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Fluorescence studies by quenching and protein unfolding on the interaction of bioactive compounds in water extracts of kiwi fruit cultivars with human serum albumin

Yong Seo Park [a,*], Martin Polovka [b], Alma Leticia Martinez-Ayala [c], Gustavo A. González-Aguilar [d], Kyung-Sik Ham [e], Seong-Gook Kang [e], Yang-Kyun Park [e], Buk-Gu Heo [f], Jacek Namiesnik [g], Shela Gorinstein [h,**,1]

[a] Department of Horticultural Science, Mokpo National University, Muan, Jeonnam, South Korea
[b] National Agricultural and Food Centre VUP, Food Research Institute, SK-824 75 Bratislava, Slovak Republic
[c] Centro de Desarrollo de Productos Bioticos, Instituto Politécnico Nacional, Carretera Yautepec-Jojutla, Km. 6, calle CEPROBI No. 8, Col. San Isidro, Yautepec, Morelos 62731, Mexico
[d] Research Center for Food & Development, A.C. (CIAD), Carretera a Ejido La Victoria, Km 0.6, Hermosillo, Sonora 83304, Mexico
[e] Department of Food Engineering, Mokpo National University, Muan, Jeonnam, South Korea
[f] Naju Foundation of Natural Dyeing Culture, Naju 520-931, South Korea
[g] Department of Analytical Chemistry, Chemical Faculty, Gdańsk University of Technology, 80 952 Gdańsk, Poland
[h] The Institute for Drug Research, School of Pharmacy, The Hebrew University, Hadassah Medical School, Jerusalem 91120, Israel

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ABSTRACT

The main aim of this investigation was to characterize new kiwi fruit cultivars after cold storage treatment and to determine the similarities and differences between them, using spectroscopic methods. The chemometric comparison of kiwi fruit cultivars based on physicochemical indices during cold storage was carried out. All kiwi fruit cultivars showed a high level of correlation between the contents of phenolic compounds (polyphenols, tannins and flavonoids) and their antioxidant capacities. The interactions of soluble polyphenols of different kiwi fruit cultivars with human serum albumin (HSA) were investigated by fluorescence. The obtained statistical and fluorescence results allow to classify the investigated kiwi fruit cultivars according to their properties. The antioxidant properties of different cultivars monitored by β-carotene assay showed that the highest percentage of antioxidant activity (%AA) at the end of the cold storage was detected for 'SKK-12' (27.61 ± 2.44) %AA with the lowest shelf life (8 weeks) and the lowest was found for 'Hayward' variety (8.33 ± 0.74) %AA with the highest shelf life (24 weeks). The averaged amount of polyphenols in 'Bidan' and 'SKK-12' 13.97 ± 1.95 mg GAE/g was much higher than in other cultivars 3.93 ± 3.26 mg GAE/g, without respect on time of cold storage. The HSA-binding capacities of these cultivars were the highest and correlated with their antioxidant capacities. To our knowledge this is the first report showing differences and similarities in new kiwi fruit cultivars, using spectroscopic techniques. The fact that fluorescence spectral methods are applied as a powerful tool to show the photophysical properties of intrinsic fluorophores in protein molecules in the presence of fruit extracts is important in this study. In conclusion, the obtained knowledge would contribute to the pharmaceutical development and clinical application of kiwi fruit extracts.

1. Introduction

Kiwi fruits with their multiple health benefits have the potential to become part of our "daily prescription for health" [1]. Comparison of the antioxidant effects *in vitro* demonstrated that kiwi fruit had stronger antioxidant effects than oranges and grapefruits, which are rich in vitamin C, and gold kiwi fruit had the strongest antioxidant effects [2]. Changes in the biochemical parameters of fruits depending on the ripening at low temperature and duration of storage and other treatments were widely studied in the last years [3–5]. There are

* Corresponding author. Tel.: +82 61 450 2376; fax: +82 61 453 2309.
** Corresponding author. Tel.: +972 2 675 8690; fax: +972 2 675 7076.
E-mail addresses: ypark@mokpo.ac.kr (Y. Seo Park), shela.gorin@mail.huji.ac.il (S. Gorinstein).

[1] This article was written in memory of my dear brother Prof. Simon Trakhtenberg, who died in November 2011, who encouraged me and our research group during all his life.

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numerous reports about 'Hayward' and 'Hort 16 A' cultivars, their properties, cold storage treatments and health benefits [5–8]. Oppositely, very little information is found about the shelf life of new kiwi fruit cultivars bred in Korea and changes in their quality during cold storage [9,10]. So, in the present study we compared the effect of long term cold storage on physicochemical properties and bioactive components of five new cultivars with the known ones such as 'Hayward' and 'Hort16A'. For this purpose texture and sensory analyses, total soluble phenols, antioxidant activity and quality parameters were determined. In order to receive the reliable results of total antioxidant capacities two generally accepted assays (FRAP and β-carotene) were used. The information of combination of spectroscopic and fluorometric methods for comparison of different kiwi fruit cultivars is limited. Human serum albumin is the drug carrier protein and serves to greatly amplify the capacity of plasma for transporting drugs. It is interesting to investigate *in vitro* how this protein interacts with polyphenols extracted from kiwi fruit samples in order to get useful information of the properties of polyphenol–protein complex. There are a number of publications showing the interaction of HSA with different substances, but not with extracts of kiwi fruit [11–15]. As far as we know no results of such investigations were published. Therefore the functional properties of new kiwi fruit cultivars were studied by the interaction of water polyphenol extracts with HSA, using 3D FL.

2. Experimental methods

2.1. Fruit samples

Kiwi fruits of seven cultivars were harvested at the optimal stage in orchard, located in Haenam county (longitude 126°15″ and latitude 34°18″), Jeonnam province, Korea, in 2013. All cultivars, except 'Hort 16 A', were bred in Korea. 'Hort 16 A' is a New Zealand gold kiwi fruit and was purchased in Jeju Island. 'Hwamei' and 'SKK-12' are green kiwi fruit cultivars of 100 g size as 'Hayward'. 'Bidan' has a smaller size of 20 g and its skin is white (flesh is green). The fruits were stored in cold room (0 °C, 90% RH) for 24 weeks. The samples were treated with liquid nitrogen in order to prevent oxidation of phenolic compounds and then lyophilized as previously described [6,16].

2.2. Chemicals and reagents

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), human serum albumin (HSA), Folin–Ciocalteu reagent (FCR), β-carotene, linoleic acid and Tween-40 (polyoxyethylene sorbitan monopalmitate) and $FeCl_3 \cdot 6H_2O$ were purchased from Sigma Chemical Co., St Louis, MO, USA. 2,4,6-Tripyridyl-*s*-triazine (TPTZ) was from Fluka Chemie, Buchs, Switzerland. All reagents were of analytical grade.

2.3. Analytical methods

The fruits were analyzed for firmness, total soluble solids (TSS), pH, total acidity (TA), starch concentration, reducing sugar, ethylene and respiration contents, and sensory quality as was previously described [9,10]. Total soluble polyphenols were extracted with water at room temperature during 1 h. The polyphenols were determined by the Folin–Ciocalteu method [17] with absorbance measurement at 750 nm (Spectrophotometer, Hewlett-Packard, model 8452 A, Rockvile, USA). The results were expressed as mg of gallic acid equivalents (GAE) per g dry weight (DW). Total flavonoid content was determined by an aluminum chloride colorimetric method with some modifications. The absorbance was measured at 510 nm. The extracts of condensed tannins (procyanidins) with 4% methanol vanillin solution were measured at 500 nm. (+)-Catechin served as a standard for flavonoids and tannins [16], and the results were expressed as catechin equivalents (CE). Ferric-reducing/antioxidant power (FRAP) reagent (2.5 mL of a 10 mmol ferric-tripiridyltriazine solution in 40 mmol HCl plus 2.5 mL of 20 mmol $FeCl_3 \cdot H_2O$ 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 kiwi fruit extract samples. The absorbance was measured at 595 nm [18]. Antioxidant assay, using β-carotene linoleate model system (β-carotene): β-carotene (0.2 mg) in 0.2 mL of chloroform, linoleic acid (20 mg), and Tween-40 (polyoxyethylene sorbitan monopalmitate) (200 mg) were mixed. Chloroform was removed at 40 °C under vacuum, and the resulting mixture was diluted with 10 mL of water. To this emulsion was added 40 mL of oxygenated water. Four milliliter aliquots of the emulsion were pipetted into different test tubes containing 0.2 mL of kiwi fruit extracts (50 and 100 ppm). The absorbance at 470 nm was taken at zero time ($t=0$) during $t=180$ min at an interval of 15 min [19].

2.4. Fluorometric measurements

Fluorometric measurements were used for the evaluation of binding properties of kiwi fruit extracts to human serum albumin. Two dimensional (2D-FL) and three-dimensional (3D-FL) fluorescence measurements were recorded on a model FP-6500, Jasco spectrofluorometer, serial N261332, Japan, equipped with 1.0 cm quartz cells and a thermostat bath and the excitation and emission slits were set at 5 nm while the scanning rate was 1200 nm min^{-1}. For the fluorescence measurement, 3.0 mL of 2.0×10^{-6} mol/L. HSA solution and various amounts of kiwi fruit extracts were added to a 1.0 cm quartz cell manually using a micro-injector. The concentrations of kiwi fruit extracts were ranged from 0 to 1.5 mg/mL, and the total accumulated volume of kiwi fruit extracts was no greater than 150 μL. The corresponding fluorescence emission spectra were then recorded in the range of 300–500 nm upon excitation at 280 nm in each case. The three-dimensional fluorescence spectra were measured under the following conditions: the emission wavelength was recorded between 200 and 795 nm, the initial excitation wavelength was set at 200 nm with an increment of 5 nm, and the others scanning parameters were just the same as those for the fluorescence emission spectra. All solutions for protein interaction were prepared in 0.05 mol/L Tris–HCl buffer (pH 7.4), containing 0.1 mol/L NaCl.

2.5. Statistical methods

Basic chemometric characterization of the investigated kiwi fruit extract samples according to their quality evaluation was carried out by summary, descriptive (normal probability, box/whisker, and dot plots) statistics and multisample median testing using the statistical package Unistat v. 6.0 (Unistat, London, United Kingdom).

3. Results and discussion

3.1. Physicochemical properties and statistical analysis of kiwi fruit cultivars

The physicochemical properties of kiwi fruit cultivars showed changes during cold storage for 8–24 weeks and the most extreme ones between them were in 'SKK-12' and 'Hayward' (Fig. 1a). Polyphenols in water extracts were the highest in 'Bidan' and 'SKK-12' and the lowest in 'Hayward' (Fig. 1a). Flavonoids and tannins for 'SKK-12' and 'Hayward' differ, but not always significantly. Relatively high content of bioactive compounds and antioxidant properties of kiwi fruit determined by the advanced analytical methods justify its use as a source of valuable antioxidants [16]. The cultivar 'Bidan' in comparison with the classic 'Hayward' showed significantly higher bioactivity

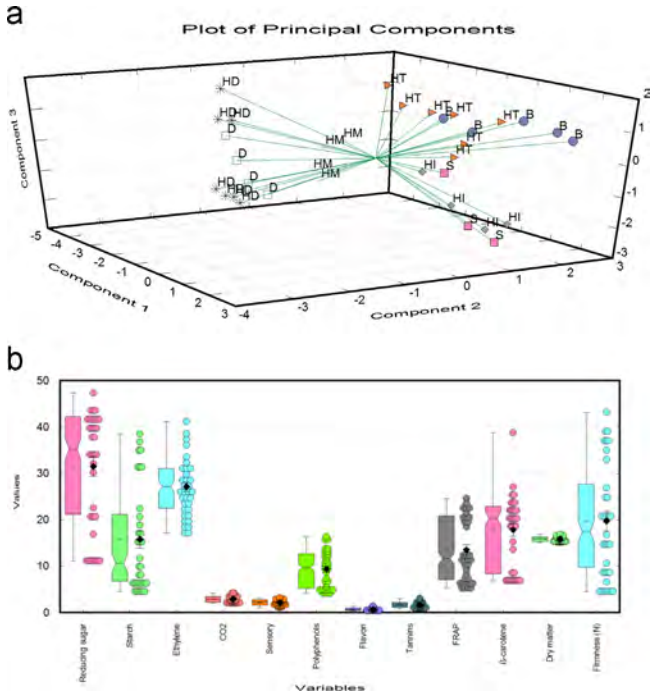

Fig. 1. (a) Principal component analysis of the seven kiwi fruit cultivars (B – 'Bidan', S – 'SKK-12', D – 'Daheung', Hm – 'Haenam', Ht – 'Hort 16 A', Hi – 'Hwamei', Hd – 'Hayward'). Variables selected for principal components construction were the following: reducing sugar, starch, ethylene, CO_2, sensory, polyphenols, flavonoids, tannins, FRAP, ß-carotene, dry matter, and firmness. (b) Box-whiskers diagram represents the distribution of individual monitored characteristics of kiwi fruit under the study without respect on time of treatment or kiwi fruit variety.

[16]. The values of β-carotene activities and ferric-reducing/antioxidant power were the highest in 'SKK-12'. The lowest results were estimated in 'Hayward'. All kiwi fruit cultivars showed a high level of correlation between the contents of phenolic compounds and their antioxidant values. The evaluation of softening characteristics [20] was similar to our results. These observations indicate that low temperature modulates the ripening of kiwi fruit in an ethylene-independent manner, suggesting that kiwi fruit ripening is inducible by either ethylene or low temperature signals [3]. Our results are in the line with Krupa et al. [21] that firmness rapidly decreased and the TSS increased for all cultivars during the first 14 days of storage at 1 °C. The obtained results of kiwi fruit bioactivity correspond with the data of other reports [8,21–23]. Our obtained results *in vitro* were similar to Lee et al. [24], where the effects of the two main kiwi fruit cultivars (gold and green kiwi fruits) and their active phenolic compound were evaluated. Our results are in full correspondence with others [21], showing a strong correlation between polyphenol contents and antioxidant activity in hardy kiwi fruits. Polyphenols in ripe fruits were similar to the ones of the of storage harvest maturity (8–10% SSC). Shelf life of new cultivars bred in Korea such as 'SKK 12', 'Hwamei', 'Daheung', and 'Bidan' considerably reduced compared to 'Hort 16 A' or 'Hayward' cultivars.

Principal component analysis (PCA) of the complete dataset proved only partial successful differentiation without respect on kiwi fruit varieties and time of treatment. Number of PC is equal or lower than the number of original variables (12 variables: reducing sugar, starch, ethylene, CO_2, sensory, polyphenols, flavonoids, tannins, FRAP, ß-carotene, dry matter, and firmness). Eigenvectors are created to represent each case in a dataset. The components are constructed in a way that the first PC describes the maximum variability of the whole dataset, the 2nd maximum of the remaining. In our case, it is obvious, that very strong parameter is the varieties of kiwi fruit, as the eigenvectors belonging to cases of the same variety are depicted close to each other in majority of cases. First three PCs cumulatively explain more than 80% of the whole dataset variability (1st PC∼42%, 1st+2nd∼69%, 1st+2nd+3rd∼81%, but PCs alone – 42%, 27% and 11%, respectively). This is satisfactory result (usually if cumulative variance is described for >75%). Looking on Eigenvalues, it can be concluded that 1st PC represents dominantly a contribution of starch content (∼40%) followed by reducing sugars and firmness (∼39%, each). Thus, these 3 parameters cover maximum of the variability. In the 2nd PC, content of polyphenols (∼45%), β-carotene and FRAP values (>44%) and in the 3rd PC, dry matter and flavonoids content (∼70%) dominates. Interesting view on the dataset offers the box-whisker plot combinig together median, quartiles and extremes in the dataset. The box-whisker diagram constracted of all the monitored kiwi fruit parameters (Fig. 1b) presents the distribution of individual experimental characteristics without respect on time of treatment and kiwi fruit variety. It illustrates the influence of last two mentioned effects on the monitored characteristics from global point of view. Based on fluorescence profiles which exhibited high binding properties with HSA the seven new kiwi fruit cultivars were classified for three groups: 'Hayward' (including 'Daheung', 'Haenam', 'Hwamei' and 'SKK12'), 'Bidan' and 'Hort 16 A' [9,10,25,26]. The properties of kiwi fruit's phenol extracts showed their ability to quench HSA, forming the complexes similar to the ones between the proteins and pure flavonoids such as quercetin [25]. Therefore it was interesting to apply fluorescence studies on some of the three kiwi fruit cultivars groups and to determine the similarities and differences in their quenching behavior.

3.2. Emission spectral studies

It was shown that all main fluorescence peaks in water extracts were located between λem from 336 to 651 nm with FI from 400 to 26.4 [9]. According to the value of FI in the main peaks of kiwi fruit cultivars water extracts were as following: 'SKK 12' (λem=340 nm and FI=404.1), following by 'Bidan' (λem=336 nm and FI=236.6), 'Hort 16 A' (λem=340 nm and FI=194.9), 'Haenam' (λem=338 nm and FI=129.9), 'Daehung' (λem=338 nm and FI=121.9) and the lowest in 'Hayward' (λem=339 nm and FI=113.4). It was the best correlation between the obtained results of polyphenols in water extracts with the data of fluorescence intensity measurements [9]. Addition of polyphenol water extract of 'Bidan' to HSA results in change in both the fluorescence intensity and a shift (blue and red) in the emission maximum of HSA (Figs. 2–4). Fluorescence quenching was observed up to 1.21 mg/mL water extract of 'Bidan' and above this concentration there was no change in the fluorescence intensity of HSA. The pattern of shift in the emission maximum of HSA during interaction of aqueous kiwi fruit extract is similar to the denaturation of urea with egg albumin, shown in our research [15]. The 3D spectrum of HSA in the absence and presence of kiwi fruit extract is provided in Fig. 2(a–e). 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, which corresponds with our previous studied on denaturation of proteins [15]. In the 3D contour spectra of HSA with 'Bidan' extract, there is a blue color in the center of each figure represents the maximum intensity which corresponds to the emission maximum resulting from tryptophan amino acid. A single contour is obtained for HSA (Fig. 2a) which corresponds to 280 nm and 300 nm as the excitation and emission wavelengths, respectively. The fluorescence intensity of HSA in the absence of kiwi fruit extract at the emission maximum is about 967.64 (Fig. 3a, line 1), then with the addition of 1.21 mg/mL kiwi fruit extract the fluorescence intensity dropped to 58.60 (Fig. 3a, line 17). Decrease in the fluorescence intensity was about 94%. The shifts in the emission maximum of HSA and the variations in the fluorescence intensity on the addition of

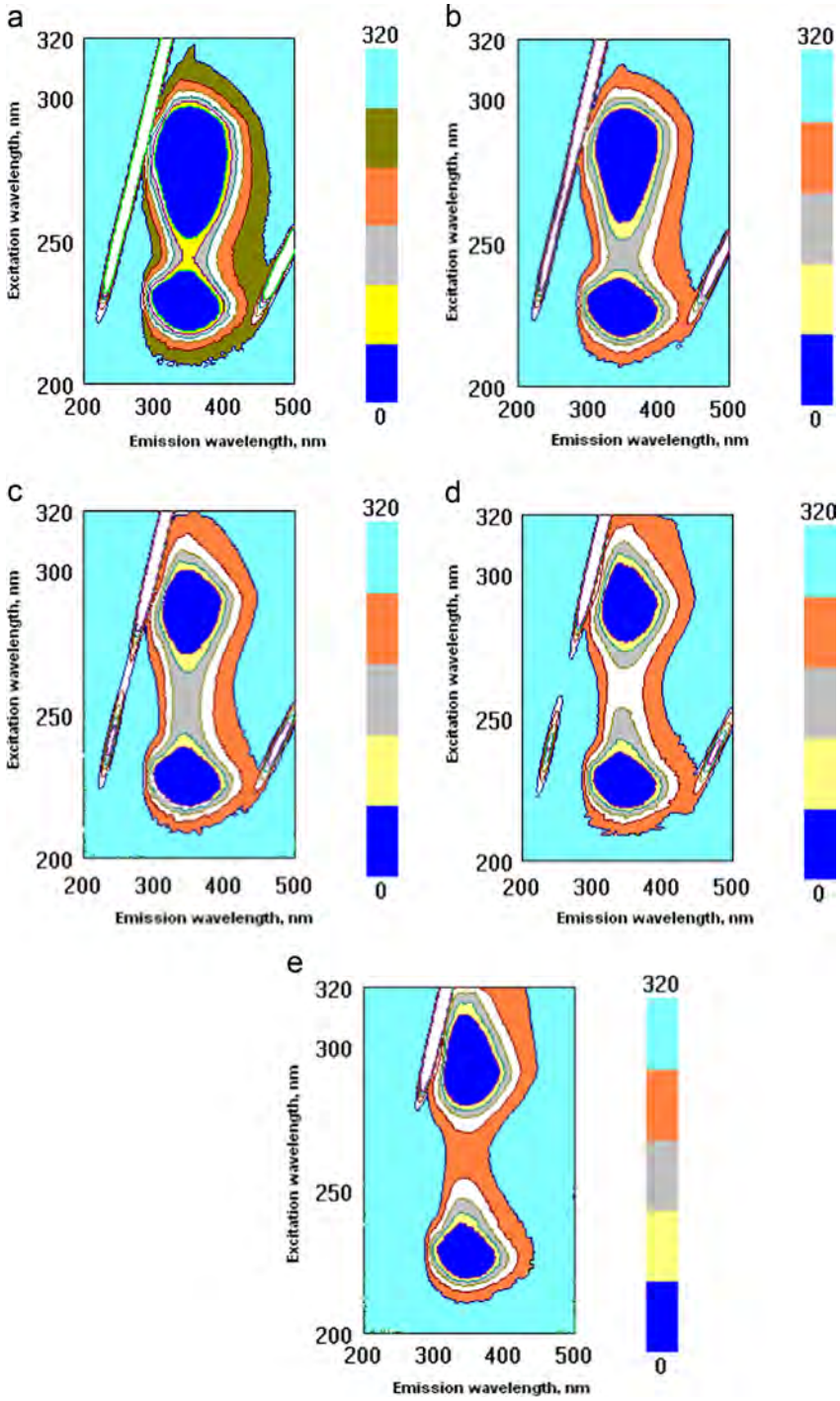

Fig. 2. 3D contour spectral studies of HSA with kiwi fruit cultivar 'Bidan' in aqueous solution. Excitation wavelength scan: 200–320 nm. Emission wavelength scan: 200–500 nm. (a) HSA, (b) HSA+0.08 mg/mL 'Bidan', (c) HSA+0.17 mg/mL 'Bidan', (d) HSA+0.24 mg/mL 'Bidan', and (e) HSA+0.40 mg/mL 'Bidan'. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article).

polyphenol water extract of 'Bidan' are shown in Fig. 3b and c. A gradual shift in the emission maximum of HSA was observed during interaction with extract of 'Bidan' up to 1.21 mg/mL (Fig. 3c). From the emission spectral studies it is understandable that kiwi fruit extracts influence the fluorescence quenching. Fig. 3 represents other cultivars such as 'Hort 16 A'. The change in the fluorescence intensity of HSA during addition of 'Hort 16 A' (Fig. 3d, e) was lower than for cultivar 'Bidan'. At the same added volume (40 μL) to HSA of aqueous extracts of 'Bidan', 'Hort 16 A' and mangosteen, which contained different amounts of soluble polyphenols, the decrease in the fluorescence intensity was 64.6% (Fig. 3a, line 6 from the top), 25.5% (Fig. 3d, line 6 from the top), and 26.1% (Fig. 3f, line 6 from the top), respectively. In the case of 'Hort 16 A' and mangosteen the change was smaller as much as twice in comparison with 'Bidan'. 3D contours of 'SKK12' and 'Hort16A' after interaction with HSA at the same added volume (different amount of polyphenols) are shown in Fig. 4a and b. The quenching properties of these cultivars are directly correlated with their antioxidant properties and the amount of polyphenols. The binding of antioxidants to HSA was also studied with the combination of another exotic fruit mangosteen, which is similar to kiwi fruit in its properties [27]. One antioxidant such as polyphenol

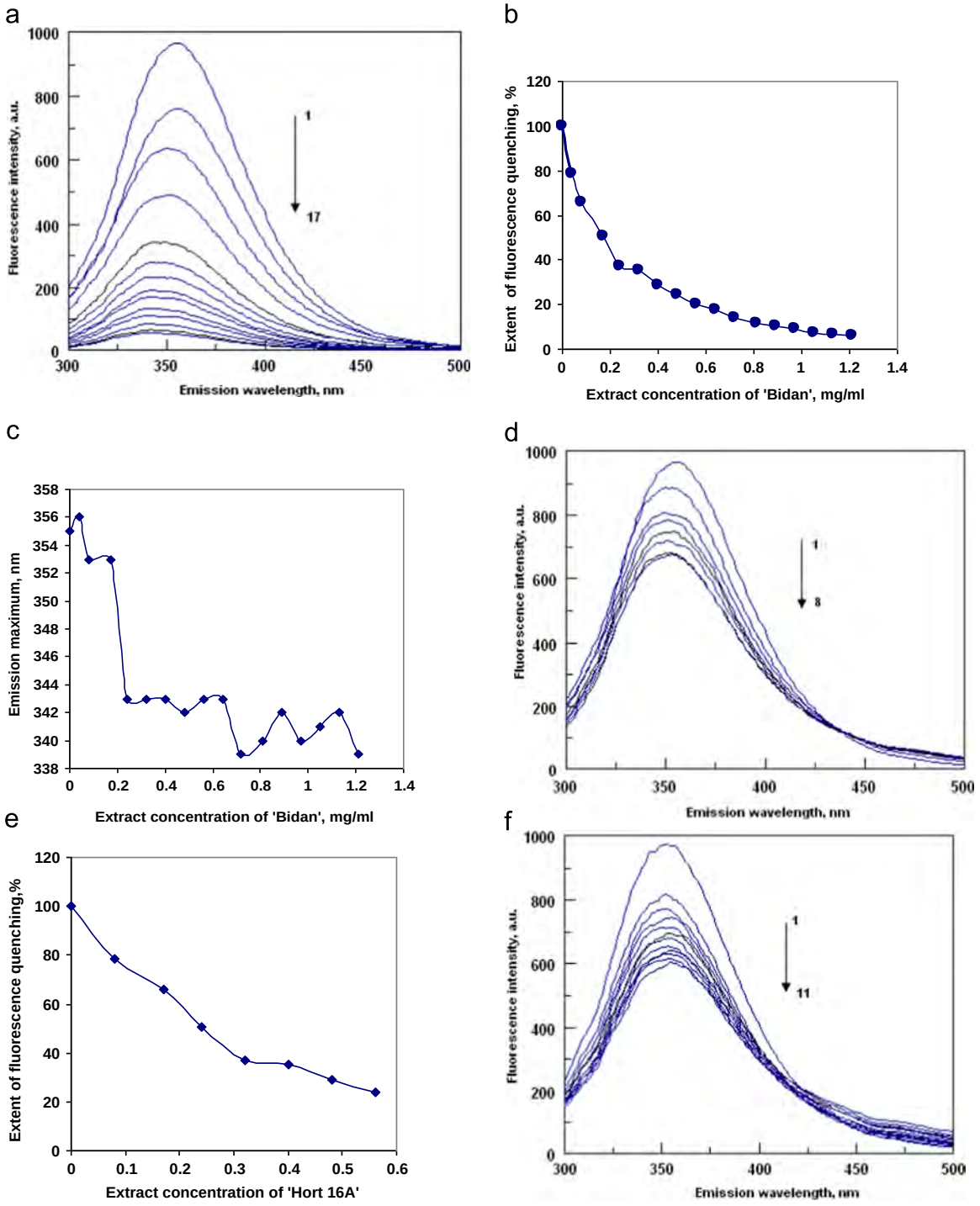

Fig. 3. (a) Emission spectra of HSA in the absence and presence of polyphenol extracts of 'Bidan' in water at λex 280 nm, and λem 300 nm: (1) HSA (2.0×10^{-6} mol/L), (2) HSA+0.04 mg/mL 'Bidan', (3) HSA+0. 08 mg/mL 'Bidan', (4) HSA+0.17 mg/mL 'Bidan', (5) HSA+0.24 mg/mL 'Bidan', (6) HSA+0.32 mg/mL 'Bidan', (7) HSA+0.40 mg/mL 'Bidan', (8) HSA+0.48 mg/mL 'Bidan', (9) HSA+0.56 mg/mL 'Bidan', (10) HSA+0.64 mg/mL 'Bidan', (11) HSA+0.72 mg/mL 'Bidan', (12) HSA+0.81 mg/mL 'Bidan', (13) HSA+0.89 mg/mL 'Bidan', (14) HSA+0.97 mg/mL 'Bidan', (15) HSA+1.05 mg/mL 'Bidan', (16) HSA+1.13 mg/mL 'Bidan', and (17) HSA+1.21 mg/mL 'Bidan'. (b) Extent of fluorescence enhancement of HSA on the addition of water extract of 'Bidan'. (c) Shift of the emission maximum of HSA on the addition of water extract of 'Bidan'. (d) Emission spectra of HSA in the absence and presence of polyphenol extracts of 'Hort16A' in water at λex 280 nm, and λem 300 nm: (1) HSA (2.0×10^{-6} mol/L), (2) HSA+0.04 mg/mL 'Hort 16 A', (3) HSA+0. 08 mg/mL 'Hort 16 A', (4) HSA+0.17 mg/mL 'Hort 16 A', (5) HSA+0.24 mg/mL 'Hort 16 A', (6) HSA+0.32 mg/mL 'Hort 16 A', (7) HSA+0.40 mg/mL 'Hort 16 A', (7) HSA+0.40 mg/mL 'Hort 16 A'. (e) Extent of fluorescence enhancement of HSA on the addition of water extract of 'Hort 16 A'. (f) Emission spectra of HSA in the absence and presence of polyphenol extracts of mangosteen in water at λex 280 nm, and λem 300 nm: (1) HSA (2.0×10^{-6} mol/L), (2) HSA+0.04 mg/mL mangosteen, (3) HSA+0.08 mg/mL mangosteen, (4) HSA+0.17 mg/mL mangosteen, (5) HSA+0.24 mg/mL mangosteen, (6) HSA+0.32 mg/mL mangosteen, (7) HSA+0.40 mg/mL mangosteen, (8) HSA+0.48 mg/mL mangosteen, (9) HSA+0.56 mg/mL mangosteen, (10) HSA+0.64 mg/mL mangosteen, (11) HSA+0.72 mg/mL mangosteen, and (12) HSA+0.81 mg/mL mangosteen. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article).

extract of kiwi fruit decreased the intensity of HSA and another fruit mangosteen extract (Fig. 4c and d) by increasing the concentration of free polyphenols at the action sites increased the decrease in comparison with the 'Bidan' extract. The change in the fluorescence intensity with addition of 0.17 mg/mL of mangosteen was about 15.52% (Fig. 4c, line 2), for 'Bidan' −49.47%, and with

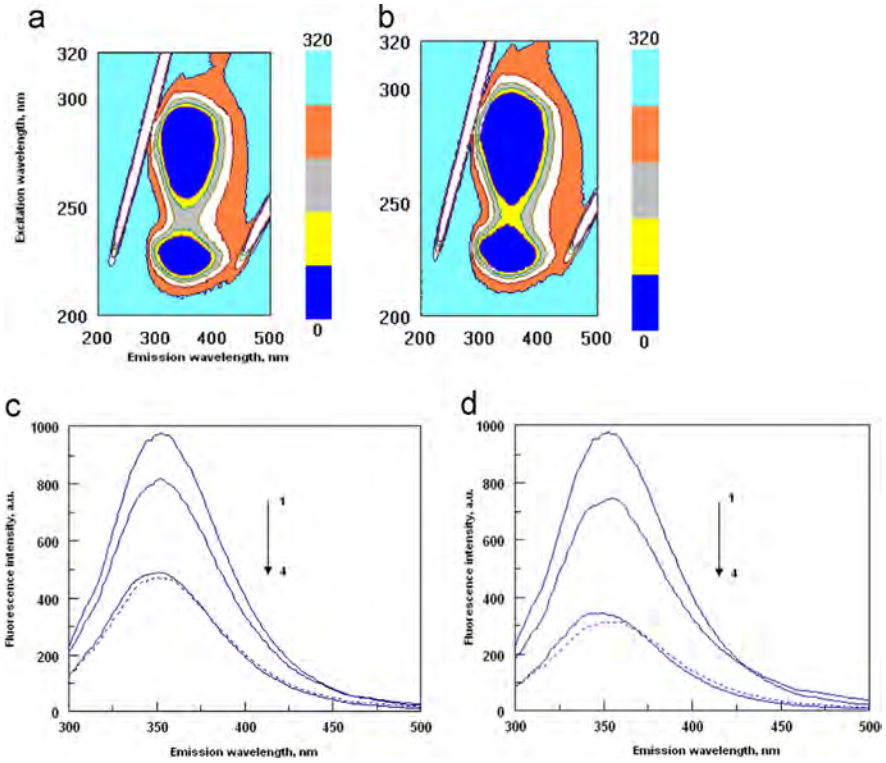

Fig. 4. 3D contour spectral studies of HSA with various kiwi fruit cultivars at 0.17 mg/mL such as 'SKK12' (a) and 'Hort 16 A'(b) in aqueous solution. Excitation wavelength scan: 200–320 nm. Emission wavelength scan: 200–500 nm. Emission spectra of HSA in the absence and presence of aqueous polyphenol extracts of 'Bidan' and mangosteeen at λex 280 nm, and λem 300 nm: (c) (1) HSA (2.0×10^{-6} mol/L), (2) HSA+0.17 mg/mL mangosteen, (3) HSA+0.17 mg/mL 'Bidan', (4) HSA+0.17 mg/mL mangosteen+0.17 mg/mL 'Bidan'. (d) (1) HSA (2.0×10^{-6} mol/L), (2) HSA+0.32 mg/mL mangosteen, (3) HSA+0.32 mg/mL 'Bidan', and (4) HSA+0.32 mg/mL mangosteen +0.32 mg/mL 'Bidan'. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article).

addition of mangosteen decreased of about 51.41%. With increase of the concentration of ' Bidan' and mangosteen as much as twice the decrease was about 67.68% (Fig. 4d, line 4).

Our presented results are in line with Liu et al. [28], who found that a combination of two drugs decreased the binding affinity of a drug with BSA, which released another drug and increased the concentration of available free drug in the blood. Our results are in agreement with others [11], where in interaction with HSA were used two antibiotics, showing synergistic effect. As it was mentioned before, HSA is the most abundant protein in blood plasma and an important carrier for many drugs. Drug interactions are very important in multidrug therapy. The efficacy of the individual antioxidants and the efficacy of a combination of two antioxidants were measured using fluorescence spectroscopy and showed a synergistic effect. It was shown that quercetin can suppress HSA. Much of the bioactivities of citrus flavanones significantly appear to impact blood and microvascular endothelial cells, therefore it was essential to investigate the interaction between kiwi fruit polyphenols and serum albumin. The binding constants ranked in the following order quercetin > rutin > -calycosin > calycosin-7-O-(sup)-D-glucoside [formononetin-7-O-(sup)-D-glucoside] [29]. These results were in direct relationship with the antioxidant properties of the extracts. Our very recent results [25,26] showed that the fluorescence is significantly quenched, because of the formation of complexes between proteins and phenolic acids and flavonoids. This interaction was investigated using tryptophan fluorescence quenching. Our result is in agreement with others that quercetin, as an aglycon, is more hydrophobic and demonstrates strong affinity toward HSA. Other reported results [30] differ from ours, probably because of the variety of antioxidant abilities of pure flavonoids and different ranges of fluorometry scanning ranges used in a similar study. The interaction of extracts with HSA is a process of complex-formations static quenching. There is approximately one binding site between HSA and the extract [11]. Our results are in agreement with [12], where in the interaction between the iron complex of deferasirox (Fe (III)–DFX) and HSA fluorescence intensity of HSA is decreased in the presence of Fe (III)–DFX complex, and the fluorescence quenching was the result of the formation of the Fe (III)–DFX–HSA complex, therefore the quenching mechanism was static. Our obtained results are in line with the binding properties of water-soluble albumin-bound curcumin, a yellow natural polyphenol extracted from turmeric (*Curcuma longa*), nanoparticles [31].

4. Conclusions

3-D fluorescence can be used as an additional tool for the characterization of the polyphenol extracts of kiwi fruit cultivars and their binding properties. We presented for the first time the results of shelf life of new cultivars bred in Korea and their comparison with the widely studied ones such as 'Hayward' and 'Hort 16 A'. Cold storage had a significant effect on physicochemical and nutritional properties of kiwi fruit and improved their quality and antioxidant activity. The use of fluorescence in various ways showed that this method is important in the study of binding properties of many fruits. This is important for medical and pharmaceutical applications. High amount of natural antioxidants such as phenolic compounds in all cultivars makes kiwi fruit even more important for daily consumption.

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