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In vitro antioxidative and binding properties of phenolics in traditional, citrus and exotic fruits☆

Yong-Seo Park [a,*], Milena Cvikrová [b], Olga Martincová [b], Kyung-Sik Ham [c], Seong-Gook Kang [c], Yang-Kyun Park [c], Jacek Namiesnik [d], Adamo Domenico Rombolà [e], Zenon Jastrzebski [f], Shela Gorinstein [g,**]

[a] Department of Horticultural Science, Mokpo National University, Muan, Jeonnam, Republic of Korea
[b] Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Prague 6, Czech Republic
[c] Department of Food Engineering, Mokpo National University, Muan, Jeonnam, Republic of Korea
[d] Department of Analytical Chemistry, Chemical Faculty, Gdańsk University of Technology, 80 952 Gdańsk, Poland
[e] Department of Agricultural Sciences, University of Bologna, 40127 Bologna, Italy
[f] Department of Pharmacology, National Medicines Institute, 00-725 Warsaw, Poland
[g] The Institute for Drug Research, School of Pharmacy, The Hebrew University, Hadassah Medical School, Jerusalem 91120, Israel

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ABSTRACT

Many polyphenols bind proteins, therefore our research was focused on the potential of protein binding to polyphenols of investigated fruits and their health-related effects. The contents of polyphenols and related antioxidant activities of traditional, citrus and exotic fruits were compared. The presence of polyphenols (flavonoids and phenolic acids) in the investigated samples and their interaction with human serum albumin (HSA) was studied by HPLC, Fourier Transform Infrared (FT-IR) and three dimensional fluorescence spectroscopy (3D-FL). The highest levels of polyphenols, antioxidant and binding capacities were found in red and blond grapefruits (citrus group), followed by strawberries and apples (traditional group) and mangosteen and kiwi fruit (exotic fruit), which also contained the highest levels of protocatechuic, p-coumaric, ferulic acids and quercetin. In conclusion, for the first time, the interaction of the polyphenols with human serum albumin was evaluated by fluorometry/FTIR. The obtained binding profiles allowed the comparison of three different groups of fruits. A mixture of these fruits can be recommended for consumption.

1. Introduction

Fruits and berries have been widely recognized as an excellent source of bioactive phenolic compounds. Consumption of a fruit- and vegetable-rich diet, the "Mediterranean diet", was epidemiologically correlated to health benefits especially for chronic diseases including diabetes and cardiovascular disease. The abundance of polyphenols in plant-rich diets, and the potent bioactivities of polyphenols, provides indirect evidence for a role for polyphenols in maintaining good health (Li & Hagerman, 2013). The health benefits of fruits are attributable to their bioactive components such as phenolics (Cano, Medina, & Bermejo, 2008; Haruenkit et al., 2007; Mikulic-Petkovsek, Slatnar, Stampar, & Veberic, 2012; Rababah et al., 2011). The consumption of citrus fruits and juices has been widely investigated for its possible role in the prevention of cardiovascular disease and cancer. These beneficial effects are mainly attributed to flavanones, the typical polyphenols of citrus species (Khan, Zill, & Dangles, 2014; Mertz et al., 2009; Sun et al., 2013). There is a number of published results on fruit properties. A variety of fruits were investigated: antioxidant potential of twelve plum cultivars, fresh and stored during 10 days at 4 °C by using different methods and even after storage could be a good source of antioxidants, which may provide health-promoting effects for humans (Mihalache et al., 2014). Eight traditional apple cultivars of Southern Italy were analyzed for phenolic composition and free radical scavenging activity in comparison with commercial cultivars (Panzella, Petriccione, Rega, Scortichini, & Napolitano, 2013). Crude extracts from 30 commonly consumed fruits were screened for their total phenolic content and antioxidant capacity (Podsedek, Majewska, Redzynia, Sosnowska, & Koziolkiewicz, 2014). Apriasari and Iskandar (2014) considered banana as one of the world's leading food crops with a high source of minerals, vitamins, carbohydrates, flavonoids and phenolic compounds. Persimmons [*Diospyros kaki Thunb.* (Ebenaceae)] are mostly considered "exotic" fruits (Giordani, Doumett, Nin, & Del Bubba, 2011). The antioxidant property of the compounds of three citrus fruits such as ascorbic acid (vitamin), cryptoxanthin (carotenoids) and flavanones (polyphenols)

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

* Corresponding author. Tel.: +82 61 450 2376; fax: +82 61 453 2309.
** Corresponding author. Tel.: +972 2 6758690; fax; +972 2 6757076.
E-mail addresses: ypark@mokpo.ac.kr (Y.-S. Park), shela.gorin@mail.huji.ac.il (S. Gorinstein).

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is established by experimental data (Rock & Fardet, 2014). Ribeiro da Silva et al. (2014) showed novel information about Brazilian tropical fruits and bioactive composition of their by-products, which is essential for the understanding of their nutraceutical potential and future application in the food industry. Judprasong, Charoenkiatkul, Thiyajai, and Sukprasansap (2013) investigated nutrients and bioactive compounds of Thai indigenous fruits. Twenty-four exotic Colombian fruits were evaluated (Contreras-Calderon, Calderon-Jaimes, Guerra-Hernandez, & Garcia-Villanova, 2011) for their antioxidant activity and total soluble phenolics and ascorbic acid contents. Fresh fruits, particularly berries, are rich in polyphenols (Pinto et al., 2013). Berries (Pinto et al., 2013) accounted for 9% of total fresh fruit intake, from which 80% were due to strawberries. Within berries, strawberries accounted for 11% of total polyphenol intake, with the other consumed berries accounting for 3% of the total polyphenol intake per day. The most common commercially available, green-fleshed kiwi fruit is the 'Hayward'cultivar, which belongs to the *Actinidia deliciosa* species (Du, Li, Ma, & Liang, 2009; Park et al., 2014; Tavarini, Degl'Innocenti, Remorini, Massai, & Guidi, 2008). As it can be concluded there are numerous reports considering different properties of fruits. Many polyphenols bind proteins, especially the proteins of human serum. HSA is a carrier for many drugs to different molecular targets. Flavonoids are powerful antioxidants and prevent DNA damage. The antioxidative protections are related to their binding modes to DNA duplex and complexation with free radicals *in vivo*. As to the binding of phenolic compounds from dietary sources, flavonoids as flavanol, flavonol, flavone, isoflavone, flavanones, and anthocyanidins are known to interact with HSA (Pal & Saha, 2014; Sinisi, Forzato, Cefarin, Navarini, & Berti, 2015). The purpose of this investigation was to compare the binding and bioactivity profiles of three groups of fruits (traditional, citrus and exotic), using HPLC, fluorometry and FTIR, based on the interaction of the fruit polyphenols with human serum albumin (HSA). Overall, the results reported on the phenolic antioxidants in fruits are just expanding the current knowledge. However, the elucidation of interaction of the polyphenols with HSA, evaluated by fluorometry/FTIR on binding profiles, could be interesting, because few literature data are available on the binding properties of the screened fruits.

2. Materials and methods

2.1. Chemicals

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), HSA, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent (FCR), lanthanum (III) chloride heptahydrate, $CuCl_2x2H_2O$, 2,9-dimethyl-1 and 10-phenanthroline (neocuproine) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All reagents were of analytical grade. Deionized and distilled water was used throughout.

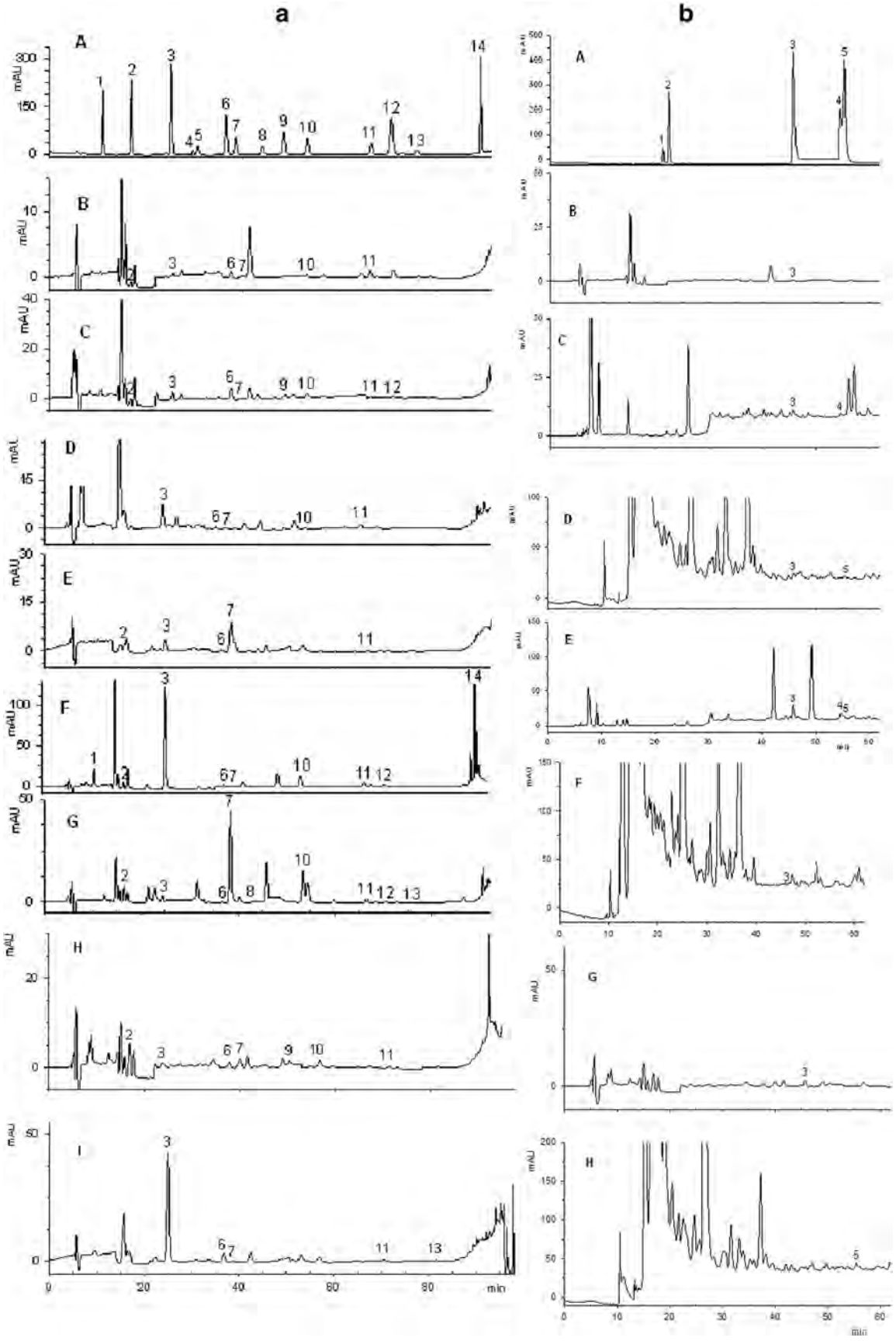
2.2. Samples

Investigated samples were divided into three groups. Traditional or commonly consumed fruits were apples (*Malus sylvestris*), bananas (*Musa acuminata*), green grapes (*Vitis riparia*) and strawberries (*Flagaria ananassa*). Blond and red grapefruits (*Citrus paradisi*); red, white and Thong Dee (TD) cultivar of pomelos (*Citrus maxima*) and lemons (*Citrus limon*) are fruits that belong to the citrus family. They share similar kinds of pulp, thick rinds, and the fact that they grow well in warm climates. Persimmons (*Diospyros kaki* L var. Triumph), mature durian (*Durio zibethinus Murr. cv Mon Thong*), mangosteen (*Garcinia mangostana L.*), and kiwi fruits (*A. deliciosa*) belong to exotic fruits which are not native and that are cultivated outside, available at their place of origin. These fruits and berries were of their respective harvest time in 2013. Randomly selected samples of each fruits and strawberries were washed in distilled water. All fruits were peeled, except green grapes and strawberries. Their edible parts were prepared manually without using steel knives. The peeled fruits were weighed, chopped and homogenized in 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 was determined. The samples were ground to pass through a 0.5 mm sieve and stored at −20 °C until their analyses.

2.3. Determination of bioactive compounds and antioxidant activity

Phenolic acids were extracted as described elsewhere (Cvikrová, Meravý, Macháčková, & Eder, 1991). Free, methanol soluble ester-bound (released after alkaline hydrolysis) and methanol soluble glycoside-bound (released after acid hydrolysis) phenolic acids were obtained from a methanolic extract of tissue ground in liquid nitrogen. Phenolic acids were analyzed by HPLC using a Dionex (Dionex Sofron GmbH, Germering, Germany) Liquid Chromatography system consisting of a P660-HPLC Pump, ASI-100 Automated Sample Injector, TCC-100 Thermostated Column Compartment and PDA-100 Photodiode Array Detector, equipped with a C18 column (Waters Spherisorb ODS-2 5 mm, 250 mm × 4.6 mm, Supelco, Ballefonte, PA, USA) and controlled by Chromeleon Software 6.5. The analytes (10 mL or 20 mL portions of the samples) were eluted with a mobile phase (flow rate, 0.5 mL min^{-1}) at 45 °C. Gradient of 0.01 M citric acid, 0.01 M $Na_2HPO_4x2H_2O$, adjusted to pH 2.4 with H_3PO_4 (solvent A) and 80% (v/v) methanol (solvent B), in percentages of solvent A: 0–40 min, 100–85%; 40–60 min, 85–65%; 60–75 min, 65–0%; 75–83 min, isocratic 0%; 83–90 min, 0–100% was used. Flavonoid compounds were extracted with 80% methanol accordingly to the method of glycoside-bound phenolic acid extraction (released after acid hydrolysis). Compounds were eluted using a gradient of acetonitrile (ACN) with phosphoric acid by the method of Xue, Zhao, Wang, and Hong (2007) with modifications. The contents of polyphenols, tannins, flavonoids and flavanols in methanol extracts of the studied fruits were determined as previously described and the details are given below (Park et al., 2014). Total polyphenols were extracted from a 50 mg aliquot of lyophilized fruit samples with 5 mL of 100% methanol. The polyphenolic contents were determined spectrophotometrically by the Folin–Ciocalteu method at 750 nm (Hewlett-Packard, model 8452A, Rockvile, USA). The results were expressed as mg of gallic acid equivalents (GAE) per g of DW (dry weight). Flavonoids were extracted with 5% $NaNO_2$. Then 10% $AlCl_3x6H_2O$ and 1 M NaOH were added to the extract, and then were measured at 510 nm (Chang, Yang, Wen, & Chern, 2002; Singleton, Orthofer, & Lamuela-Raventos, 1999). The total flavanols were estimated using the *p*-dimethylaminocinnamaldehyde (DMACA) method, and then the absorbance at 640 nm was read (Arnous, Makris, & Kefalas, 2001). The extracts of condensed tannins (procyanidins) with 4% methanol vanillin solution were measured at 500 nm (Broadhurst & Jones, 1978). (+)-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, Guclu, Bektasoglu, & Apak, 2007) in water extract (100 mg of lyophilized sample and 5 ml of water). The absorbance of the formed bis (Nc)-copper (I) chelate was measured at 450 nm. For determination of antioxidant activity, two complementary assays were used: DPPH and CUPRAC (Apak,

Fig. 1. HPLC analysis of methanol soluble ester-bound phenolic acids (a) and flavonoids (b) extracted from traditional and exotic fruits. (a) Each profile represents an equivalent amount of extract, normalized on a volume of extract per 5 mg of tissue basis. Chromatograms are showing the separation: A — standard mixture (1 — gallic acid; 2 — protocatechuic acid; 3 — *p*-hydroxybenzoic acid; 4 — *m*-hydroxybenzoic acid, 5 — 2,3 dihydroxybenzoic acid, 6 — vanillic acid; 7 — caffeic acid; 8 — chlorogenic acid; 9 — syringic acid; 10 — *p*-coumaric acid; 11 — ferulic acid; 12 — anisic acid; 13 — sinapic acid; 14 — cinnamic acid); B — Banana; C — Persimmon; D — Mature durian; E — Green grape; F — Strawberry; G — Apple; H — Kiwi fruit 'Hayward'; and I — Mangosteen. (b) Each profile represents an equivalent amount of extract, normalized on a volume of extract per 10 mg of tissue basis. A — standard mixture (1 — epicatechin; 2 — esculetin; 3 — quercetin; 4 — kaempferol; 5 — apigenin); B — Banana; C — Persimmon; D — Green Grape; E — Strawberry; F — Mature durian; G — Kiwi fruit 'Hayward'; and H — Mangosteen.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Guclu, Ozyurek, & Karademir, 2004; Brand-WIlliams, Cuvelier, & Berset, 1995). In its radical form, 1-diphenyl-2-picrylhydrazyl (DPPH) has an absorption band at 515 nm, which disappears upon reduction by antiradical compounds. Methanol solution of DPPH (3.9 mL, 25 mg/L) was mixed with the sample extracts (0.1 mL). The reaction progress was monitored at 515 nm until the absorbance was stable. 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 fruit sample (or standard) solution (x mL) and H_2O [(1.1-x) mL] were added to produce the final volume of 4.1 mL. The absorbance at 450 nm was recorded against a reagent blank.

2.4. Fluorometric and FTIR measurements

Fluorometric measurements were used for the evaluation of binding properties of fruit extracts to human serum albumin (HSA). 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 of HSA solution and various amounts of fruit extracts were added to a 1.0 cm quartz cell manually using a micro-injector. The concentrations of fruit extracts were ranged from 0 to 1.5 mg/mL, and the total accumulated volume of 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 other 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 of NaCl (Park et al., 2014). The presence of polyphenols in the investigated fruit samples and their interaction with HSA was studied by Fourier transform infrared (FT-IR) spectroscopy. A Nicolet iS 10 FT-IR nSpectrometer (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).

2.5. Statistical analysis

To verify the statistical significance, mean ± SD of five independent measurements was calculated. Differences between groups were tested by one-way ANOVA. In the assessment of the antioxidant activity, Spearman's correlation coefficients (R) were used. Linear regressions were also calculated. P-values of <0.05 were considered significant.

3. Results and discussion

3.1. Antioxidative compounds in fruits

Some phenolic acids and flavonoid compounds were extracted from all fruits, followed by HPLC determination in order to evaluate the differences in the bioactive compounds in investigated samples. Fig. 1a and b presents only some of the phenolic acids and flavonoids in order to illustrate the results of HPLC analysis. Protocatechuic, p-hydroxybenzoic, vanillic, caffeic, *p*-coumaric, and ferulic acids were detected in all fruit samples. In commonly consumed (traditional) fruits the significantly highest amount of these acids (μg g^{-1} DW, Fig. 1a, positions F, G, B, E, respectively; Table 1a) was in strawberry (286.37 ± 28.54), followed by apple pulp (173.27 ± 16.53), then in banana (24.93 ± 2.51) and the lowest was in green grapes (22.34 ± 2.05). The total polyphenols (mg GAE/g DW, Table 2a) were in the similar order as for main phenolic acids, with the highest for strawberry (14.60 ± 1.34), but with the lowest one in banana (1.60 ± 0.16). In exotic group of fruits (μg g^{-1} DW, Fig. 1a, positions H, I, C, D, respectively, Table 1a) the highest amount of phenolic acids was in kiwi fruit (47.46 ± 4.69), followed by mangosteen (29.43 ± 3.09), persimmon (24.34 ± 2.21) and mature durian (7.35 ± 0.51). The amount of polyphenols (mg GAE/g DW, Table 2a) was the highest in mangosteen (10.59 ± 1.45) and the lowest in mature durian (1.83 ± 0.14). The significantly highest amount of flavonoids (quercetin, kaempferol and apigenin, μg g^{-1} DW) was in strawberry (30.69 ± 3.17, Table 1a, Fig. 1b, position E). Quercetin represents the highest percentage (46–100%) among flavonols in most analyzed berries (Tables 1a and 2a), as well as in other fruits. In wild strawberry and gooseberry (Mikulic-Petkovsek et al., 2012) the prevailing flavonols belong to the group of isorhamnetins (50–62%) and kaempferols, which represent the major part of flavonols in currants (49–66%). Persimmons are also a good source of polyphenolic compounds such as *p*-coumaric acid, catechin, epicatechin, epigallocatechin, and condensed proanthocyanidins (Giordani et al., 2011). Some of these compounds (μg g^{-1}) were detected in our study (Table 1a), such as *p*-coumaric acid (4.02 ± 0.43) and flavonoids such as quercetin (2.04 ± 0.02) and kaempferol (1.47 ± 0.15). These chemical compounds, together with *in vivo* and *in vitro* studies, suggest a relevant role of persimmon in the protection against free radicals and in the prevention of some human diseases, where the antioxidant activity was relatively high in comparison with studied fruits. Protocatechuic, p-hydroxybenzoic, vanillic, caffeic, and ferulic acids were detected in all citrus fruit samples. The highest amount of these phenolic acids (μg g^{-1} DW, Table 1b) was in red grapefruit (498.29 ± 48.28), following by blond grapefruit, white and red pomelo (176.62 ± 17.31; 165.47 ± 16.23; 156.92 ± 14.63, respectively) and with the significantly lowest in lemon (45.89 ± 4.3). *p*-Coumaric acid was only found in red and blond grapefruits. The highest amount of flavonoids was in red pomelo (333.76 ± 32.49) and the lowest one was in TD pomelo (11.36 ± 1.19). The highest amount of quercetin was in lemon, followed by blond grapefruit. Our results agree with those of Sun et al. (2013), where hydroxycinnamic acids, particularly ferulic and caffeic acids, were the main phenolic acids in citrus fruits. There was a greater amount of free (extractable) than bound (insoluble) phenolic acids. For citrus fruits the significantly highest content of polyphenols (mg GAE/g DW, Table 2b) was in red grapefruit (15.08 ± 1.61), followed by blond grapefruit with the lowest in white pomelo (2.92 ± 0.29). Hesperetin and its derivatives are characteristic flavanones of sweet orange, tangelo, lemon and lime (Tables 1b, 2b), while naringenin and its derivatives are those of grapefruit and sour orange (Khan et al., 2014).

3.2. Antioxidant activity of phenolics

Electron transfer (ET) assays (Apak et al., 2007) include the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)/Trolox Equivalent Antioxidant Capacity (ABTS/TEAC), CUPRAC, DPPH, Folin–Ciocalteu and Ferric-reducing/antioxidant power (FRAP) methods, each using different chromogenic redox reagents with different standard potentials. CUPRAC assay offers advantages over other ET-based assays, such as the selection of working pH at physiological pH (as opposed to the Folin and FRAP methods, which work at alkaline and acidic pHs, respectively), applicability to both hydrophilic and lipophilic antioxidants (unlike Folin and DPPH), completion of the redox reactions for most common flavonoids (unlike FRAP). According to two used assays (DPPH and CUPRAC), the significantly highest level of antioxidant activity (μMTE/g DW, Table 2a, $p < 0.05$) was registered in strawberries (26.14 ± 2.52 and 32.38 ± 3.08), followed by apple pulp and green grapes, with the lowest value in banana (9.25 ± 0.83 and 5.37 ± 0.48). Our results of the evaluation of strawberry were in accordance with Rababah et al. (2011), where the fresh strawberry had the highest contents of total phenolics (8503.1 mg GAE kg^{-1}), the highest antioxidant activity (54.88% inhibition), followed by the other fruits. Mauli

Table 1
Contents[1,2] of methanol soluble individual phenolic acids and flavonoids of (a) traditional and exotic and (b) citrus fruits.

a

Phenolic acids	Banana	Persimmon	M. durian	G. grape	Strawberry	Apple pulp	Kiwi fruit	Mangosteen
Gallic	–	–	–	–	82.43 ± 8.14^{a}	–	–	–
Protocatechuic	4.05 ± 0.31^{c}	6.75 ± 0.64^{bc}	–	0.55 ± 0.06^{cd}	12.82 ± 1.15^{b}	24.70 ± 2.26^{a}	14.14 ± 1.42^{b}	0.16 ± 0.02^{d}
p-Hydroxybenzoic	0.60 ± 0.06^{d}	2.16 ± 0.02^{cd}	0.98 ± 0.07^{d}	3.83 ± 0.31^{c}	131.77 ± 13.12^{a}	5.05 ± 0.53^{c}	0.66 ± 0.07^{d}	25.16 ± 2.41^{b}
Vanillic	5.41 ± 0.52^{bc}	8.94 ± 0.83^{b}	0.56 ± 0.04^{cd}	0.76 ± 0.07^{cd}	11.24 ± 1.14^{a}	0.29 ± 0.02^{d}	5.41 ± 0.51^{bc}	3.60 ± 0.31^{c}
Caffeic	7.91 ± 0.72^{cd}	2.04 ± 0.14^{de}	0.84 ± 0.08^{de}	16.03 ± 1.61^{c}	5.59 ± 0.53^{d}	106.18 ± 10.14^{a}	21.70 ± 2.14^{b}	0.51 ± 0.05^{e}
Chlorogenic	–	–	–	–	–	1.34 ± 0.12^{e}	–	–
Syringic	–	1.82 ± 0.18^{a}	–	–	–	–	0.66 ± 0.07^{b}	–
p-Coumaric	1.73 ± 0.16^{cd}	4.02 ± 0.43^{c}	0.79 ± 0.07^{d}	–	92.60 ± 9.42^{a}	33.18 ± 3.25^{b}	4.05 ± 0.43^{c}	–
Ferulic	7.71 ± 0.73^{b}	1.02 ± 0.15^{d}	4.18 ± 0.32^{c}	1.17 ± 0.12^{d}	32.35 ± 3.18^{a}	3.87 ± 0.33^{c}	1.50 ± 0.12^{d}	3.12 ± 0.25^{c}
Sinapic	–	–	–	–	–	0.39 ± 0.03^{b}	–	0.57 ± 0.05^{a}
Anisic	–	0.39 ± 0.03^{b}	0.04 ± 0.01^{c}	–	114.18 ± 11.14^{a}	0.05 ± 0.01^{ec}	–	–
Cinnamic	–	–	–	–	92.65 ± 9.18^{a}	–	–	–
Quercetin	0.29 ± 0.02^{c}	2.04 ± 0.02^{b}	–	1.40 ± 0.14^{b}	15.45 ± 1.42^{a}	–	–	Trs
Kaempferol	–	1.47 ± 0.15^{b}	–	–	10.10 ± 1.22^{a}	–	–	–
Apigenin	–	–	–	0.83 ± 0.09^{b}	5.14 ± 0.53^{a}	–	–	4.55 ± 0.47^{a}

b

Phenolic acids	R. pomelo	W. pomelo	TD. pomelo	R. grapefruit	B. grapefruit	Lemon
Protocatechuic	5.41 ± 0.42^{a}	0.09 ± 0.01^{c}	–	0.62 ± 0.05^{bc}	2.38 ± 0.22^{b}	0.07 ± 0.01^{c}
p-Hydroxybenzoic	30.63 ± 3.11^{a}	8.79 ± 0.82^{c}	14.26 ± 1.43^{b}	20.35 ± 2.42^{ab}	8.75 ± 0.75^{c}	15.70 ± 1.48^{b}
Vanillic	72.53 ± 6.43^{a}	65.05 ± 6.41^{b}	33.00 ± 3.25^{bc}	66.81 ± 6.43^{b}	26.46 ± 2.48^{c}	14.77 ± 1.39^{d}
Caffeic	5.59 ± 0.52^{cd}	22.17 ± 2.18^{b}	3.58 ± 0.24^{cd}	72.70 ± 7.24^{a}	14.00 ± 1.43^{c}	0.44 ± 0.04^{d}
Chlorogenic	–	–	–	0.22 ± 0.03^{a}	–	–
Syringic	–	–	–	–	0.25 ± 0.02^{a}	–
p-Coumaric	–	–	–	24.73 ± 2.35^{a}	22.40 ± 2.18^{a}	–
Ferulic	42.76 ± 4.15^{c}	69.37 ± 6.81^{bc}	9.25 ± 0.87^{d}	337.81 ± 32.14^{a}	125.03 ± 12.43^{b}	14.91 ± 1.38^{cd}
Sinapic	27.65 ± 2.64^{a}	3.29 ± 0.31^{d}	11.71 ± 1.18^{c}	6.36 ± 0.58^{cd}	10.19 ± 1.04^{c}	19.25 ± 1.85^{b}
Quercetin	3.44 ± 0.31^{c}	0.75 ± 0.07^{cd}	0.01 ± 0.01^{d}	4.69 ± 0.48^{bc}	8.28 ± 0.83^{b}	47.53 ± 4.27^{a}
Kaempferol	–	–	–	–	19.81 ± 1.84^{a}	–
Apigenin	330.32 ± 32.18^{a}	151.06 ± 14.82^{b}	11.35 ± 1.18^{d}	46.14 ± 4.53^{bc}	29.98 ± 2.83^{c}	29.05 ± 2.83^{c}

[1] Represented by the sum of free, ester- and glycoside-bound forms extracted from fruits expressed as μg g^{-1} of lyophilized dry matter.
[2] Data expressed as means ± SEM. Means within rows sharing the same letter are not significantly different according to Tukey ($p < 0.05$). Abbreviations: M. durian, mature durian; G. grape, green grape; R. pomelo, red pomelo; W. pomelo, white pomelo; TD. pomelo, Thong Dee pomelo; R. grapefruit, red grapefruit; W. grapefruit, white grapefruit; –, not found.

bananas stem extracts contain a bioactive compound with the highest level of tannin and followed by ascorbic acid and total flavonoid (Apriasari & Iskandar, 2014). The antioxidant activity in exotic group was highest in mangosteen (17.00 ± 1.65 and 26.86 ± 2.67), followed by kiwi fruit and persimmon, with the lowest in mature durian (6.90 ± 0.51 and 10.00 ± 1.11). Our results are in line with others

Table 2
Contents[1,2] of methanol soluble bioactive compounds and their antioxidant activities of (a) traditional and exotic and citrus (b) fruits (g^{-1}, dry matter).

a

Indices	Banana	Persimmon	M. Durian	G. Grape	Strawberry	Apple pulp	Kiwi fruit	Mangosteen
Polyph (mg GAE)	1.60 ± 0.16^{d}	4.52 ± 0.41^{cd}	1.83 ± 0.14^{d}	6.19 ± 0.55b^{c}	14.60 ± 1.34^{a}	8.04 ± 0.72^{b}	6.20 ± 0.54^{c}	10.59 ± 1.45^{ab}
Flavon (mgCE)	1.16 ± 0.14b^{c}	1.02 ± 0.08^{c}	1.15 ± 0.11^{c}	1.78 ± 0.14^{ab}	3.21 ± 0.28^{a}	3.19 ± 0.34^{a}	1.13 ± 0.11^{c}	1.42 ± 0.12^{b}
Flavan (mgCE)	0.03 ± 0.07^{c}	0.04 ± 0.01^{c}	0.14 ± 0.09^{b}	0.15 ± 0.02^{b}	0.71 ± 0.11^{a}	0.67 ± 0.18^{a}	0.51 ± 0.14^{ab}	0.12 ± 0.07^{b}
Vit. C (mgAA)	0.64 ± 0.09^{d}	3.16 ± 0.32^{ab}	1.48 ± 0.12^{c}	1.81 ± 0.14b^{c}	3.26 ± 0.35^{ab}	4.89 ± 0.49^{a}	2.92 ± 0.28^{b}	4.25 ± 0.45^{a}
Tannin (mgCE)	0.03 ± 0.01^{d}	2.04 ± 0.18^{a}	0.84 ± 0.11^{c}	0.64 ± 0.05^{c}	2.15 ± 2.52^{a}	1.50 ± 0.12^{b}	1.62 ± 0.14^{ab}	1.84 ± 0.12^{ab}
DPPH (μMTE)	9.25 ± 0.83^{bc}	15.13 ± 1.43^{ab}	6.90 ± 0.51^{c}	15.77 ± 1.43^{ab}	26.14 ± 2.52^{a}	21.65 ± 2.14^{ab}	16.21 ± 1.54^{b}	17.00 ± 1.65^{b}
CUPRAC (μMTE)	5.37 ± 0.48^{d}	13.36 ± 1.24^{bc}	10.00 ± 1.11^{c}	12.39 ± 1.25^{bc}	32.38 ± 3.08^{a}	23.78 ± 2.25^{ab}	19.67 ± 1.84^{b}	26.86 ± 2.67^{ab}
Binding (%)	1.29 ± 0.21^{cd}	2.23 ± 0.32^{cd}	0.69 ± 0.09^{d}	6.20 ± 0.83^{c}	25.93 ± 2.65^{a}	12.52 ± 1.45^{b}	7.35 ± 0.63^{c}	25.39 ± 2.43^{a}

b

Indices	R. pomelo	W. pomelo	TD. pomelo	R. grapefruit	B. grapefruit	Lemon
Polyph (mg GAE)	4.86 ± 0.47^{bc}	2.92 ± 0.29^{c}	10.96 ± 1.14^{b}	15.08 ± 1.61^{a}	14.56 ± 1.15^{a}	4.80 ± 0.45^{bc}
Flavon (mgCE)	0.91 ± 0.11^{a}	0.70 ± 0.09^{ab}	0.65 ± 0.09^{ab}	1.05 ± 0.12^{a}	1.05 ± 0.07^{a}	0.58 ± 0.09^{b}
Flavan (mgCE)	0.03 ± 0.01^{bc}	0.01 ± 0.01^{c}	0.09 ± 0.01^{a}	0.09 ± 0.02^{a}	0.07 ± 0.01^{ac}	0.02 ± 0.01^{c}
Vit. C (mg AA)	1.32 ± 0.18^{bc}	1.36 ± 0.16^{bc}	0.98 ± 0.14^{c}	5.54 ± 0.65^{a}	5.45 ± 0.61^{a}	4.56 ± 0.44^{b}
Tannin (mgCE)	0.01 ± 0.01^{b}	0.01 ± 0.01^{b}	0.01 ± 0.01^{b}	0.13 ± 0.02^{a}	0.13 ± 0.07^{a}	0.01 ± 0.01^{b}
DPPH (μMTE)	6.88 ± 0.65^{c}	6.44 ± 0.54^{c}	12.93 ± 1.44^{b}	16.52 ± 1.64^{a}	14.82 ± 1.35^{b}	6.33 ± 0.71^{c}
CUPRAC (μMTE)	12.15 ± 1.22^{bc}	8.15 ± 0.82^{c}	20.22 ± 2.33^{b}	32.62 ± 3.18^{a}	30.59 ± 3.24^{a}	10.05 ± 1.03^{bc}
Binding (%)	10.19 ± 1.28^{b}	6.50 ± 0.87^{c}	10.35 ± 1.23^{b}	29.01 ± 3.00^{a}	27.41 ± 2.84^{a}	9.23 ± 1.18^{b}

Means within rows sharing the same letter are not significantly different according to Tukey ($p < 0.05$). Abbreviations: M. durian, mature durian; G. grape, green grape; R. pomelo, red pomelo; W. pomelo, white pomelo; TD. pomelo, Thong Dee pomelo; R. grapefruit, red grapefruit; B. grapefruit, blond grapefruit; cupric reducing antioxidant capacity (CUPRAC), 1, 1-diphenyl-2-picrylhydrazyl (DPPH); Polyph, polyphenols; Flavon, flavonoids; Flavan, flavanols; Vit. C, vitamin C; GAE, gallic acid equivalent; CE, catechin equivalent; TE, trolox equivalent; AA, ascorbic acid.
[1] Extracted at room temperature in a concentration of 25 mg of lyophilized sample in 1 mL of methanol.
[2] Data expressed as means ± SEM.

(Malta, Tessaro, Eberlin, Pastore, & Liu, 2013): so, gabiroba fruit showed the highest amounts of total phenolics (851.0 ± 40.7 mg GAE/100 g fruit) and the highest antioxidant activity for oxygen radical absorbance capacity 8027.5 ± 378.6 μmol TE/100 g of fruit and peroxyl radical scavenging capacity, 2342.5 ± 48.1 μmol AAE/100 g fruit. The ABTS, FRAP, total phenolics and ascorbic acid values in 24 exotic fruits from

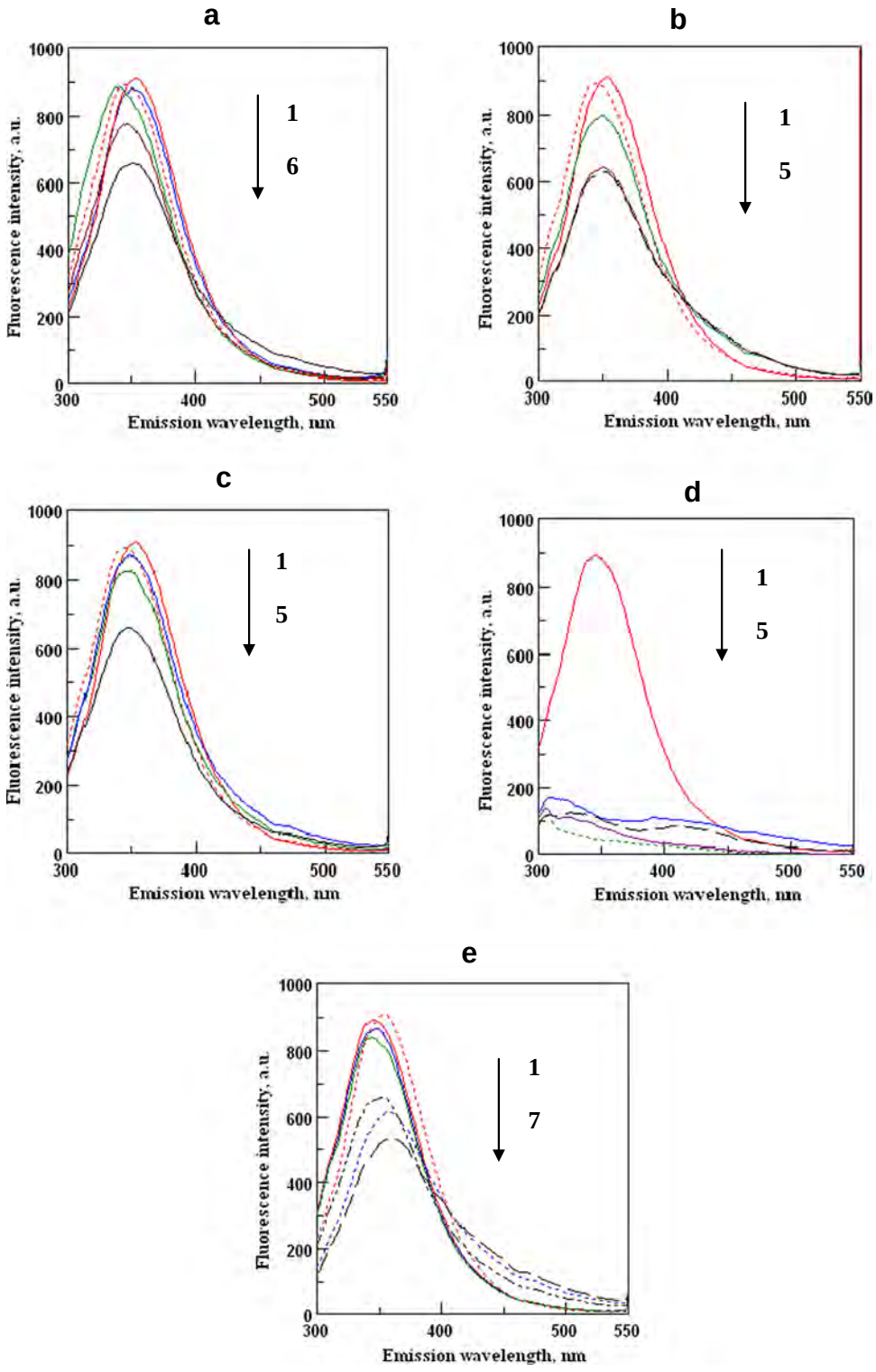

Fig. 2. Emission spectra of HSA in the absence and presence of fruit polyphenol extracts in methanol at λex 280 nm and λem 300 nm: a, (1) HSA in buffer (2.0×10^{-6} mol/L), (2) HSA in methanol (2.0×10^{-6} mol/L), (3) HSA + banana, (4) HSA + mature durian, (5) HSA + strawberry, (6) HSA + apple; b, (1) HSA in buffer (2.0×10^{-6} mol/L), (2) HSA in methanol (2.0×10^{-6} mol/L), (3) HSA + TD pomelo, (4) HSA + blond grapefruit, (5) HSA + red grapefruit; c, (1) HSA in buffer (2.0×10^{-6} mol/L), (2) HSA in methanol (2.0×10^{-6} mol/L), (3) HSA + persimmon, (4) HSA + kiwi fruit 'Hayward', (5) HSA + mangosteen; d, fluorescence spectra of different extracts (1) methanol extract HSA [λem = 350 nm, fluorescence intensity (FI) = 893)], (2) methanolic solution of strawberry (λem = 310 nm, 392 nm, FI = 172, 110), (3) methanolic solution of red grapefruit (λem = 308 nm, 327 nm, FI = 119, 125), (4) methanolic solution of kiwi fruit 'Hayward' (λem = 306 nm, FI = 138), (5) pure methanol (λem = 307 nm, FI = 117); e, fluorescence spectra of (1) HSA in buffer, (2) HSA + 20 μl of methanol, (3) HSA + 40 μl methanol, (4) HSA + 60 μl methanol, (5) HSA + 20 μl of strawberry polyphenol methanol extract (0.17 mg/ml), (6) HSA + 40 μl of strawberry polyphenol methanol extract (0.34 mg/ml), (7) HSA + 60 μl of strawberry polyphenol methanol extract (0.51 mg/ml).

Colombia (Contreras-Calderon et al., 2011) in the edible part were 3.25 to 175 μM TE/g FW, 6.29 to 144 μM TE/g FW, 15.7 to 1018 mg GAE/100 g FW, and 0.53 to 257 mg ascorbic acid/100 g FW, respectively, which corresponded to the present results (Table 2a). In the citrus group the highest antioxidant activities were by DPPH and CUPRAC (μM TE/g DW, Table 2b, $p < 0.05$); the highest were in red grapefruit (16.52 ± 1.64 and 32.62 ± 3.18), followed by blond grapefruit, with the lowest in white pomelo (6.44 ± 0.54 and 8.15 ± 0.82). The used two different antioxidant assays gave different results, but the correlation with other bioactive compounds was similar to one another. This is the biological significance of two different methods and the evidence of the real results in spite of the fact that these methods used different physiological pHs.

3.3. Binding properties of antioxidants with HSA evaluated by fluorometry

Phenolic acids and their derivatives are abundant in traditional, exotic and citrus fruits. HSA interacts with the compounds with high affinity. We have studied by fluorescence spectroscopy the specific binding of HSA with polyphenol extracts of all studied fruits.

Addition of fruit polyphenol methanol extract to HSA results in change in the fluorescence intensity and in a shift (blue and red) in the emission maximum of HSA (Figs. 2, 3). As a control for binding properties, HSA was dissolved in the same amount of methanol as the added fruit polyphenol methanol extracts. The fluorescence intensity of HSA in buffer in the absence of fruit extract at the emission maximum was

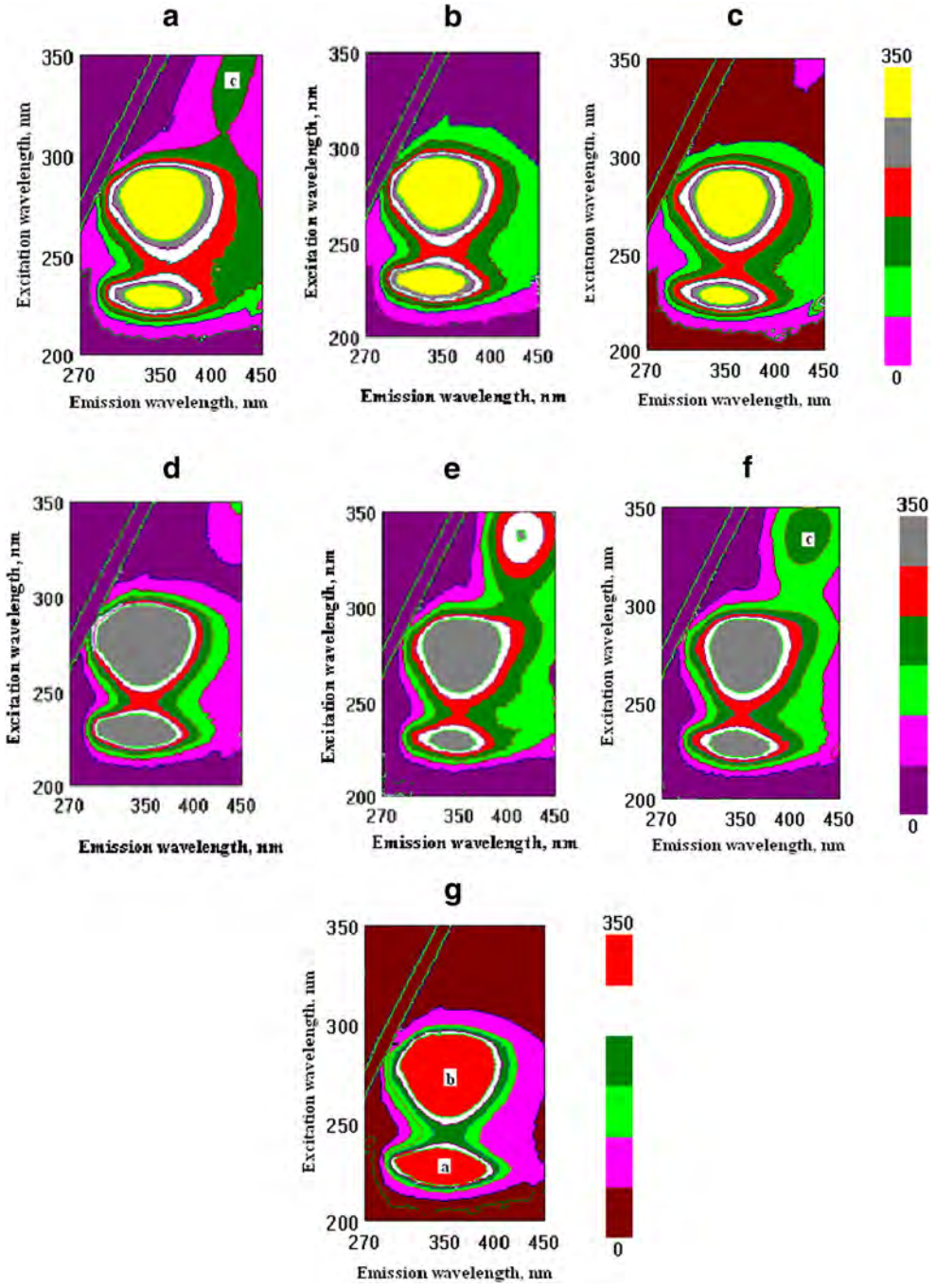

Fig. 3. 3D-cross spectral studies of human serum albumin (HSA) with fruits in methanol solution. Excitation wavelength scan: 200–350 nm. Emission wavelength scan: 270–450 nm. (a) HSA + strawberry; (b) HSA + apple; (c) HSA + mangosteen; (d) HSA + kiwi fruit 'Hayward'; (e) HSA + red grapefruit; (f) HSA + blond grapefruit; (g) HSA + methanol. To 20 μl HSA were added 20 μl of 0.17 mg/ml of fruit methanolic extract. The reaction was for 1 h at room temperature. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

about 910.51 (Fig. 2a, b, c, e, line 1). Fluorescence of HSA in methanol (Fig. 2a, b, c, e, line 2) decreased to 893.00. Methanol decreased the FI of about 1.92% in comparison with buffered extract. It is better shown when the FI of HSA with different amounts of methanol (Fig. 2e, lines 2–4) was compared with the same amounts of strawberry methanol extracts (Fig. 2e, lines 5–7). The binding of methanol itself was 1.92% at addition of 20 μl of methanol (Fig. 2e, line 2), then after addition of 20 μl of strawberry methanol extract (Fig. 2e, line 5) the binding was about 26.44%. At addition of 40 μl of methanol, and 60 μl of methanol the binding was 4.71% and 7.88% (Fig. 2e, lines 3–4) in comparison with 40 μl, 60 μl strawberry extracts (Fig. 2e, lines 6–7) of 29.19 and 36.01%. With the addition of 0.17 mg/mL of fruit extracts the fluorescence intensity dropped for durian, banana, persimmon, kiwi fruit, TD pomelo, mangosteen, strawberry, blond grapefruit and red grapefruit to 886.76, 881.30, 872.72, 826.15, 798.78, 661.90, 656.93, 643.44, and 628.88, respectively (Fig. 2a, b, c). The highest decrease in the fluorescence intensity between the studied fruits was in red grapefruit (Fig. 2b, line 5). The shifts in the emission maximum of HSA changed from 353 nm to 340 nm during the addition of polyphenol methanol extracts of durian (Fig. 2a, line 4). The protein is excited at 280 nm, which is precisely the wavelength of maximal absorption by the phenols themselves. In order to prevent the influence of this factor different maximal wavelengths were shown on Fig. 2d, lines 1–4 (for HSA $\lambda_{ex} = 350$ nm; for strawberry $\lambda_{ex} = 310$ and 392 nm; red grapefruit $\lambda_{ex} = 308$ and 327 nm; for kiwi fruit $\lambda_{ex} = 306$–307 nm). The 3D-FL spectra of HSA in the absence and presence of methanol fruit extracts is provided in Fig. 3 (a–g) with fruits of three groups. Some of the obtained cross images were similar; therefore we showed only two spectra from each group. In traditional group spectrum of apple with two peaks (Fig. 3b, peak a [(λ_{ex}/λ_{em}) = 227/344 nm/nm; intensity (F_0) = 535.01]; peak b (λ_{ex}/λ_{em} = 279/350 nm/nm; $F_0 = 685.61$), banana and green grapes (not shown) were similar in their configurations, where a color in the center of peaks a and b represents the maximum intensity, which corresponds to the emission maximum. The size of the color (intensity) reversely corresponds with the value of binding capacity (Tables 2–3). The spectra of strawberry were different (Fig. 3a), showing three peaks: peak a ($\lambda_{ex}/\lambda_{em} = 226/345$ nm/nm; $F_0 = 403.95$); peak b ($\lambda_{ex}/\lambda_{em} = 280/349$ nm/nm; $F_0 = 633.55$), which were similar to apple, banana and green grapes and peak c ($\lambda_{ex}/\lambda_{em} = 339/427$ nm/nm; $F_0 = 120.52$), which is characteristic only for berries. The exotic group with mangosteen [Fig. 3c, peak a ($\lambda_{ex}/\lambda_{em} = 228/349$ nm/nm; $F_0 = 439.28$)]; peak b ($\lambda_{ex}/\lambda_{em} = 279/355$ nm/nm; $F_0 = 580.22$), durian and persimmon (not shown) revealed similar configurations with two peaks, except kiwi fruit, where the third small peak was found [Fig. 3d, peak a ($\lambda_{ex}/\lambda_{em} = 228/341$ nm/nm; $F_0 = 611.05$)]; peak b ($\lambda_{ex}/\lambda_{em} = 279/345$ nm/nm; $F_0 = 855.95$); peak c ($\lambda_{ex}/\lambda_{em} = 275/454$ nm/nm; $F_0 = 167.21$). Three dimensional (Fig. 4A) and the cross spectrum (Fig. 3e, 4B) of red grapefruit showed three peaks: peak a [($\lambda_{ex}/\lambda_{em}$) = 229/345 nm/nm; intensity (F_0) = 410.39]; peak b ($\lambda_{ex}/\lambda_{em} = 278/352$ nm/nm; $F_0 = 598.61$); peak c ($\lambda_{ex}/\lambda_{em} = 337/414$ nm/nm; $F_0 = 331.92$). Blond grapefruits (Fig. 3d, peak a [($\lambda_{ex}/\lambda_{em}$) = 226/350 nm/nm; $F_0 = 467.00$]; peak b ($\lambda_{ex}/\lambda_{em} = 278/354$ nm/nm; $F_0 = 569.73$); peak c ($\lambda_{ex}/\lambda_{em} = 335/419$ nm/nm; $F_0 = 201.24$). The other citrus fruits, such as pomelos and lemon, had the same shape, but with less prominent peaks than red and blond grapefruits. Peak c corresponds to *p*-coumaric acid (Fig. 4C, peak c with $\lambda_{ex}/\lambda_{em} = 338/440$ nm/nm, $F_0 = 208.42$). The highest change in the fluorescence intensity during quenching of HSA with polyphenols occurred in peak a. 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. A single contour is obtained for HSA [Fig. 3g, peak a ($\lambda_{ex}/\lambda_{em} = 227/349$ nm/nm; $F_0 = 766.27$); peak b ($\lambda_{ex}/\lambda_{em} = 279/352$ nm/nm; $F_0 = 875.28$)], 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. From the emission spectral studies it is understandable that 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). Our research is in accordance Sheng et al. (2014), where the binding

Table 3
Correlation coefficients between bioactive compounds and the overall antioxidant and binding activities in methanol extracts[1] of investigated fruits.

a

Correlation	Banana	Persimmon	M. durian	G. grape	Strawberry	Apple pulp	Kiwi fruit	Mangosteen
Polyph × DPPH	0.8331	0.9513	0.8941	0.9182	0.9312	0.9803	0.9021	0.9307
Flavon × DPPH	0.8842	0.8642	0.9013	0.8768	0.8768	0.8936	0.9194	0.9215
Vit. C × DPPH	0.6543	0.8842	0.6550	0.8753	0.8814	0.8834	0.8843	0.8704
Polyph × CUPRAC	0.8716	0.8176	0.7584	0.9521	0.8911	0.9174	0.8918	0.9549
Flavon × CUPRAC	0.9105	0.8636	0.7183	0.9411	0.9115	0.9289	0.9196	0.9102
Vit. C × CUPRAC	0.6896	0.9015	0.8357	0.8714	0.9025	0.9315	0.8993	0.8868
Polyph × binding	0.7714	0.7299	0.9366	0.8857	0.9237	0.9493	0.9041	0.9718
Vit. C × binding	0.6254	0.6989	0.5865	0.8715	0.9042	0.9014	0.9035	0.8639
DPPH × binding	0.9062	0.7536	0.8952	0.8937	0.8890	0.9032	0.9390	0.8812
CUPRAC × binding	0.8187	0.7439	0.7234	0.8992	0.8969	0.9285	0.9331	0.9097

b

Correlation	R. pomelo	W. pomelo	TD. pomelo	R. grapefruit	B. grapefruit	Lemon
Polyph × DPPH	0.8675	0.8612	0.8915	0.9237	0.9445	0.9478
Flavon × DPPH	0.7413	0.7402	0.8362	0.9269	0.8962	0.9332
Vit. C × DPPH	0.7503	0.7691	0.6768	0.8841	0.6796	0.8741
Polyph × CUPRAC	0.8114	0.8223	0.8615	0.8432	0.9182	0.8563
Flavon × CUPRAC	0.8237	0.5118	0.8578	0.7195	0.8480	0.7651
Vit. C × CUPRAC	0.7807	0.7563	0.5890	0.9642	0.8127	0.9038
Polyph × binding	0.9457	0.8249	0.8393	0.9012	0.9198	0.9274
Vit. C × binding	0.8878	0.7806	0.8383	0.8409	0.8714	0.9241
DPPH × binding	0.9377	0.9352	0.8887	0.8197	0.7884	0.9065
CUPRAC × binding	0.8915	0.9195	0.8915	0.8765	0.8314	0.9117

[1] Extracted at room temperature in a concentration of 25 mg of lyophilized sample in 1 mL of methanol. Abbreviations: M. durian, mature durian; G. grape, green grape; R. pomelo, red pomelo; W. pomelo, white pomelo; TD. pomelo, Thong Dee pomelo; R. grapefruit, red grapefruit; B. grapefruit, blond grapefruit; cupric reducing antioxidant capacity (CUPRAC), 1, 1-diphenyl-2-picrylhydrazyl (DPPH); Polyph, polyphenols; Flavon, flavonoids; Flavan, flavanols; Vit. C, vitamin C; GAE, gallic acid equivalent; CE, catechin equivalent; TE, trolox equivalent; AA, ascorbic acid.

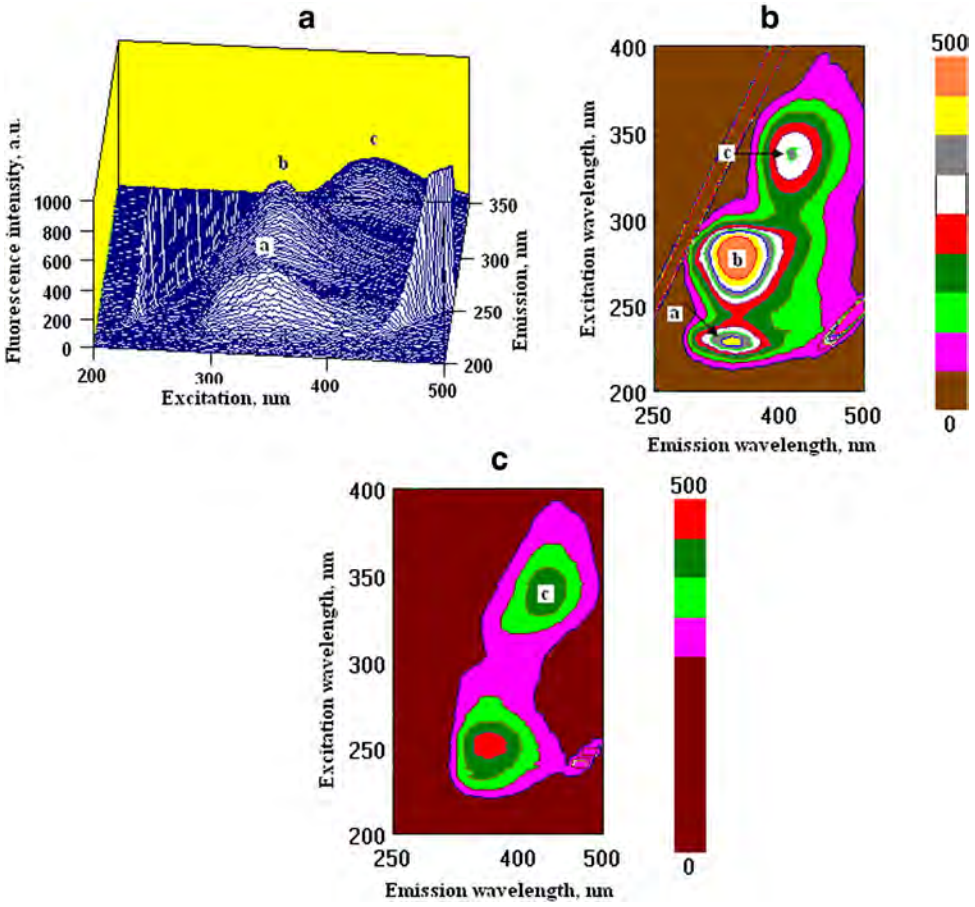

Fig. 4. A, 3D-image with three peaks a, b, c, of human serum albumin (HSA) with red grapefruit in methanol solution. Excitation wavelength scan: 200–500 nm. Emission wavelength scan: 200–350 nm; B, cross image of HSA + red grapefruit with three peaks a, b, c. C, cross image of HSA + *p*-coumaric acid with one peak c. To 20 μl HSA were added 20 μl of 0.17 mg/ml of fruit methanolic extract. The reaction was for 1 h at room temperature. *p*-Coumaric acid is 0.01 mM. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

characteristics of keracyanin (purple pigments from mulberry extracts) with HSA showed that HSA fluorescence quenched by keracyanin follows a static mode. To explore binding behavior of ellagic acid to protein, fluorescence titration results indicated that ellagic acid effectively quenched the intrinsic fluorescence of HSA.

3.4. Correlations between antioxidative compounds and their bioactive properties

Correlations between antioxidative compounds with their respective bioactive properties (antioxidant activities and binding properties with HSA) were calculated and presented in Table 3. It was of interest to know which of the two bioactive compounds mentioned (polyphenols or ascorbic acid) are the main contributors to the antioxidant activity of all screened fruits in this research. As it was calculated, a very good correlation was between the antioxidant activity as determined by two antioxidant assays and phenolic compounds. So, high correlations were found between total polyphenols and flavonoids and antioxidant activities with DPPH (R^2 in range 0.83–0.98 and 0.74–0.93) and CUPRAC (R^2 in range 0.76–0.93 and 0.61–0.94). The correlation with vitamin C was slightly lower and was estimated between antioxidant activities with DPPH (R^2 in range 0.66–0.88) and CUPRAC (R^2 in range 0.59–0.90). Ascorbic acid appeared to have higher correlation in apple, kiwi fruit, red grapefruit and lemon (Table 3). There were positive correlations between the antioxidant activity and total polyphenols and ascorbic acid as it is shown in our results. The correlation between the binding properties and polyphenols was $R^2 = 0.73–0.97$; vitamin C and binding was $R^2 = 0.59–0.92$; DPPH and binding was $R^2 = 0.75–0.94$; and CUPRAC and binding was R^2 from 0.72 to 0.93 (Table 3). So, high correlations were found between total polyphenols and antioxidant activities in Thai indigenous fruits ($R^2 > 0.9$) as potentially good sources of nutrients, bioactive compounds, and antioxidant activities (Judprasong et al., 2013). Our results are in line with Gomes, Tessaro, Eberlin, Pastore, and Liu (2013), where it was shown that phenolics, including flavonoids, are suggested to be the major bioactive compounds, contributing to the health benefits of fruits. A good correlation was observed between the percentage of reduced DPPH and the total phenol content ($R^2 = 0.79$). Similar results were obtained when FRAP, total phenolics and anthocyanin content in a variety of Chilean berry fruits (blueberries, blackberries, raspberries and strawberries) and apples (cv. Fuji, Granny Smith, Pink Lady, Red Delicious and Royal Gala) were investigated (Henriquez, Lopez-Alarcon, Lutz, & Speisky, 2011). Our results were as well similar to Moo-Huchin et al.'s (2014), where the physicochemical characteristics varied between Mexican tropical fruits and were good sources of bioactive compounds. Total soluble phenol content between the tropical fruits from Yucatan, Mexico showed a correlation with antioxidant activity by ABTS ($R^2 = 0.52$) and DPPH ($R^2 = 0.43$). Most fruit extracts showed significant correlations between the antioxidant capacity and the incubation time in berries and apples, although in some cases the FRAP indexes did not correlate with the total phenolics and/or anthocyanins content. Among all phenol classes, the flavan-3-ol content showed the highest correlation ($R^2 = 0.77$) in traditional apple cultivars (Panzella et al., 2013). Antioxidant capacity ranged from 0.66 to 124.66 μmol TE/g of fruit and strongly correlated with phenolic content, while the enzyme inhibition was poorly correlated with total phenolic and antioxidant capacity in commonly used fruits (Podsedek et al.,

2014). Our results are in accordance with Kaulmann, Jonville, Schneider, Hoffmann, and Bohn's (2014), where it was shown that for plums the best predictors of antioxidant capacity as assessed by FRAP were chlorogenic acid and vitamin C ($R^2 = 0.853$), and chlorogenic acid and flavonoids for FRAP ($R^2 = 0.711$). The quenching properties of these fruits are directly correlated with their antioxidant properties and the amount of polyphenols (Table 2a and b). The binding properties (Figs. 2–4) of the investigated fruit polyphenols and HSA, based on the data of fluorescence intensity measurements and the obtained results of polyphenols and antioxidant activities showed the best correlation (Table 3).

3.5. Binding properties of antioxidants with HSA evaluated by FTIR

The IR spectra of methanol fruit extracts after interaction with HSA were compared between them and also with the standard (HSA without the addition of the fruit polyphenol extracts) in the range of common peaks (Fig. 5a and b). The frequencies of the bands correlated with the standard in some regions, but completely differed for citrus samples (Goormaghtigh, Ruysschaert, & Raussens, 2006; Liang, Zhao, Meng, Liu, & Lin, 2013). In strawberries and durian (Fig. 5a, from the top lines 2, 3), kiwi fruit and grapefruit red (Fig. 5b from the top lines 1, 2) appeared two peaks at 2400 cm^{-1}. The sharpest one is in red grapefruit. The correlation (Fig. 5a) and (Fig. 5b) on the 3600–2800 and 1800–1000 cm^{-1} regions was done. Spectra have been scaled to an identical area under amides I (with the 1655 and 1630 amide I components) and II [enlarged over the amide I–II region (1720–1480 cm^{-1})]. The maximum of amide I (appears to be shifted toward; 1655 cm^{-1}). The comparison of these results for the first time showed that IR spectra can be used for a rapid estimation of binding properties of the extracted fruit polyphenols in interaction with HSA. Our results of FTIR and fluorescence binding of HSA to polyphenols were in line with Khan, Rakotomanomana, Dufour, and Dangles (2011), showing the binding of flavanoids from citrus fruits. Our results were in accordance with Derenne et al.'s (2013), where cancer cells exposed *in vitro* to 6 polyphenols: 3 natural well documented polyphenols (curcumin, epigallocatechin gallate (EGCG) and quercetin) and 3 synthetic molecules with a very closely related chemical structure. Metabolic changes induced by polyphenols closely related from a chemical point of view were identified. Our results by FTIR spectra showed that fruit polyphenol extracts altered HSA secondary structure, which was in line with the results of Tang, Liang, Zheng, and Lian (2013). As the fluorometric methods also IR spectroscopy can be used as an additional indication of similarity and differences between the cultivars.

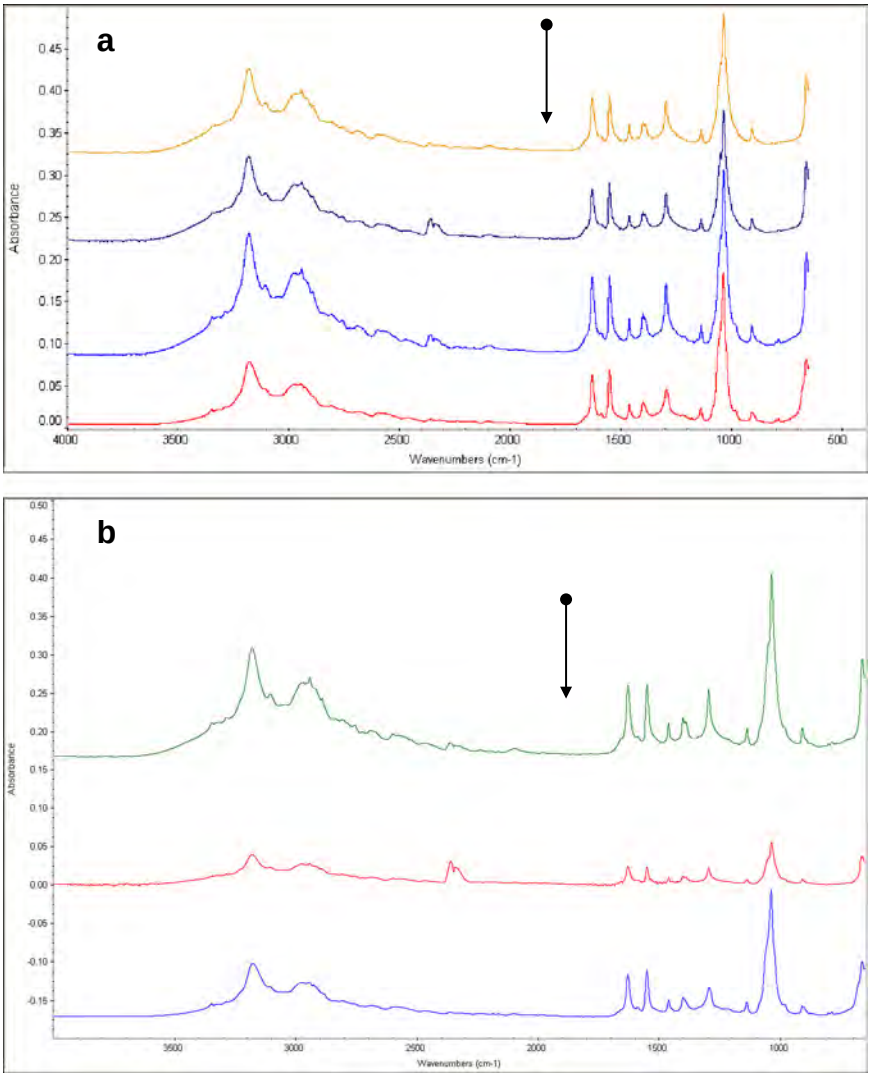

Fig. 5. Infrared spectra of methanol extracts of (a): from the top lines the following samples are HSA + apple, HSA + strawberry, HSA + mature durian, HSA; (b) from the top lines of the following samples are HSA + kiwi fruit 'Hayward', HSA + red grapefruit, HSA. A Nicolet iS 10 FT-IR Spectrometer with the smart iTRTM ATR (attenuated total reflectance) accessory was used to record IR spectra. Comparison and matching was done on the basis of wavenumber peaks of HSA as a standard and between the different fruit extracts in the region from 4000 to 650 cm^{-1}.

4. Conclusions

We have studied by fluorescence spectroscopy the specific binding of HSA with investigated fruit polyphenols. The contents of bioactive compounds, antioxidant and binding properties are higher in strawberries, followed by apple, and to a less degree in other studied fruits could be a valuable addition and new source to known disease preventing diets. Our results by FTIR spectra showed that fruit polyphenol extracts altered HSA secondary structure. As the fluorometric methods also IR spectroscopy can be used as an additional indication of similarity and differences between the binding properties of extracted polyphenols. Fluorometry was used as an indicator for comparison of methanol extracts from the studied fruits. The similarity and differences between these fruits were based on the data of quenching of their extracts with HSA in the polyphenol region. Our data may be interesting to better understanding the effects of fruit consumption on the human body, as to the binding to albumin and potential competition with drugs. Using fluorescence it is possible to detect the polyphenols and their binding abilities in the consumed fruits.

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