edible peel of hardy kiwis can significantly increase the healthful properties. The antioxidative activity of these fruits is strongly correlated with the content of vitamin C and polyphenols (Krupa, Latocha, & Liwińska, 2011; Latocha, Krupa, Wołosiak, Worobiej, & Wilczak, 2010). The attractiveness to consumers of hardy kiwis (Latocha, Jankowski, & Radzanowska, 2011), makes it likely to become popular on the Polish market. The aim of this study was to assess the health-promoting properties of the six varieties of *A. arguta* family, cultivated in Poland, and to compare with *A. eriantha* cultivar 'Bidan' (new one) and *A. deliciosa* 'Hayward' (traditional), grown in South Korea. This assessment was made on the basis of a comprehensive study of the chemical composition, nutritional value and bioactivity of fruits. The binding properties of the investigated fruits were studied by the interaction of their polyphenols with human serum albumin (HSA). FTIR as a quantitative tool was used with KBr pellet and potassium ferricyanide, $K_3Fe(CN)_6$ (2117 cm^{-1}), as an internal standard, to get characteristic parameters of chemical groups in the samples (Barth, 2007; Joshi, Kalyanasundaram, & Balasubramanian, 2013). As far as we know, no results of such investigations have been published previously.

2. Material and methods

2.1. Chemicals

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picryl-hydrazl (DPPH), 2,9-dimethyl-1,10-phenanthroline (neocuproine), Folin–Ciocalteu reagent 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.

2.2. Samples

2.2.1. A collection of mini kiwis

Mini kiwis [*A. arguta* (Siebold et. Zucc) Planch. ex. Miq.] were cultivated in 2013, on sandy loam soil, on the experimental field (ecological) of the Department of Environmental Protection, (SGGW). Plants were pruned regularly and irrigated during the drought periods. 'Bingo' (hybrid of *arguta* and *purpurea*), 'M1' (select *arguta*), 'Anna', 'Weiki', 'Jumbo' and 'Geneva' were picked at a stage when they were ripe enough to eat randomly. This is the basic method used for these species with collection of 3 kg of fruit of six varieties. Fruits were washed and freeze-dried together with the peel (ELENA company, Kokanin 86). Before the freeze-drying process (lyophilizer Baujar 1974 DDR, Typ – TG 15.4, Reg – Nr 094), physicochemical analyses were performed.

2.2.2. Analysis of fresh fruits A. arguta

A. arguta fruits were analyzed for the average weight (determined mass of 10 pieces randomly selected fruits in triplicates). Firmness was done on 30 fruits in triplicates of 10 pieces (Intron 5542, with pin 4.5 mm and head 500 N). Acidity was measured by titration with 0.1 M NaOH to pH = 8.1, according to PN-EN 12147. Extract content was done according to PN-EN 12143–2000 (Abbe refractometer at 20 °C).

2.2.3. Analysis of freeze-dried A. arguta

In all samples of *A. arguta* the total numbers of bacteria at 30 °C (PN-EN ISO 4833:2004 + Ap1:2005), bacterial counts of *Escherichia coli* (PN-ISO 4832:2007), contents of fungi and molds (PN-ISO 21527-2:2009) were measured, according to methods accredited by WESSLING, Poznań, Poland.

2.2.4. A collection of A. deliciosa 'Hayward' and A. eriantha 'Bidan'

'Hayward' and 'Bidan' were grown under organic conditions in an orchard in Heanam county (longitude 126° 15″ and latitude 34° 18 ″) in Jeonnam province in Korea, 2013. For the investigation five replicates of five fruits, where each at their commercial maturity stage [the degree of soluble solids content (SSC) and in the range of 6.8–7.5%], were used. 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 lyophilized for 48 h (Virtis model 10–324), and the dry weight (DW) was determined. The samples were ground to pass through a 0.5 mm sieve and stored at −80 °C.

2.2.5. Analysis of freeze-dried A. arguta, A. deliciosa and A. eriantha

2.2.5.1. Determination of bioactive compounds and total antioxidant capacity. Polyphenols were extracted from lyophilized fruits with two solvents: water and ethanol (concentration 25 mg/ml) at room temperature and were determined by Folin–Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventos, 1999) with measurements at 750 nm using a spectrophotometer (Hewlett–Packard, model 8452A, Rockvile, USA).

Flavonoids, extracted with 5% $NaNO_2$, 10% $AlCl_3 \times H_2O$ and 1 M NaOH, were measured at 510 nm. Total flavanols were estimated using the *p*-dimethylaminocinnamaldehyde method, and the absorbance was measured at 640 nm. The extracts of condensed tannins (procyanidins) with 4% vanillin solution in MeOH were measured at 500 nm. (+)Catechin served as a standard for flavonoids, flavanols and tannins as previously was described in details (Park et al., 2013, 2014).

Total Antioxidant Capacity (TAC) was determined by four complementary assays: (1) 2,2′-Azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{\cdot+}$) was generated by the interaction of ABTS (7 mM) and $K_2S_2O_8$ (2.45 mM) (Re et al., 1999). (2) 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+}) (Benzie & Strain, 1996). (3) Scavenging free radical potentials were tested in a methanolic solution (3.9 ml) of 1,1-diphenyl-2-picrylhydrazyl (DPPH) with the samples extracts in water or ethanol (0.1 ml) (Brand-Williams, Cuvelier, & Berset, 1995). (4) Cupric reducing antioxidant (CUPRAC) is based on utilizing the copper (II) – neocuproine reagent as the chromogenic oxidizing agent (Apak, Guclu, Ozyurek, & Karademir, 2004).

2.2.5.2. Determination of pigments, vitamin C, total dietary fiber and its fractions. For the extraction of carotenoids, 2 g of freeze-dried fruits were weighed and homogenized with 50 ml of acetone/petroleum ether, according to de Sa and Rodriguez-Amaya (2004). HPLC was carried out on an Agilent Technologies 1200 Rapid Resolution column using Poroshell 120 EC-C18 column (4.6 mm × 100 mm, 2.7 μm), reverse phase, at a flow rate of 0.5 ml/min at room temperature. Total chlorophylls, chlorophylls *a* and *b* and total carotenoids were extracted with 100% acetone and determined spectrophotometrically at the following absorbances (nm): 661.6, 644.8 and 470, respectively. Total anthocyanins were estimated by a pH differential method. Absorbance was measured on Beckman spectrophotometer at 510 nm and at 700 nm in buffers at pH 1.0 and 4.5, as previously described (Park et al., 2013, 2014). Total ascorbic acid was determined by

CUPRAC assay in water extract (100 mg of lyophilized sample and 5 ml of water) (Ozyurek, Guclu, Bektasoglu, & Apak, 2007). The contents of total dietary fiber (TDF) and its fractions [soluble dietary fiber (SDF) and insoluble dietary fiber (IDF)] were performed by an enzymatic–gravimetric method using a set of enzymes Megazyme, according to AOAC, Official Method 991.43 (1995).

2.2.5.3. Fluorometric and FTIR measurements. Binding properties of kiwi fruit extracts to human serum albumin (HSA) were determined by two (2D-FL) and three dimensional (3D-FL) fluorescence (model FP-6500, Jasco spectrofluorometer, serial N261332, Japan). For the fluorescence measurements, 3.0 mL of 2.0×10^{-6} mol/L HSA solution and various amounts of kiwi fruit ethanolic or water extracts were added to a 1.0 cm quartz cell manually using a micro-injector. The emission spectra were recorded in the range of 300–500 nm upon excitation at 280 nm. The 3D-FL were measured at the emission wavelengths between 200 and 795 nm, and the initial excitation wavelength at 200 nm. All solutions for protein interaction were prepared in 0.05 mol/L Tris–HCl buffer (pH 7.4), containing 0.1 mol/L NaCl (Park et al., 2014). The samples after fluorescence measurements were lyophilized and the interaction of polyphenol fruit extracts with HSA was also studied by Fourier transform infrared (FT-IR) spectroscopy in dry state. A Nicolet iS 10 FT-IR Spectrometer (Thermo Scientific Instruments LLC, Madison, WI, USA), with the smart iTRTM ATR (Attenuated Total Reflectance) accessory, was used to record IR spectra (Derenne, Van Hemelryck, Lamoral-Theys, Kiss, & Goormaghtigh, 2013). KBr pellets were made by mixing 3 mg of internal standard $K_3Fe(CN)_6$, 10 mg of the investigated samples and 150 mg KBr. Samples of this type were used for quantitative comparisons among the functional groups (Joshi et al., 2013). The standard curve was done with different concentrations of the internal standard, with absorption band at 2117 cm^{-1}. The relative concentrations of the investigated samples were calculated from the calibration equation using the height of peaks at specific absorption bands.

2.2.5.4. MS analysis. The ethanolic extracts were submitted to MS analysis for determination of bioactive compounds and were processed exactly as described in Park et al. (2014). A mass spectrometer, a TSQ Quantum Access Max (Thermo Fisher Scientific, Basel, Switzerland) was used. All samples were done by direct infusion in the mass spectrometer by electrospray ionization (ESI) in negative mode, full scan analysis, range of 100–900 m/z. For optimization of the acquisition parameters and for identity confirmation, only a part of standards was employed, not for all compounds that were found in the investigated samples (Gomez-Romero et al., 2011; Mikulic-Petkovsek, Slatnar, Stampar, & Veberic, 2012; Park et al., 2014).

2.3. Statistical analysis

To verify the statistical significance, means ± SD of five independent measurements were calculated. One-way analysis on variance (ANOVA) for statistical evaluation of results was used, following by Duncan's new multiple range test to asses differences between group's means. *P* values of <0.05 were considered to be significant.

3. Results and discussion

3.1. Physicochemical parameters and microbiological tests of A. arguta

The physicochemical parameters of *A. arguta* kiwis include average weight, fruit firmness, extract content, and acidity. *A. arguta* fruits are relatively small, comparable to the grapes or gooseberries. Their average weight was from 4.5 g ('M1') to 12.5 g ('Jumbo'). The average weight of fruits of three cultivars: 'Ananasnaya', 'Weiki' and 'Bingo' were similar and amounted to 6.8, 7.1 and 7.4 g, respectively. The fruits of certain varieties were uniformly green ('Jumbo', 'M1'), the others on the sunlit side had a clear red-cherry blush ('Weiki', 'Ananasnaya'), or pink ('Bingo') or brownish-red ('Geneva'). Most varieties of fruits after harvest had similar firmness (3.36–3.93 N). The highest firmness had fruits of cultivar 'Ananasnaya' (4.94 N). The extract was amounted from 11.8% to 15.8%. The highest content of the extract was found in 'Geneva' and the lowest was in 'M1'. No differences were noted between 'Weiki', 'Bingo' and 'Jumbo'. The acidity of the fruits amounted from 1.15 to 1.59%, and the smallest was in 'M1', and the highest was in 'Bingo'. Microbiological tests in freeze-dried fruits of *A. arguta* showed that the total number of microorganisms (colony forming unit (cfu)/g) varied from the lowest in 'Bingo' (150) to the highest in 'Jumbo' (760). *E. coli* (cfu/g) in all cultivars were <10; amount of yeast (cfu/g) was <10 in 'Bingo' and 'Jumbo'. The highest amount was in 'Anna' of 220. Molds (cfu/g) varied from 110 for 'Geneva' to 760 in 'M1'. These results point out that all fruits had low contents of the total number of microorganisms, *E. coli*, molds and yeasts. This indicates good growing conditions on the plantations of fruits and good preparation for their research.

3.2. Polyphenols, flavonoids, flavanols and tannins

The bioactive compounds in *A. arguta* and kiwi fruits 'Hayward' and 'Bidan' are shown in Table 1A. As can be seen, the contents of polyphenols in water extracts were higher than in ethanolic extracts. The highest content of polyphenols was found in 'M1'. In other cultivars of *A. arguta* polyphenols were higher than in 'Hayward', but lower than in 'Bidan'. The significantly lowest content of polyphenols was found in *A. deliciosa* 'Hayward' and in *A. arguta* 'Ananasnaya' (Kim et al., 2009; Latocha et al., 2010). According to Kim et al. (2009) the phenolic content is strongly correlated with astringency taste. A great difference in polyphenol contents among studied cultivars of the same species indicates the possibility of selecting the cultivars with the highest concentration of these compounds, and as a consequence, stronger pro-healthy properties. Their pro-health influence is stronger when they occur along with vitamin C (Nishiyama et al., 2004; Yeomaus, Linseisen, & Wolfram, 2005). Krupa et al. (2011) and showed a strong correlation between polyphenol contents and vitamin C in hardy kiwi fruit. The contents of flavonoids in ethanolic extracts were higher than in water extracts. Flavonoids in *A. arguta* were also the highest in 'M1' and the lowest in 'Hayward'. As was shown, the content of flavonoids in kiwi fruit 'Bidan' and in other varieties of hardy kiwi was similar. Flavanols in water extracts were higher than in ethanol extracts, but also the highest were estimated in 'M1'. It should be underlined that the amount of flavanols was higher in all varieties of hardy kiwis than in 'Hayward' and 'Bidan'. The contents of tannins in ethanolic extracts were higher than in water extracts, and the highest were in 'M1'. The content of tannins was also higher in other varieties than in 'Hayward' and 'Bidan'. In our previous and cited reports were determined bioactive compounds and among them flavonoids, flavanols and phenolic acids (Gorinstein and Poovarodom et al., 2011; Park et al., 2011). As can be seen, the contents of the bioactive compounds extracted with water and ethanol differ significantly, however, the highest amount of polyphenols was in 'M1', followed by 'Bingo', 'Geneva' and 'Anna' ('Ananasnaya'). Our results are in line with other reports, which discussed the influence of various solvents on the composition of extracted bioactive compounds and different extraction conditions. Dahmoune, Nayak, Moussi, Remini, and Madani (2015) showed that it was observed that total phenolics, tannins, flavonoids and antioxidant activities in microwave-assisted extracts

Table 1
(A) The content of total polyphenols, flavonoids, flavanols and tannins; (B) antioxidant capacities (μM TE); (C) correlation between vitamin C, polyphenols and antioxidant capacities of six *Actinidia arguta* cultivars and kiwi fruit 'Hayward' and 'Bidan' in water (W) and ethanol (Et) extracts (per g DW).

A

Kiwi fruit	POL(mg GAE)	FLAV0 N (mg CE)	FLAV (mg CE)	TAN (mg CE)
Bingo^{W}	15.00 ± 1.45^{bc}	2.97 ± 0.24^{c}	1.40 ± 0.12^{b}	4.61 ± 0.37^{b}
M1^{W}	19.26 ± 1.83^{b}	5.11 ± 0.51^{a}	2.03 ± 0.17^{a}	9.32 ± 0.82^{a}
Anna^{W}	14.16 ± 1.36^{c}	3.00 ± 0.32^{c}	1.04 ± 0.09^{c}	3.76 ± 0.28^{c}
Weiki^{W}	10.19 ± 1.12^{d}	2.28 ± 0.21^{d}	0.65 ± 0.04^{d}	2.50 ± 0.18^{d}
Jumbo^{W}	11.11 ± 1.23^{cd}	3.76 ± 0.31^{c}	0.63 ± 0.05^{d}	2.32 ± 0.19^{d}
Geneva^{W}	14.25 ± 1.43^{c}	4.06 ± 0.28^{b}	1.43 ± 0.12^{b}	4.69 ± 0.32^{b}
Hayward^{W}	5.47 ± 0.52^{e}	1.65 ± 0.14^{e}	0.11 ± 0.07^{e}	1.96 ± 0.12^{de}
Bidan^{W}	48.12 ± 4.65^{a}	3.24 ± 0.24^{bc}	0.05 ± 0.01^{f}	0.82 ± 0.07^{e}
Bingo^{Et}	9.05 ± 0.86^{bc}	3.38 ± 0.31^{c}	0.54 ± 0.06^{b}	5.10 ± 0.42^{b}
M1^{Et}	11.16 ± 1.14^{b}	7.19 ± 0.68^{a}	0.97 ± 0.08^{a}	7.56 ± 0.65^{a}
Anna^{Et}	8.90 ± 0.81^{c}	3.14 ± 0.28^{c}	0.53 ± 0.03^{b}	5.27 ± 0.42^{b}
Weiki^{Et}	7.87 ± 0.74^{cd}	2.95 ± 0.21^{cd}	0.39 ± 0.01^{c}	5.75 ± 0.48^{b}
Jumbo^{Et}	8.42 ± 0.81^{c}	4.93 ± 0.44^{b}	0.45 ± 0.02^{bc}	5.00 ± 0.43^{b}
Geneva^{Et}	9.52 ± 0.92^{bc}	4.75 ± 0.43^{b}	0.57 ± 0.06^{b}	4.09 ± 0.34^{bc}
Hayward^{Et}	5.41 ± 0.51^{d}	1.09 ± 0.11^{d}	0.17 ± 0.03^{d}	1.80 ± 0.12^{c}
Bidan^{Et}	56.23 ± 5.47^{a}	3.58 ± 0.31^{c}	0.08 ± 0.05^{e}	4.96 ± 0.35^{b}

B

Kiwi fruit	FRAP	ABTS	DPPH	CUPRAC
Bingo^{W}	16.79 ± 1.54^{c}	80.00 ± 7.54^{c}	19.01 ± 1.54^{bc}	64.98 ± 5.32^{b}
M1^{W}	22.34 ± 2.14^{b}	112.25 ± 10.18^{b}	39.32 ± 3.21^{b}	104.48 ± 9.14^{a}
Anna^{W}	17.19 ± 1.62^{c}	84.72 ± 7.54^{c}	16.03 ± 1.34^{cd}	61.62 ± 6.12^{b}
Weiki^{W}	11.76 ± 1.09^{d}	45.51 ± 3.28^{e}	11.69 ± 1.16^{d}	45.42 ± 3.67^{c}
Jumbo^{W}	13.12 ± 1.21^{cd}	51.57 ± 4.32^{d}	14.71 ± 1.32^{cd}	42.74 ± 3.98^{c}
Geneva^{W}	14.90 ± 1.23^{cd}	52.22 ± 5.04^{d}	18.33 ± 1.54^{c}	54.48 ± 5.42^{bc}
Hayward^{W}	8.60 ± 0.76^{e}	14.82 ± 1.32^{f}	7.20 ± 0.65^{e}	11.49 ± 1.23^{d}
Bidan^{W}	83.71 ± 7.42^{a}	127.81 ± 11.23^{a}	139.90 ± 12.65^{a}	112.25 ± 10.11^{a}
Bingo^{Et}	12.82 ± 1.32^{b}	17.60 ± 1.54^{bc}	12.17 ± 1.15^{bc}	38.57 ± 3.54^{c}
M1^{Et}	13.68 ± 1.43^{b}	27.52 ± 2.42^{b}	14.48 ± 1.26^{b}	70.35 ± 6.56^{b}
Anna^{Et}	11.96 ± 1.23^{bc}	12.96 ± 1.12^{cd}	10.34 ± 1.11^{c}	36.70 ± 3.34^{c}
Weiki^{Et}	10.12 ± 0.87^{c}	11.81 ± 1.04^{d}	9.73 ± 0.76^{cd}	34.67 ± 3.23^{c}
Jumbo^{Et}	11.55 ± 1.12^{bc}	15.34 ± 1.42^{c}	10.54 ± 1.05^{c}	34.07 ± 3.12^{c}
Geneva^{Et}	13.08 ± 1.21^{b}	16.92 ± 1.32^{bc}	12.39 ± 1.12^{bc}	31.90 ± 2.98^{cd}
Hayward^{Et}	9.03 ± 0.84^{d}	12.31 ± 1.18^{cd}	6.49 ± 0.45^{ed}	23.55 ± 2.19^{d}
Bidan^{Et}	95.37 ± 8.65^{a}	147.00 ± 13.23^{a}	78.80 ± 6.86^{a}	130.73 ± 12.65^{a}

C

Correlation	Correlation coefficient
Vit. C × POL^{W}	0.9764
Vit. C × POL^{Et}	0.9028
Vit. C × FRAP^{W}	0.9342
Vit. C × FRAP^{Et}	0.8819
Vit. C × ABTS^{W}	0.6703
Vit. C × ABTS^{Et}	0.9065
Vit. C × DPPH^{W}	0.9474
Vit. C × DPPH^{Et}	0.9054
Vit. C × CUPRAC^{W}	0.7013
Vit. C × CUPRAC^{Et}	0.9289

Mean ± SD (standard deviation) of 5 measurements. Average in rows marked with different letters differ significantly ($P < 0.05$).
Abbreviations: A, *Actinidia*, CE, catechin equivalent; GAE, gallic acid equivalent; DW, dry weight; TE, Trolox equivalent; POL, polyphenols; FLAVON, flavonoids; FLAV, flavanols; TAN, tannins; ABTS, 2,2-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid); DPPH, 1,1-diphenyl-2-picrylhydrazyl; CUPRAC, Cupric reducing antioxidant capacity; FRAP, Ferric-reducing/antioxidant power; Vit. C, vitamin C.

were higher than the others: ultrasound-assisted and conventional solvent extracts in *Myrtus* leaves. Ethanol concentration (42%) and liquid-to-solid ratio (32 mL/g solvent to material ratio) were the significant parameters for the extraction process. Dorta, Lobo, and Gonzalez (2012) showed that among optimized factors, extraction solvent was the most important. Antioxidant capacities, flavonoids, tannins and proanthocyanidins were the highest with methanol, ethanol:water, or acetone:water. From the perspective of food security, it is advisable to choose ethanol (which also has a notable antioxidant content). Ethanol:water, or acetone:water are solvents that can be used in compliance with good manufacturing practice. Therefore we also proposed ethanol as a solvent for the extraction of bioactive compounds. Our results are in agreement with Qian, Liu, and Huang (2004), where the extracts of *Lycium chinense* Mill fruits with water, 50% ethanol and 95% ethanol were compared in antiradical efficiency. The results showed that water infusion was similar to 50% ethanol extract in flavonoids, and 95% ethanol extract contained more flavonoids. In the present work we used analytical spectrophotometric methods for the determination of total polyphenol content and other bioactive compounds belonging to this group. Similar methods were used in our previous studies (Park et al., 2011, 2013, 2014). This allows a comparison of their contents in a variety of fruits, coming from different continents, growing conditions and stages of maturity.

3.3. Total antioxidant capacity

The total antioxidant capacity of studied kiwi fruit cultivars are shown in the Table 1B.

In order to receive reliable data, the total antioxidant capacities in fruits were determined by four complementary assays: ABTS, DPPH, FRAP and CUPRAC. The obtained results by these four methods were different, but the relationship between these methods and the polyphenols was similar to our previous reports (Park et al., 2011, 2013, 2014). As can be seen, according to four assays, the significantly highest level of antioxidant capacity was registered in less popular new cultivar 'Bidan', and then in 'M1' (*A. arguta*) ($P < 0.05$), following by 'Bingo', 'Anna' and 'Geneva'. These relationships are concerned in both aqueous and alcoholic extracts. As was shown above, these cultivars have also the highest content of polyphenols among studied samples (Table 1A). 'Bidan' has the highest antioxidant activity, is a new Asiatic cultivar, which is less tasty and less popular in the market than other kiwi fruits. 'Bidan' contains the highest content of vitamin C, so it is less sweet than 'Hayward'.

3.4. Vitamin C, pigments, carotenoids, total dietary fiber

A. arguta contains a high level of vitamin C, and the highest content was estimated in 'M1', 'Geneva' and 'Bingo' (Table 2A). The lowest content of vitamin C was found in 'Hayward' (Samadi-Maybodi & Shariat, 2003), and the highest was in new cultivar 'Bidan' ($P < 0.05$). Latocha et al. (2010) found that the contents of vitamin C in studied kiwis *A. arguta* and in *A. deliciosa* 'Hayward' were similar. Nishiyama et al. (2004) underlined that the concentration of vitamin C in fruits *A. arguta*, which were growing in Japan, was higher. Our study also showed a higher vitamin C content in fruit *A. arguta*, but it was lower than in the new cultivar 'Bidan'. It should be emphasized that the kiwi fruit 'Bidan' with the highest antioxidant activity also had the highest content of vitamin C, as well as 'M1' cultivar. The correlation between the vitamin C, polyphenols and antioxidant capacities is presented in Table 1C. Nearly all kiwi fruit cultivars with high vitamin C and polyphenols have as well high antioxidant capacities. Water extracts showed higher correlation coefficients than ethanolic extracts, except two antioxidant methods ABTS and CUPRAC (Table 1C). In general, vitamin C showed the highest correlation in water extracts with polyphenols, FRAP and DPPH values (0.98–0.93). This correlation allows better documentation of kiwi fruit health-promoting properties.

Actinidia fruits characterized by the green color of flesh, as in the maturity stage of consumption, which is associated with high levels of chlorophylls. Chlorophyll, the green blood product, structurally related to heme, is an antioxidant, protects cells against the

Table 2
(A) The amount of chlorophyll *a* and *b* and xantophylls + carotenes (per g DW), anthocyanins (per kg DW) and vitamin C (AA mg per g DW); (B) The amount of carotenoids: lutein, zeaxanthin and β -carotene (μg) from HPLC assay (per g DW); (C) The amount of TDF and its fractions IDF and SDF (% DW); (D) Relative amounts of chemical groups [μM $K_3Fe(CN)_6$] determined by FTIR internal standard method, of six *Actinidia arguta* cultivars and kiwi fruit 'Hayward' and 'Bidan'.

A

Kiwi fruit	Chlorophyll *a*, (μg)	Chlorophyll *b*, (μg)	Total chlorophylls, (μg)	Xanthophylls + Carotenes (μg)	Anthocyanins, (mg CGE)	Vitamin C (mg AA)
Bingo	103.5 ± 9.45^{d}	17.5 ± 1.54^{d}	121.0	38.0 ± 3.54^{c}	60.1 ± 5.87^{b}	14.91 ± 1.18^{bc}
M1	99.5 ± 8.15^{d}	18.3 ± 1.83^{cd}	117.8	38.0 ± 3.43^{c}	NF^{e}	18.78 ± 1.54^{b}
Anna	120.0 ± 11.54^{c}	22.0 ± 2.67^{c}	142.0	46.0 ± 3.87^{b}	22.0 ± 2.18^{c}	11.14 ± 1.09 ^{c}
Weiki	169.9 ± 15.63^{b}	32.5 ± 3.43^{b}	202.4	59.0 ± 4.62^{a}	7.2 ± 0.54^{d}	7.12 ± 0.67^{cd}
Jumbo	166.0 ± 15.52^{b}	24.5 ± 2.51^{c}	190.5	58.0 ± 5.43^{a}	70.2 ± 6.23^{a}	8.34 ± 0.74^{cd}
Geneva	115.5 ± 9.87^{c}	23.5 ± 1.87^{c}	139.0	46.0 ± 4.18^{b}	20.1 ± 1.87^{c}	15.02 ± 1.34^{bc}
Hayward	60.5 ± 6.09^{e}	24.0 ± 2.21^{c}	84.5	30.0 ± 2.18^{d}	NF^{e}	5.98 ± 0.48^{d}
Bidan	209.0 ± 18.43^{a}	119.8 ± 9.54^{a}	328.8	37.8 ± 3.45^{c}	NF^{e}	41.20 ± 4.34^{a}

B

Kiwi fruit	Lutein	Zeaxanthin	β-carotene	Total carotenoids
Bingo	3.98 ± 0.04^{d}	0.26 ± 0.07^{c}	0.96 ± 0.16^{ab}	5.21 ± 0.27^{d}
M1	5.44 ± 0.67^{cd}	0.21 ± 0.06^{c}	0.59 ± 0.22^{c}	6.23 ± 0.95^{cd}
Anna	7.01 ± 0.31^{bc}	0.24 ± 0.03^{c}	1.10 ± 0.36^{b}	8.35 ± 0.70^{c}
Weiki	11.61 ± 0.96^{b}	0.57 ± 0.11^{b}	2.47 ± 0.39^{a}	14.65 ± 1.46^{b}
Jumbo	8.58 ± 0.47^{bc}	1.03 ± 0.06^{a}	1.51 ± 0.09^{ab}	11.11 ± .62^{bc}
Geneva	8.30 ± 0.74^{bc}	0.58 ± 0.26^{b}	1.18 ± 0.34^{b}	10.06 ± 1.34^{bc}
Hayward	6.73 ± 0.67^{c}	NF^{d}	NF^{d}	6.73 ± 0.67^{cd}
Bidan	24.15 ± 3.06^{a}	NF^{d}	1.11 ± 0.24^{b}	25.26 ± 3.30^{a}

C

Kiwi fruit	TDF	IDF	SDF	IDF/SDF
Bingo	25.86 ± 0.07^{ab}	20.43 ± 0.06^{a}	5.43 ± 0.01^{ab}	3.76 ± 0.00^{a}
M1	27.11 ± 0.01^{a}	21.40 ± 0.06^{a}	5.71 ± 0.04^{ab}	3.75 ± 0.04^{a}
Anna	24.75 ± 0.01^{ab}	18.26 ± 0.07^{ab}	6.50 ± 0.07^{a}	2.81 ± 0.04^{b}
Weiki	24.58 ± 0.01^{ab}	17.61 ± 0.13^{b}	6.97 ± 0.13^{a}	2.53 ± 0.06^{b}
Jumbo	19.78 ± 0.08^{b}	14.02 ± 0.01^{c}	5.76 ± 0.09^{ab}	2.43 ± 0.04^{b}
Geneva	19.86 ± 0.14^{b}	14.09 ± 0.14^{c}	5.77 ± 0.01^{ab}	2.44 ± 0.02^{b}
Hayward	12.09 ± 0.04^{c}	6.97 ± 0.13^{d}	5.12 ± 0.09^{b}	1.36 ± 0.05^{c}
Bidan	13.72 ± 0.04^{bc}	9.76 ± 0.11^{cd}	3.96 ± 0.07^{c}	2.47 ± 0.08^{b}

D

Kiwi fruit water extracts + HSA	Relative amounts [μM $K_3Fe(CN)_6$] of chemical groups at following absorption bands (cm^{-1})				
	1650	1550	1455	1400	1300
Bingo	18.99 ± 1.7^{c}	14.66 ± 1.4^{c}	6.08 ± 0.4^{bc}	9.76 ± 0.7^{b}	11.75 ± 1.0^{c}
M1	18.85 ± 1.7^{c}	13.75 ± 1.2^{cd}	5.55 ± 0.3^{c}	9.09 ± 0.6^{b}	7.95 ± 0.6^{cd}
Anna	19.52 ± 1.8^{bc}	14.59 ± 1.2^{c}	6.23 ± 0.6^{b}	9.60 ± 0.6^{b}	11.57 ± 0.7^{c}
Weiki	19.96 ± 1.7^{bc}	18.63 ± 1.8^{b}	6.87 ± 0.5^{b}	10.20 ± 0.8 ^{ab}	13.08 ± 1.3^{b}
Jumbo	19.74 ± 1.8^{bc}	16.64 ± 1.5^{bc}	6.65 ± 0.6^{b}	10.20 ± 0.8 ^{ab}	12.20 ± 1.0 ^{bc}
Geneva	19.74 ± 1.9^{bc}	15.08 ± 1.3^{bc}	6.43 ± 0.4^{b}	10.10 ± 0.9 ^{ab}	12.20 ± 0.9 ^{bc}
Hayward	20.63 ± 1.9^{b}	19.30 ± 1.9^{b}	7.09 ± 0.5^{ab}	10.42 ± 0.7^{ab}	13.30 ± 1.1^{ab}
Bidan	11.86 ± 1.1^{d}	8.21 ± 0.6^{d}	2.22 ± 0.1^{d}	5.32 ± 0.4^{c}	6.21 ± 0.5^{d}
HSA	31.50 ± 2.8^{a}	27.06 ± 2.5^{a}	8.87 ± 0.7^{a}	11.31 ± 0.9^{a}	22.18 ± 2.1^{a}

Mean ± SD (standard deviation) of 3 measurements. Average in rows marked with different letters differ significantly ($P < 0.05$).
Abbreviations: DW – dry weight; NF – not found; CGE – cyanidin-3-glucoside equivalent; TDF – total dietary fiber; IDF – insoluble dietary fiber; SDF - insoluble dietary fiber. In Table 2D lyophilized water polyphenols extracts after interaction with HSA were measured. $K_3Fe(CN)_6$ is an internal standard in FTIR spectroscopic method. The relative amounts of chemical groups were calculated from the standard curve Fig. 2D, according to the calibration equation: $y = 0.4508x$, where y is the height of the internal standard peak at different concentrations and x, is the concentration of the internal standard in μM.

effects of free radicals, increases the excretion of toxins from the body, has properties such as bactericides, anti-inflammatory, anti-cancer, enhancing immunity, prevents aging and atherosclerosis (El-Sayed, Hussin, Mahmoud, & Al Fredan, 2013; McGhie & Ainge, 2002; Nishiyama, Fukuda, & Oota, 2005). The content of pigments (chlorophylls *a* and *b*, xanthophylls, and anthocyanins) found in six *A. arguta* cultivars, *A. deliciosa* 'Hayward' and *A. eranthia* 'Bidan' are presented in Table 2A. The highest quantities of chlorophylls (*a* and *b*) and xanthophylls was found in cultivar 'Bidan', and the lowest was in 'Hayward' ($P < 0.05$). The quantities of chlorophylls and xanthophylls in all species of *A. arguta* were higher than in 'Hayward'. The highest content of anthocyanins was noted in the case of 'Weiki' and 'Bingo'. The amounts of carotenoids (lutein, zeaxanthin and β-carotene) in six cultivars of *A. arguta* and in 'Hayward' and 'Bidan' are shown in the Table 2B. Zeaxanthin was not found in 'Hayward' and 'Bidan', and β-carotene was not estimated in 'Hayward'. The content of lutein in cultivar 'Bidan' was significantly higher than in 'Hayward'. *A. arguta* fruits, grown in Polish conditions, contained lutein, especially 'Weiki', 'Jumbo' and 'Geneva', compared to 'Hayward'. Such relation also concerns the total sum of carotenoids. Consumption of the pigments lutein and zeaxanthin is very important for human health, and the lowest contents of these pigments were in *A. deliciosa* 'Hayward', and the highest amount was in *A. arguta* 'Weiki' (Latocha et al., 2010). In our studies the highest levels of total carotenoids were found in 'Bidan' and 'Weiki'. Some authors pointed out that the contents of carotenoids and phenolic compounds in kiwi fruit can have a big impact on climate and soil conditions (fertilization). Park et al. (2013) found significant differences in the content of carotenoids and chlorophylls in 'Bidan' obtained from organic crop. Incomplete transformation of chloroplast into chromoplast in fruit of these species might be

the other reason, which was mentioned by McGhie and Ainge (2002). Nevertheless, considerable oscillations of lutein and other carotenoids contents may be present even for the same species, which is indicated by Nishiyama et al. (2005). Therefore, it is advisable to make a selection of cultivars also in the view of their chemical composition. As it was described above, the cultivars contain also significant amounts of pigments, including chlorophylls and carotenoids (McGhie & Ainge, 2002; Nishiyama et al., 2005; Park et al., 2014). Among the carotenoids (which are the precursors of vitamin A), lutein and zeaxantin characterized by antioxidant properties (Fraser & Bramley, 2004).

The amount of total dietary fiber (TDF) and its fractions (IDF and SDF) and IDF/SDF of six *A. arguta* cultivars and *A. eriantha* and *A. deliciosa* 'Bidan' and 'Hayward' are shown in the Table 2C. All cultivars of *A. arguta* have a high content of TDF, particularly in its insoluble fraction IDF (from 14.02% to 21.40% DM in 'Jumbo' and 'M1', respectively). IDF content in 'Hayward' and 'Bidan' was significantly lower (from 6.97% to 9.76% DM), respectively. This fraction of dietary fiber has a significant impact on the normal motility of intestinal. Also fraction SDF was significantly higher in all fruits of *A. arguta* and ranged from 5.43% to 6.97% DW for 'Bingo' and 'Weiki', respectively. This fraction in the fruits of *A. deliciosa* was lower and ranged from 3.96% to 5.12% DW for 'Bidan' and 'Hayward', respectively. The fraction of soluble fiber has a significant effect on lipid metabolism, and therefore plays an important role in the prevention of cardiovascular diseases. Relation between the IDF to SDF fractions was less favorable in 'Bingo' and in 'M1' and calculated as 3.76:1. In other fruits this relation was more favourable, and the best was in 'Hayward' (1.36:1). It was shown previously that amount of TDF in kiwi fruits was high (80–84 mg/100 g DW) (Park et al., 2011, 2013), and the relation between its fractions IDF to SDF was worthwhile (1.8–2.3:1).

3.5. Binding properties of antioxidants with HSA evaluated by fluorometry and FTIR

Phenolic acids and their derivatives are abundant in kiwi fruits. HSA interacts with the compounds with high affinity. We have studied the specific binding of HSA with polyphenol extracts of all samples by fluorescence spectroscopy. Addition of kiwi fruit polyphenol ethanol extract to HSA results in the change in the fluorescence intensity and in a shift (blue and red) in the HSA emission maximum (Figs. 1 and 2). As a control for binding properties, HSA was dissolved in the same amount of ethanol as the added fruit polyphenol ethanol extracts. The fluorescence intensity of HSA in buffer in the absence of kiwi fruit extract at the emission maximum was about 1000 (Fig. 2A, line 1). Fluorescence of HSA in ethanol (Fig. 2A, line 2, Aa, Ab) decreased to 970.79. Ethanol decreased the FI of HSA of about 2.92%. With the addition of 0.17 mg/mL of kiwi fruit extracts the fluorescence intensity (FI) dropped for 'Weiki', 'Hayward', 'M1' and 'Bidan' to 897.89, 857.25, 752.27, and 152.28, respectively (Fig. 2A, lines 3–6). The highest decrease in the fluorescence intensity between the presented four fruits was in 'Bidan' (Fig. 2A, line 6). The 3D-FL spectra of HSA in the presence of kiwi fruit extracts is provided in Fig. 2(A–D). The polyphenol extracts of presented kiwi fruits were similar and two peaks were shown in the spectra. The binding properties are correlated to tryptophan amino acid as the excitation wavelength is centered largely around 280–285 nm and not on 275 nm, which supports our observation that the fluorescence results from tryptophan and not from tyrosine and phenylalanine. In the 3D-FL contour spectra of HSA with fruit extracts, there are blue and green colors in the center of each figure represent the maximum intensity, which corresponds to the emission maximum resulting from tryptophan amino acid. A single contour is obtained for HSA (Fig. 2 A), which corresponds to 280 nm and 320 nm as the excitation and emission wavelengths, respectively. There are only some examples of 3D-contour spectra to show the results of the experiment. The images are similar, therefore only some of them are shown. Kiwi fruits and their aqueous extracts are not toxic, because all determined compounds have health benefits: they are antioxidative, direct scavenge of free radicals, stimulate immune activity, anti-inflammator, hypolipidemic and have protective effects on the digestive system, and inhibit the growth of cancer cells (Amer, Eid, & Hamad, 2014). We have presented the ethanol polyphenol extracts of fruits in interaction with HSA, because they included a part of denaturation of ethanol itself in the protein (Fig. 1). Therefore the quenching in ethanol solution was compared with HSA in ethanol. The obtained calculations of the binding in ethanol was compared in water solution (Fig. 2 B). HSA showed two peaks: peak a with $\lambda_{ex}/\lambda_{em}$ (nm/nm)=227/349 with FI = 672.15 and peak b with $\lambda_{ex}/\lambda_{em}$ (nm/nm)=280/352, FI = 902.51 (Fig.2Aa, Ab). After interaction of HSA with 'Bidan' only one peak b is shown (Fig. 1A, Aa, $\lambda_{ex}/\lambda_{em}$ (nm/nm) = 278/357, FI = 152.18). First peak a disappeared after interaction with kiwi fruit 'Bidan', having high antioxidant capacity. In case of 'Hayward' (Fig. 1B, Bb, peak a $\lambda_{ex}/\lambda_{em}$ (nm/nm) = 227/349, FI = 567.69 and peak b $\lambda_{ex}/\lambda_{em}$ (nm/nm) = 279/354, FI = 743.36); 'AM1' (Fig. 1C, Cc, peak a $\lambda_{ex}/\lambda_{em}$ (nm/nm) = 227/354, FI = 263.42 and peak b $\lambda_{ex}/\lambda_{em}$ (nm/nm) = 278/355, FI = 783.31); 'Weiki' (Fig. 1D, Dd, peak a $\lambda_{ex}/\lambda_{em}$ (nm/nm) = 227/354, FI = 324.65 and peak b $\lambda_{ex}/\lambda_{em}$ (nm/nm) = 277/358, FI = 850.67) two peaks changed their intensities, depending on the bioactivity of the kiwi fruit extracts. As an example of the investigated samples the order in binding properties was the following: 'Bidan' (84.31%) > 'M1' (22.51%) > 'Hayward' (11.70%) > 'Weiki' (7.51%). The results between the ethanolic and water extracts (Fig. 2B) were slightly different with the following binding properties: 'Bidan' (78.25%) > 'M1' (20.93%) > 'Hayward' (10.56%) > 'Weiki' (10.05%). The peaks a and b for water extracts were in the same position as for ethanolic extracts with a difference for 'Bidan' (Fig. 2Bb, peak a with $\lambda_{ex}/\lambda_{em}$ (nm/nm) = 227/349, FI = 297.15 and peak b $\lambda_{ex}/\lambda_{em}$ (nm/nm) = 290/359, FI = 423.14).

From the emission spectral studies it is understandable that kiwi fruit extracts influence the fluorescence quenching. Our results are in line with other reports, which demonstrated chemical classes of natural polyphenols, their bioactivities and bioavailability and metabolism (Li & Hagerman, 2013).

The IR spectra of some kiwi fruit water extracts after interaction with HSA were compared between them and also with HSA without the addition of polyphenol extracts in the range of common peaks (Fig. 2C). The internal standard approach allowed the direct comparison of the intensities of IR peaks among different spectra and therefore between protein-phenol substances in the investigated samples. The IR data obtained by this method was used for quantitative comparison of the absorption bands assigned to specific functional groups [Amide I ($\sim$1650 cm^{-1}); Amide II ($\sim$1550 cm^{-1}); Amide III ($\sim$1400–1200^{-1})]. The peak at 2117 cm^{-1} was chosen, because this region of the spectrum contained no absorbance for the investigated substances, but only for the internal standard The peaks in the spectra have been assigned based on data previously reported for interaction of HSA with polyphenols (Barth, 2007; Park et al., 2013). The standard band was established in the calibration curve and also in all spectra with samples (Fig. 2, at 2117 cm^{-1}). The height of the standard curve was compared with the height of chemical groups in the samples in order to calculate the relative amounts in different samples (insert in Fig. 2C). In 'Weiki', 'AM1', 'Bidan' appeared peak at 2360 cm^{-1}. The maximum of amide I (appears to be shifted toward 1650 cm^{-1}). In 'Bidan' appeared peaks at 864, 813, 777 cm^{-1} sharper than in 'Weiki' and 'Hayward'. Peak at 1205 cm^{-1} was the highest in 'Weiki' (not shown). The quantitative comparison of investigated samples is presented in Fig. 2C and Table 2D.

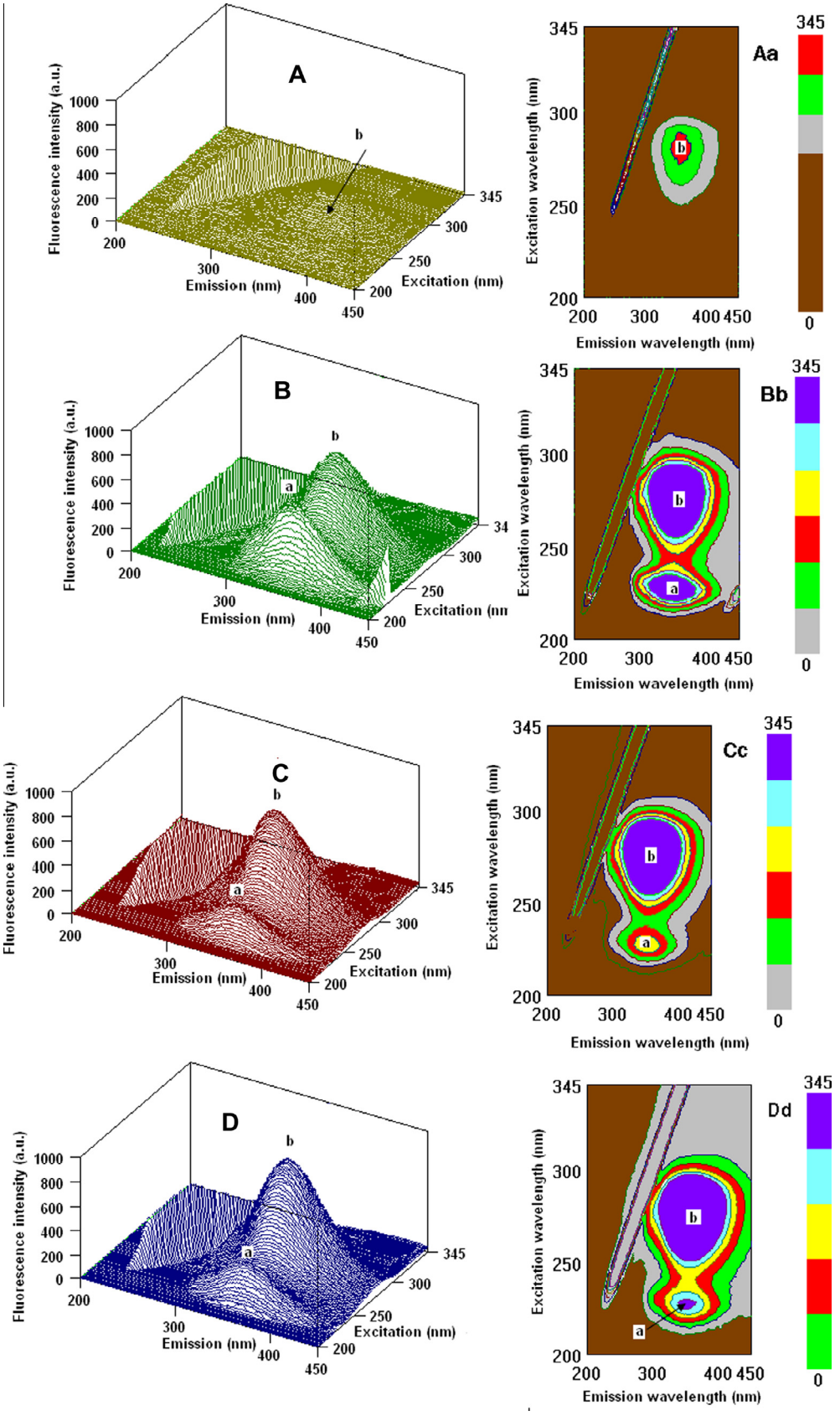

Fig. 1. 3D-FL cross spectral studies of human serum albumin (HSA) with kiwi fruit in ethanol solution. Excitation wavelength scan: 200–345 nm. Emission wavelength scan: 210–450 nm. (A, Aa) HSA + 'Bidan'; (B, Bb) HSA + 'Hayward'; (C, Cc) HSA + 'M1'; (D, Dd) HSA + 'Weiki'. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article). To 20 μl HSA were added 20 μl of 0.17 mg/ml of kiwi fruit ethanol extract. The reaction was during 1 h at room temperature.

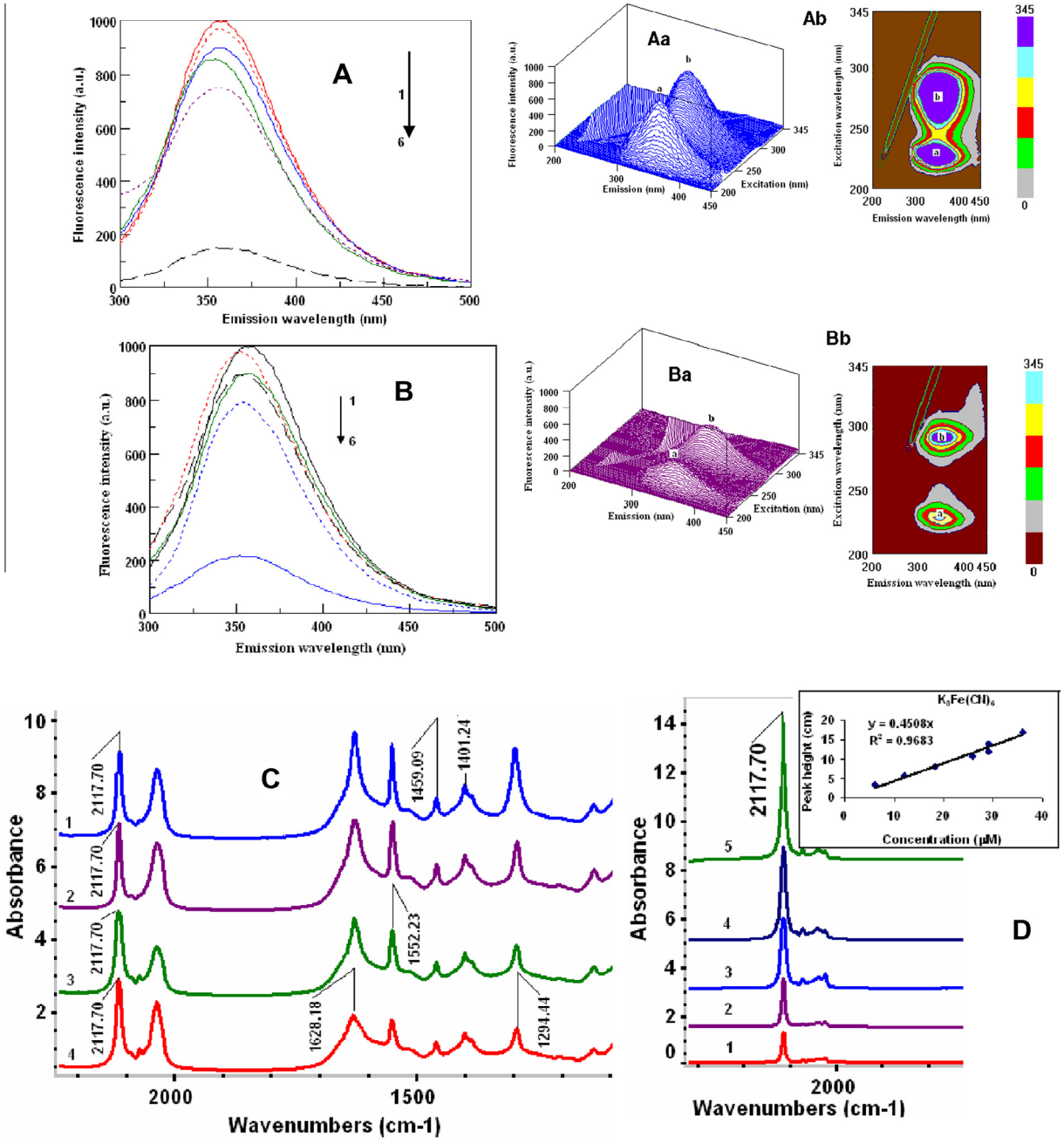

Fig. 2. Emission spectra of HSA in the absence and presence of fruit polyphenol extracts in ethanol (Et) and water (W) at λ_{ex} 280 nm and λ_{em} 300 nm: (A) (1) HSA in buffer [$(2.0 \times 10^{-6}$ mol/L, λ_{em} = 358 nm, fluorescence intensity (FI) = 1000)], (2) HSA in ethanol [$(2.0 \times 10^{-6}$ mol/L, λ_{em} = 355 nm, FI = 970.79)], (3) HSA + 'Weiki^{Et}' (λ_{em} = 359 nm, FI = 897.89), (4) HSA + 'Hayward^{Et}' (λ_{em} = 354 nm, FI = 857.25), (5) HSA + 'M1^{Et}' (λ_{em} = 356 nm, FI = 752.27), (6) HSA + 'Bidan^{Et}' (λ_{em} = 354 nm, FI = 152.28); (Aa) 3D-FL of HSA in ethanol (2.0×10^{-6} mol/L), **Ab**, cross spectral image of HSA. (B) (1) HSA in buffer [$(2.0 \times 10^{-6}$ mol/L, λ_{em} = 358 nm, fluorescence intensity (FI) = 1000)], (2) HSA^{W} [$(2.0 \times 10^{-6}$ mol/L, λ_{em} = 355 nm, FI = 980.20)], (3) HSA + 'Weiki^{W}' (λ_{em} = 359 nm, FI = 899.48), (4) HSA + 'Hayward^{W}' (λ_{em} = 354 nm, FI = 894.40), (5) HSA + 'M1^{W}' (λ_{em} = 353 - nm, FI = 790.74), (6) HSA + 'Bidan^{W}' (λ_{em} = 352 nm, FI = 217.48); Ba, 3D-FL of 'Bidan ^{W}' (2.0×10^{-6} mol/L), Bb, cross spectral image of 'Bidan^{W}'. C, Infrared spectra recorded from KBr pellets containing $K_3Fe(CN)_6$ as an internal standard with the peak at 2117 cm^{-1}. From the top lines 1, HSA^{W}; 2, HSA ^{W} + 'Hayward^{W}'; 3, HSA^{W} + 'M1^{W}'; 4, HSA^{W} + 'Bidan^{W}'. D, Spectral peaks of internal standard at 2117.70 cm^{-1} with different concentrations μM $K_3Fe(CN)_6$: 1 2, 3, 4, 5, 6; 12; 18; 28; 36, respectively. A Nicolet iS 10 FT-IR Spectrometer with the smart iTRTM ATR (attenuated total reflectance) accessory was used to record IR spectra.

During interaction of HSA with polyphenols the lowest decrease in comparison with the HSA sample of 62% was with 'Bidan' water extracts (11.86 ± 1.1 μM) in the range of Amide I bands. Similar relationship was also repeated for Amide II of 70% for 'Bidan', at 1455 cm^{-1} about 75%, then for Amide III at 1400 cm^{-1} was about 53% and at 1300 cm^{-1} – 72%. The calculated average binding property in all Amides for Bidan was 66.4%, which was slightly lower than found by fluorescence (78.25%). According to the results of FTIR measurement and calculations the highest binding ability was in 'Bidan', following by 'M1', 'Bingo', 'Anna', 'Geneva', 'Jumbo', 'Weiki' and 'Hayward' (Table 2D). Our results were in accordance with Derenne et al. (2013), where cancer cells exposed *in vitro* to 6 polyphenols: 3 natural well documented polyphenols (curcumin, epigallocatechin gallate (EGCG) and quercetin) and 3 synthetic

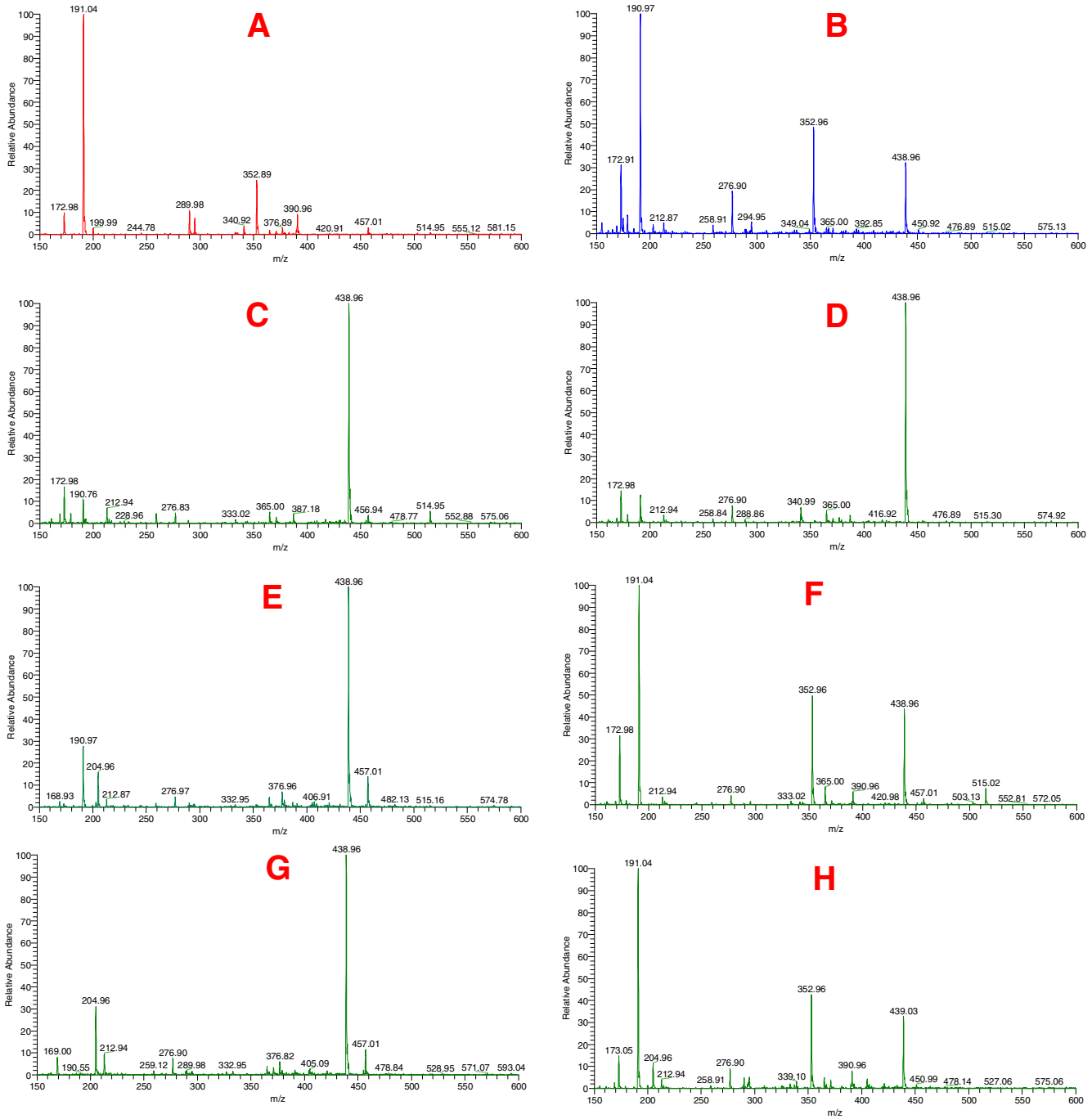

Fig. 3. ESI-MS spectra in negative ion mode of kiwi fruit cultivar groups ethanolic extracts. (A–H) 'Hayward', 'Bidan', ' Jumbo', 'Geneva', 'Weiki', 'Bingo', 'Anna', 'M1'.

molecules with a very closely related chemical structure. As the fluorometric methods also IR spectroscopy can be used as an additional indication of similarity and differences between the cultivars.

MS spectra of three different families of *Actinidia* were determined and compared (Table 3, Fig. 3). In cultivars 'Hayward', 'Bidan', 'Bingo' and 'M1' the main peak was at m/z 190.97 (100%). Only in cultivar 'Geneva' the same peak appeared with 30% in MS. This peak corresponded to quinic acid, which was found in all previously studied kiwi fruit cultivars of *A. deliciosa* and *A. eriantha* (Park et al., 2014). Second average peak at m/z 352.9 (caffeoyl-quinic acid) was with different sizes (% in MS): 'Hayward' (25), 'Bidan' (50), 'Bingo' (50) and 'M1' (40). All cultivars from *A. arguta* group and *A. eriantha* kiwi fruit have a peak at m/z 438.9. 'Jumbo', 'Geneva', 'Weiki' and 'Anna' are characterized with this peak as the biggest (100%). Interesting that the same peak appeared in two cultivars of *A. arguta* ('Bingo' and 'M1') and in *A. eriantha* ('Bidan') in the size of 45–30% MS. From the spectra data it can be concluded that 'Bidan', 'Bingo' and 'M1' have similar peaks composition. The recorded spectra were in the same scale (in the range between 150 and 600 m/z) for comparison. We choose negative mode for the MS method, because in many publications was described that this mode is the best for analysis of low- molecular phenolic compounds (Chen, Inbaraj, & Chen, 2012; Clifford, Wu, &

Table 3
Mass spectral data of polyphenol extracts from different kiwi fruit samples.

Kiwi fruits	$[M-H]^-$(% in MS)	Compound
Hayward	172.9(10)	[di-caffeoylquinic acid – caffeoyl-quinic]
	191 (100)	Quinic acid
	352.9 (25)	Caffeoyl-quinic acid
Bidan	172.9 (20)	[di-caffeoylquinic acid – caffeoyl-quinic]
	191 (100)	Quinic acid
	352.9 (50)	Caffeoyl-quinic acid
	438.9 (30)	Methyl (epi)afzelechin-3-O-gallate
Jumbo	172.9 (18)	[di-caffeoylquinic acid – caffeoyl-quinic]
	438.9 (100)	Methyl (epi)afzelechin-3-O-gallate
Geneva	172.9 (18)	[di-caffeoylquinic acid – caffeoyl-quinic]
	438.9 (100)	Methyl (epi)afzelechin-3-O-gallate
Weiki	191 (30)	Quinic acid
	438.9 (100)	Methyl (epi)afzelechin-3-O-gallate
	457 (15)	Epigallocatechin gallate
Bingo	172.9 (30)	[di-caffeoylquinic acid – caffeoyl-quinic]
	191 (100)	Quinic acid
	352.9 (50)	Caffeoyl-quinic acid
	438.9 (45)	Methyl (epi)afzelechin-3-O-gallate
	515 (10)	Di-*O*-caffeoylquinic acid
Anna	438.9 (100)	Methyl (epi)afzelechin-3-O-gallate
	457 (10)	Epigallocatechin gallate
M1	172.9 (15)	[di-caffeoylquinic acid – caffeoyl-quinic]
	191 (100)	Quinic acid
	352.9 (40)	Caffeoyl-quinic acid
	438.9 (30)	Methyl (epi)afzelechin-3-O-gallate

Kuhnert, 2006; Gomez-Romero et al., 2011). The main peaks which are presented in Fig. 3 and Table 3 can be used as fingerprint for characterization of different kiwi fruit cultivars of different families, based on the percentage of the main peaks and for *A. arguta* presented for the first time.

In recent study of Park et al. (2014) have been reported results only for kiwi fruit 'Hayward' and 'Bidan' and other cultivars, grown in Asia. In the present report fruits of *A. arguta*, which were cultivated in Poland, for the first time were compared with widely consumed kiwi fruits, using advanced analytical methods.

4. Conclusions

We examined the fruit of *A. arguta,* derived from Polish ecological plantations, which are generally a better sources of bioactive compounds, especially polyphenols and also vitamin C, than kiwi fruit 'Hayward' and inferior to kiwi fruit 'Bidan'. The highest bioactivities among the studied *A. arguta* have varieties 'M1', 'Bingo' and 'Geneva'. Varieties 'Weiki', 'Jumbo' and 'Geneva' have the highest content of total carotenoids, particularly, lutein and β-carotene, zeaxanthin also, which does not contain the tested fruits 'Bidan' and 'Hayward'. These varieties also have the highest content of chlorophylls and xanthophylls. Hardy kiwi fruits contain higher dietary fiber content than kiwi fruit 'Bidan' and 'Hayward'. IDF fiber fraction is also higher, especially in the 'Bingo' and 'M1' in comparison with 'Hayward' and 'Bidan'. The high bioactivity, binding ability with HSA, nutritional value and also pro-healthy action of fruits of *A. arguta* from Polish ecological plantation allows recommend them for the consumption and dissemination.

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