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Chemistry and biological properties of berry volatiles by two-dimensional chromatography, fluorescence and Fourier transform infrared spectroscopy techniques

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ABSTRACT

In this study, three-dimensional fluorescence spectroscopy in combination with ultraviolet visible (UV–Vis) absorption spectroscopy, Fourier transform infrared spectroscopy (FTIR) and two-dimensional chromatography techniques were employed to investigate the main compounds in gooseberries, blueberries and cranberries. The determination of the terpenes (the main group of secondary metabolites) in the three berries was done by headspace solid-phase microextraction coupled with comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (HS-SPME/GC × GC–TOFMS). Main volatiles were assigned in each of the three berries' chromatograms. The compounds were organized in different groups: monoterpene hydrocarbons and monoterpene oxygen-containing compounds (oxides, alcohols, aldehydes, and ketones). The highest amount of alcohol and ester compounds (85%) was estimated in blueberry; carboxylic acids, ketones and aldehydes were found in cranberry (62%) and terpenes in cape gooseberry (8%). Human serum albumin (HSA) has been used as a model protein to study drug–protein interaction. Specific binding of polyphenols from berries to HSA under the physiological conditions was a result of the formation of a polyphenol–HSA complex. The berries' extracts interact with HSA before and after incubation with different binding affinities which are related to their antioxidant properties. The effect of the complexation on the secondary protein structure was verified in the changes of amide bands. Principal component analysis (PCA) was applied to discriminate the differences among the samples' compositions.

1. Introduction

The health benefits of berries are well documented due to their rich content in bioactive phytochemicals (pigments, phenolics and vitamins) as well as volatiles responsible for specific flavors (Arancibia-Avila et al., 2011; Caprioli et al., 2016; Dembitsky et al., 2011; Gorinstein et al., 2013; Namiesnik et al., 2014a, 2014b). There are a few reports on the properties of cranberry. The antioxidant, radical scavenging, antibacterial, antimutagen and anticarcinogen properties of cranberry's major bioactive compounds (anthocyanins, flavonols, flavan-3-ols, proanthocyanidins, and phenolic acid derivatives) were investigated by Cote, Caillet, Doyon, Sylvain, and Lacroix (2010). European cranberry is rich in biologically active substances, making it valued by both the phyto-pharmaceutical and food industries (Cesoniene, Jasutiene, & Sarkinas, 2009). Overall results by Kim, Jung, Kim, and Kwak (2008) suggested that freeze-dried cranberry powder might have the serum lipid improving and antioxidative effects demonstrated by their protection against protein and lipid oxidation. At present cape gooseberry (*Physalis peruviana*) fruit is one of the less consumed raw materials of plant origin for human nutrition. This fruit, as well as alimentary products made of it, was used by healers in folk medicine in the distant past (Rop, Mlcek, Jurikova, & Valsikova, 2012). The volatile compounds are good biomarkers of berry freshness, quality and authenticity (Caprioli et al., 2016; Dragović-Uzelac et al., 2008; Gutiérrez, Sinuco, & Osorio, 2010; Hanene et al., 2012; Rodriguez-Saona, Parra, Quiroz, & Isaacs, 2011). There are some reports

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about determination of volatile substances in different berries (Carvalho, 2014; Croteau & Fagerson, 2006; Mayorga, Knapp, Winterhalter, & Duque, 2001; Wang, Wang, & Chen, 2008; Yilmaztekin, 2014a; Yilmaztekin & Sislioglu, 2015). Application of a headspace solid-phase microextraction (HS-SPME) method for analysis of volatiles by comprehensive two-dimensional gas chromatography (GC × GC) time-of-flight mass spectrometry (TOFMS) is presented in a number of reports (Dymerski et al., 2015; Nicolotti et al., 2013; Yilmaztekin, 2014b). Some recent reports proposed as well different procedures for volatile substances determination in different berries. A fast and efficient GC–MS method including a minimal sample preparation technique for the discrimination of sea buckthorn varieties based on their chromatographic volatile fingerprint was proposed by Socaci, Socaciu, Tofana, Rati, and Pintea (2013). Fast gas chromatography–surface acoustic wave detection (FGC–SAW) was employed to characterize blueberry volatile profiles according to genotypes and fruit maturity (Du, Olmstead, & Rouseff, 2012; Du, Plotto, Song, Olmstead, & Rouseff, 2011; Du & Rouseff, 2014), which was effective for major blueberry volatiles, but could not determine many mid- and low-level volatiles as they were often coeluted with higher concentration volatiles. The information of a combination of spectroscopic and fluorometric methods for the comparison of different berries is limited. Evaluation of the antioxidant properties of gooseberries, cranberries and blueberries was done in our recent reports (Namiesnik et al., 2014a, 2014b). Based on the cited data the main purpose of this study was to determine the volatile and bioactive substances in cape gooseberry (*Physalis peruviana*) and to compare them with those from blueberry (*Vaccinium corymbosum*) and cranberry (*Vaccinium macrocarpon*). For this purpose the volatile substances were determined by headspace solid-phase microextraction coupled with comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (HS-SPME/GC × GC–TOFMS) as was shown in other reports as well (Kupska, Chmiel, Jedrkiewicz, Wardencki, & Namiesnik, 2014; Ozel, Gogus, & Lewis, 2008). Pharmaceutical interactions with human serum albumin (HSA) are of great interest, because HSA is a pharmacokinetic determinant and a good model for exploring the protein–ligand interactions. Naturally occurring flavones due to their hydrophobic nature possess various pharmacological activities and bind to HSA in human plasma (Liu, Bao, Ding, Jang, & Zou, 2010; Singh, Ghosh, & Dasgupta, 2013; Xiao et al., 2011). It is well known (Caruso, Vilegas, Fossey, & Cornelio, 2012; Poor et al., 2012) that natural flavonoids can also bind to HSA at the same binding site as achratoxin A does (site I, subdomain IIA). The bioactivity of the berry extracts and monoterpenes was determined by two antioxidant methods ABTS and CUPRAC (Apak, Guclu, Ozyurek, & Karademir, 2004; Apak, Özyürek, KGüçlü, & Çapanoğlu, 2016). The polyphenol extracts of berries were submitted to the interaction with HSA. Such interaction was studied at natural conditions and during incubation of the protein–polyphenol complex by fluorimetry and FTIR spectroscopy and radical scavenging assays (Magalhaes, Segundo, Reis, & Lima, 2008; Shi, Dai, Liu, Xie, & Xu, 2003; Simões, Esteves da Silva, & Leitão, 2014; Tang, Zuo, & Shu, 2014). To our knowledge, there has been no study reporting a combination of the volatile and antioxidant contents of these kinds of berries. Therefore, the characterization of biological properties of berries will be done by radical scavenging assays, two-dimensional chromatography, three-dimensional fluorescence and FTIR techniques.

Table 1
Volatiles identified using chromatograms obtained after analysis of blueberry, cranberry and cape gooseberry by SPME–GC × GC–TOFMS in TIC mode.

No.	Compounds
Blueberry	
1	Linalyl butyrate
2	Nonanal
3	Phenylethyl alcohol
4	α-Ethylcaproic acid
5	Isomethyl ionone
6	Acetic acid phenethyl ester
7	n-Hexyl acetate
8	1-Hexanol
9	*cis*-3-Hexen-1-ol
10	3-Nonyne
11	Isopentyl alcohol
12	Isopentyl alcohol, acetate
13	Isovaleric acid
14	Methyl isovalerate
15	Ethyl butanoate
Cranberry	
1	Oxalic acid, 2-methylphenyl pentadecyl ester
2	Propanoic acid
3	Benzeneethanol
4	(E)-2-Octen-1-ol
5	1-Hepten-3-ol
6	(E)-2-Octen-1-al
7	n-Caproaldehyde
8	Benzyl formate
9	*trans*-2-*trans*-4-Heptadienal
10	Propenal
11	*trans*-2-Pentenal
12	2-Methyl-1-butanol
13	Pentyl alcohol
14	*cis*-3-Hexen-1-ol
15	Benzaldehyde
Cape gooseberry	
1	Ethyl dodecanoate
2	Caryophylene oxide
3	Octanoic acid, 3-methylbutyl ester
4	Dodecanoic acid, methyl ester
5	Ethyl caprate
6	Capric acid methyl ester
7	n-Dodecane
8	γ-Butyl-γ-butyrolactone
9	Methyl-2-methoxyoct-2-enoate
10	Caprylic acid methyl ester
11	Butyl 3-hydroxybutyrate
12	Ethyl caproate
13	Methyl benzoate
14	γ-Caprolactone
15	Benzyl alcohol
16	3-Methyl-3-vinyl-1-cyclopropene
17	Benzaldehyde
18	6-Methyl-5-heptene-2-one
19	3-Methyl-1-penten-3-ol
20	Methyl β-methylcrotonate
21	n-Butyl acetate
22	Hexanal
23	Pentyl alcohol
24	γ,γ-Dimethylallyl alcohol
25	3,4-Pentadienal

2. Materials and methods

2.1. Reagents and materials

Analytical terpene standards were used to confirm the identity of selected compounds (Sigma-Aldrich, St. Louis, MO, USA). The standards of 19 in quantity included: β-pinene, camphene, β-myrcene, α-pinene, α-phellandrene, terpinolene, *p*-cymene, eucalyptol, limonene, α-ocimene, γ-terpinene, fenchone, (E)-linalool oxide, linalool, camphor, terpinen-4-ol, α-terpineol, β-cyclocitral, and α-ionone. As an internal standard the borneol substance was used (Sigma-Aldrich, St. Louis, MO, USA). A high purity deionized water from MilliQ A10 Gradient/Elix System (Millipore, Bedford, MA, USA) and GC grade sodium chloride (Sigma-Aldrich, St. Louis, MO, USA) were used throughout the experiment.

2.2. Sample preparation

All berries were from West Pomerania Province, harvested in late June, Poland. During the studies, three types of the fruit samples

Table 2
Volatiles identified using chromatograms obtained after analysis of samples of berries by SPME-GC × GC-TOFMS in TIC mode.

No.	Compound	RT1 (s) ± NMP[a]	Average RT2 ± SD[b]	Quantification (Y = AX + B)	S_A	S_B	R^2	Similarity[c]	Unique mass	C_J[d] (μg/kg^{-})	C_Z[d] (μg/kg)	C_M[d] (μg/kg)
1	α-Pinene	1032 ± 1	2.05 ± 0.03	Y = 0.0437 * X + 0.1134	0.0007	0.0323	0.9991	920	93	17	4.2	98
2	Camphene	1056 ± 1	2.06 ± 0.02	Not quantified	–	–	–	891	91	–	–	–
3	β-Pinene	1110 ± 1	2.07 ± 0.01	Not quantified	–	–	–	952	93	–	–	–
4	β-Myrcene	1134 ± 1	2.05 ± 0.03	Not quantified	–	–	–	901	93	–	–	–
5	α-Phellandrene	1164 ± 1	2.12 ± 0.02	Not quantified	–	–	–	905	93	–	–	–
6	*p*-Cymene	1188 ± 0	2.08 ± 0.03	Not quantified	–	–	–	909	119	–	–	–
7	Eucalyptol	1212 ± 1	2.12 ± 0.02	Not quantified	–	–	–	962	154	–	–	–
8	Limonene	1220 ± 1	2.14 ± 0.02	Not quantified	–	–	–	941	93	–	–	–
9	α-Ocimene	1236 ± 1	2.10 + 0.01	Not quantified	–	–	–	934	93	–	–	–
10	γ-Terpinene	1272 ± 1	2.13 ± 0.02	Y = 0.1283 * X + 0.1453	0.0019	0.0435	0.9921	952	93	19	3.0	95
11	Fenchone	1302 ± 1	2.30 ± 0.03	Not quantified	–	–	–	911	81	–	–	–
12	(E)-linalool oxide	1312 ± 1	2.27 ± 0.03	Not quantified	–	–	–	892	59	–	–	–
13	Terpinolene	1320 ± 0	2.10 ± 0.02	Y = 0.1234 * X − 0.1390	0.0034	0.0357	0.9987	922	93	1.2	6.8	180
14	Linalool	1332 + 1	2.35 ± 0.01	Y = 0.1879 * X + 0.0281	0.0025	0.0262	0.9996	939	71	23	14	73
15	Camphor			Not quantified	–	–	–	902	95	–	–	–
16	Terpinen-4-ol	1500 ± 0	2.47 ± 0.01	Y = 0.2957 * X − 0.0623	0.0100	0.1042	0.9977	958	71	21	140	50
17	α-Terpineol	1528 ± 1	2.81 ± 0.03	Y = 0.1354 * X + 0.0992	0.0032	0.0359	0.9988	936	93	0.88	0.95	3.7
18	β-Cyclocitral	1572 ± 1	2.34 ± 0.01	Not quantified	–	–	–	904	152	–	–	–
19	α-Ionone	1820 ± 1	1.99 ± 0.02	Not quantified	–	–	–	912	121	–	–	–

[a] RT1 (s) ± NMP: 1st dimension retention times with the variation in modulation period (MP) among the samples, where a compound was detected.
[b] SD: standard deviation of 2nd dimension retention times among the samples, where a compound was detected.
[c] Forward similarity; value out of 1000.
[d] Concentration of compounds for the following samples — C_J for blueberry, C_Z for cranberry, C_M for cape gooseberry.

blueberries, cranberries and cape gooseberries (*Vaccinium corymbosum*, *Vaccinium macrocarpon* and *Physalis peruviana*) were washed and homogenized before each analysis. After this step, 8.0 g of a blended sample was placed into a 20 mL vial and 2.0 g of oven-dried sodium chloride was also transferred. Subsequently, the borneol internal standard was added with a concentration of 10 μg/kg into each sample. The vial was sealed with a cap with PTFE-lined silicone septum to prevent the loss of volatiles. Five replicates were done for the analysis of each type of fruit, and triplicate analysis for each standard was carried out. The total number of 162 samples was prepared including 15 fruit analyses. Standards (57 analyses) were used for positive identification of 19 terpene compounds and for quantification of six selected terpenes (90 runs). For bioactive compounds the following procedure was used. The edible parts of berries were prepared manually without using steel knives. The berries were weighed, chopped and homogenized under liquid nitrogen in a high-speed blender (Hamilton Beach Silex professional model) for 1 min. A weighed portion (50–100 g) was then lyophilized for 48 h (Virtis model 10-324), and the dry weight was determined. The samples were ground to pass through a 0.5 mm sieve and stored at − 20 °C until the bioactive substances were analyzed.

2.3. Extraction of the analytes

Volatile compounds from the fruit samples were extracted using headspace solid-phase microextraction (HS-SPME). Prior to the extraction process, the samples were incubated at 50 °C for 10 min and agitated at 700 rpm. Extraction at the same temperature was carried out for 30 min using a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) SPME fiber of 50/30 μm thickness and 2 cm length (Sigma–Aldrich) was used. Subsequently the fiber was removed from the vial and transferred to the injector of a two-dimensional gas chromatograph for thermal desorption of the analytes at 250 °C for 3 min.

2.4. Instrumentation

The GC × GC system was an Agilent 6890A gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a liquid nitrogen-based dual stage cryogenic modulator and a split/splitless injector, coupled with Pegasus IV time-of-flight mass spectrometer (LECO Corp., St. Joseph, MI, USA). The column set consisted of a 30 m × 0.25 mm × 0.25 μm primary column (1D) with an Equity 1 stationary phase (Supelco, Bellefonte, PA, USA) and a 1.6 m × 0.10 mm × 0.10 μm secondary column (2D) with a SolGel-Wax stationary phase (SGE Analytical Science, Austin, TX, USA). A modulation period of 6 s was employed with the cryogenic trap cooled to − 196 °C using liquid nitrogen. The sample components were separated using the following optimized temperature program for the primary GC oven: initial temperature of 40 °C maintained for 3 min, then ramped at 5 °C/min to 150 °C and at 10 °C/min to 250 °C, and finally kept for 2 min. The optimized temperature program for the secondary GC oven was with the shift of + 5 °C regarding the program of the primary GC oven. The total analysis time was 37 min. The injector was carried out in splitless mode at 250 °C. Helium was used as the carrier gas at a constant flow of 1.0 mL/min. The temperatures for the transfer line and ion source were maintained at 250 °C. The detector voltage was set to 1600 V. Ions in the m/z 33–400 range were analyzed with a data acquisition rate of 125 spectra/s. The entire extraction process was carried out using an MPS autosampler (GERSTEL Co., Mülheim, Germany).

2.5. Data analysis

Data processing was performed using the algorithm for peak deconvolution included in the Chroma TOF software (LECO Corp., version 4.44). Tentative identification was accomplished through MS library search using the NIST 2011 and Willey 11 mass spectral library. The similarity parameter was set up to 850 values to assure correct identification. Positive identification of 19 analytes (β-pinene, camphene, β-myrcene, α-pinene, α-phellandrene, terpinolene, *p*-cymene, eucalyptol, limonene, α-ocimene, γ-terpinene, fenchone, (E)-linalool oxide, linalool, camphor, terpinen-4-ol, α-terpineol, β-cyclocitral, and α-ionone) was confirmed by the comparison of retention times (in ^{1}D and ^{2}D) with authentic standards (Tables 1–2). Furthermore a fiber blank run was done every 10 analyses to consider the influence of column or SPME fiber degradation.

2.6. Software for chemometric data analysis

The principal component analysis (PCA) was carried out using the open source R software (version 3.0.2; Free Software Foundation,

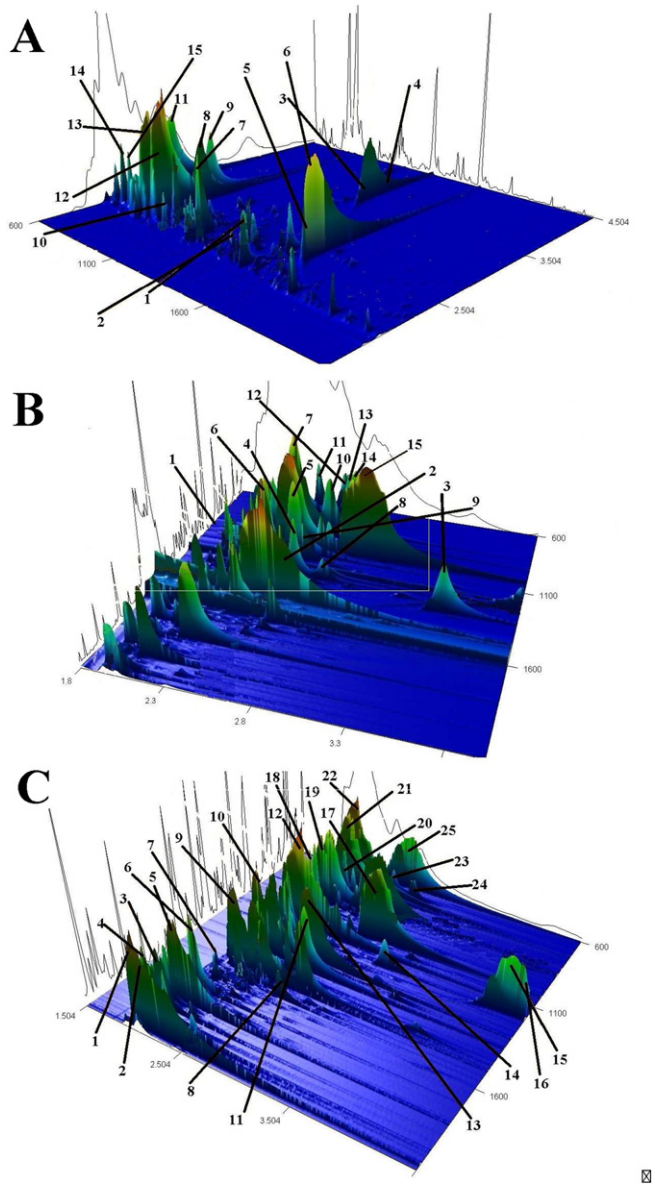


Fig. 1. Chromatograms of volatile compounds from: A — blueberry, B — cranberry, and C — cape gooseberry, obtained using the SPME–GC × GC–TOFMS approach in TIC (*Total Ionic Current*) mode. Compounds numbers correspond to Table 1.

Boston, MA, USA) to discriminate samples regarding the differences in volatile fraction composition of selected fruit types. Two types of graphs were applied for the above-mentioned discrimination. In the first approach the object classification was achieved by presenting samples in two PCs using the total area of peaks belonging to the specific chemical classes. The second presentation of the dataset was done by the use of a PCA biplot in R, which allows defining the correlations between variables (peak areas of selected terpenes) and objects (samples). In both cases the input data were mean-centered and autoscaled.

2.7. Determination of bioactive compounds and antioxidant activities

2.7.1. Chemicals

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), human serum albumin (HSA), Tris, tris(hydroxymethy1) aminomethane, Folin–Ciocalteu reagent, 2,2′-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{\bullet+}$), lanthanum (III) chloride heptahydrate, $CuCl_2 \times 2H_2O$, and 2,9-dimethyl-1,10-phenanthroline (neocuproine) were used.

2.7.2. Extraction of phenolic compounds

The lyophilized samples of berries (1 g) were extracted with 40 mL of ethanol/water (50%:50%) at 40 °C during 4 h. Ultrasound-assisted extraction was carried out with an Ultrasonic Cleaner Delta DC-80H, with an operating frequency of 40 kHz, an output power of 80 W, and a 45 W heater. The extracts were filtered through the Buchner funnel. These extracts were submitted for determination of bioactive compounds (Da Porto, Porretto, & Decorti, 2013; Haruenkit et al., 2010). The polyphenols were determined by the Folin–Ciocalteu method with measurement at 750 nm with a spectrophotometer (Hewlett-Packard, model 8452A, Rockville, USA). The results were expressed as mg of gallic acid equivalents (GAE) per g DW (Singleton, Orthofer, & Lamuela-Raventos, 1999).

The total antioxidant capacity (TAC) was determined by the following assays:

1. Cupric reducing antioxidant capacity (CUPRAC): this assay is based on utilizing the copper (II)–neocuproine [Cu (II)–Nc] reagent as the chromogenic oxidizing agent. To the mixture of 1 mL of copper (II)–neocuproine and NH_4Ac buffer solution, acidified and nonacidified methanol extracts of berry (or standard) solution (x, in mL) andH_2O [(1.1 − x) mL] were added to make a final volume of 4.1 mL. The absorbance at 450 nm was recorded against a reagent blank (Apak et al., 2004; Apak et al., 2016).
2. 2,2′-Azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt (ABTS): $ABTS^{\bullet+}$ was generated by the interaction of ABTS (7 mM) and $K_2S_2O_8$ (2.45 mM). This solution was diluted with ethanol until the absorbance in the samples reached 0.7 at 734 nm (Re et al., 1999).

2.8. Fluorometric measurements

Fluorometric measurements were used for the evaluation of the binding properties of berry 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. For the fluorescence measurement, 3.0 mL of 2.0×10^{-6} mol/L HSA solution and various amounts of berry extracts were added to a 1.0 cm quartz cell manually using a micro-injector. The concentrations of berry extracts were ranged from 0 to 1.5 mg/mL, and the total accumulated volume of berry 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, and the initial excitation wavelength was set at 200 nm with an increment of 5 nm. 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 NaCl. The interaction of polyphenols from berries and standards with HSA was also measured before and after incubation for 24 h at 37° C.

2.9. IR spectra

Interaction of total phenols with HSA was studied by IR spectroscopy. A Nicolet iS 10 FT-IR Spectrometer (Thermo Scientific Instruments LLC, Madison, 105 WI, USA), with the smart iTRTM ATR (attenuated total reflectance) accessory was used to record IR spectra (Shi et al., 2003). KBr pellets were made by mixing 10 mg of the investigated samples and 150 mg KBr.

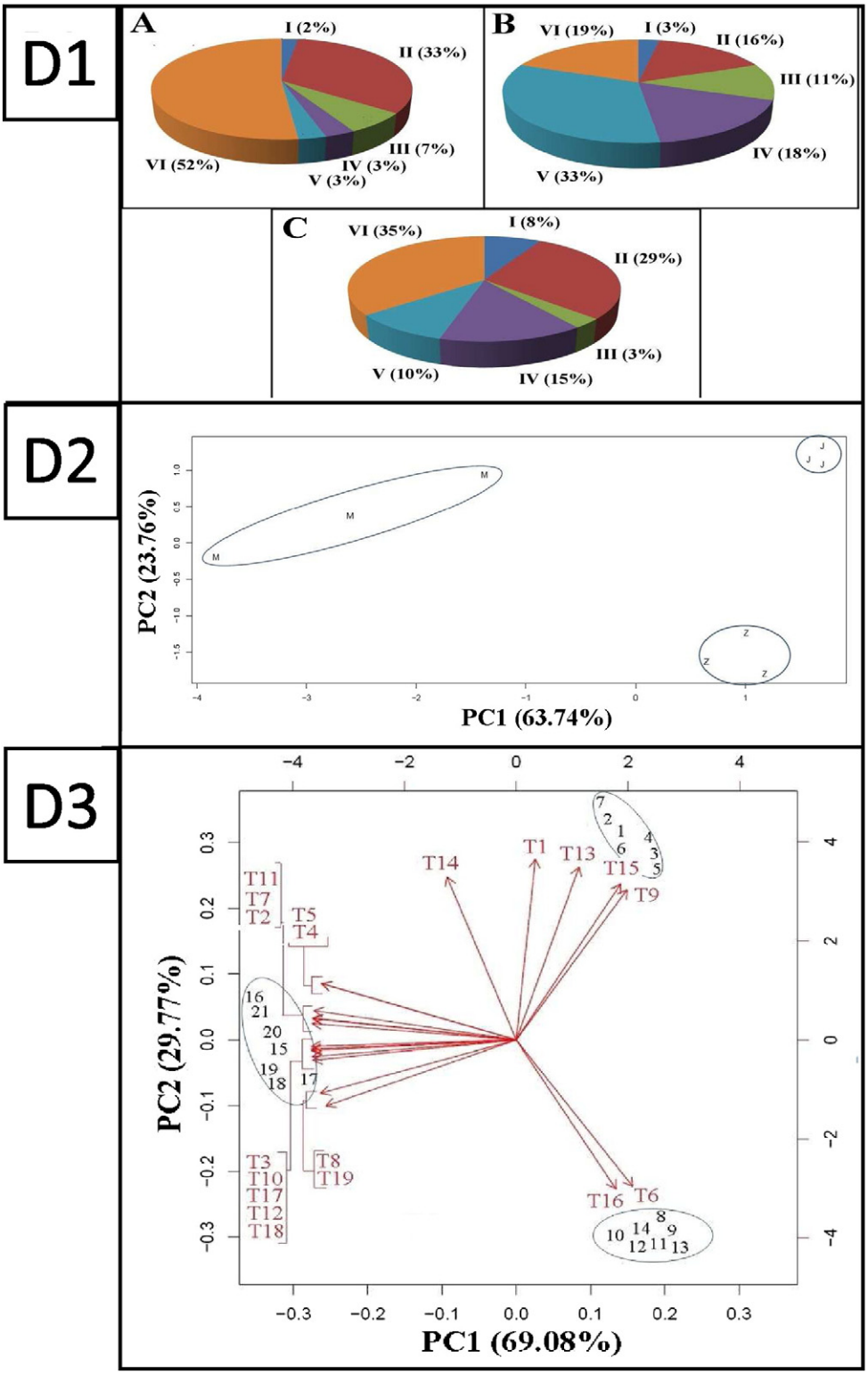


Fig. 2. Distribution of volatiles by chemical families for three types of fruits (D1): A — blueberry, B — cranberry, and C — cape gooseberry. The values indicate the total relative peak area of each chemical family: I — terpenes, II — esters, III — carboxylic acids, IV — ketones, V — aldehydes, and VI — alcohols; (D2), PCA results for samples of selected berries using summary area of peaks belonging to the terpene, ester, carboxylic acid, ketone, aldehyde and alcohol group of compounds as an input data. J — blueberry, Z — cranberry, M — cape gooseberry; (D3), PCA biplot of terpenes on the first two PCs. Vectors indicate different compounds, which are responsible for separation of objects on the PC1 PC2 plane. Numbers show the position of reference for a specific sample on a PCA biplot graph; T1 — β-pinene, T2 — camphene, T3 — β-myrcene, T4 — α-pinene, T5 — α-phellandrene, T6 — terpinolene, T7 — *p*-cymene, T8 — eucalyptol, T9 — limonene, T10 — α-ocimene, T11 — γ-terpinene, T12 — fenchone, T13 — (E)-linalool oxide, T14 — linalool, T15 — (−)-camphor, T16 — terpinen-4-ol, T17 — α-terpineol, T18 — β-cyclocitral, T19 — α-ionone, 1–7 — blueberry, 8–14 — cranberry, 15–21 — cape gooseberry.

Table 3
Bioactive compounds in ethanol–water (50:50) extracts of gooseberries (*Physalis peruviana*), cranberries (*Vaccinium macrocarpon*) and blueberries (*Vacciniumcorymbosum*)[1,2].

Extracts	Polyphenols	ABTS	CUPRAC
Gooseberry	12.89 ± 1.15^{c}	61.15 ± 5.43^{c}	33.74 ± 3.18^{c}
Cranberry	24.67 ± 2.21^{b}	216.98 ± 18.32^{b}	119.39 ± 9.87^{b}
Blueberry	45.54 ± 3.54^{a}	327.76 ± 28.17^{a}	191.83 ± 16.54^{a}
α-Pinene	6.20 ± 0.63^{cd}	42.85 ± 3.25^{cd}	24.12 ± 2.12^{cd}
Terpinolene	5.47 ± 0.42^{d}	37.81 ± 3.18^{d}	21.21 ± 2.03^{d}
Linalool	1.73 ± 0.14^{e}	11.96 ± 1.09^{e}	6.73 ± 0.61^{e}

Means within a column with the different superscripts or without superscript are statistically different ($p < 0.05$; Student's *t*-test).
Abbreviations: GAE, gallic acid equivalent; ABTS, 2,2′-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt; CUPRAC, cupric reducing antioxidant capacity; TE, trolox equivalent; polyphenols, mg GAE; ABTS and CUPRAC, μM TE.
[1] Values are means ± SD of 3 measurements.
[2] Per gram dry weight.

3. Results and discussion

3.1. Volatile substances

The composition of cape gooseberry volatile fraction has been described by chromatographic techniques (Gutiérrez et al., 2010; Mayorga et al., 2001; Yilmaztekin, 2014a, 2014b). The first approach in this issue was to compare the unextracted chromatogram (in TIC mode — *Total Ionic Current mode*) of cape gooseberry with the ones of blueberry and cranberry. Analyzing the results (Fig. 1, Table 1) it can be observed that the main constituents of 15 volatiles in each sample of blueberry and cranberry and 25 compounds in gooseberry are similar and belong to the same type of compounds from the chemical families of terpenes, esters, carboxylic acids, ketones, aldehydes and alcohols. The total amount of peaks on each chromatogram was within the range from 2000 to 3000, however those from 600 to 900 peaks were not tentatively identified and were treated as unknown peaks, regarding the complied similarity criteria. For three types of samples all identified compounds were grouped according to their membership to the chemical family (Fig. 2D1). As it can be observed, the volatile fraction of these berries differs regarding to the specific type. The highest amount of alcohol and ester compounds is contained in blueberry, carboxylic acids, ketones and aldehydes in cranberry and terpenes in cape gooseberry. Dragović-Uzelac et al. (2008) found higher alcohols, esters, aldehydes, phenols, monoterpenes and acids, among which esters and alcohols were found to be the major compounds in all investigated blueberry cultivars. The same results were found also by us: esters (33%, Fig. 2D1, A) and alcohols (52%, Fig. 2D1, A). The following compounds probably contributed to the blueberry aroma: α-terpineol (peak 5, Fig. 1A; Table 1) and n-hexyl acetate (peak 7, Fig. 1A). Gutiérrez et al. (2010), found that the compounds with the highest flavor dilution factor value were hexanal, 3-hydroxy-2-butanone, 2-methylpropanol, ethyl 2-hydroxybutanoate, ethyl octanoate, and butyl 3-hydroxybutanoate. In our study one of the main compounds was hexanal (peak 22, Fig. 1C). β-Pinene, limonene, (Z)- and (E)-dihydrolinalool oxide, linalool, R-terpineol, and geranyl acetone were present in all of the studied blueberry cultivars (Du & Rouseff, 2014; Du et al., 2011; Rodriguez-Saona et al., 2011). In cranberry (Fig. 1B, Table 1) were found 4-penten-2-ol, 3-*cis*-hexenyl formate, benzaldehyde, α-1-terpineol, butyric acid, and benzyl alcohol (Ruse et al., 2012). The main component was 3-methylbutanoic acid (Yilmaztekin & Sislioglu, 2015) and terpenes such as α- and β-pinene, myrcene, limonene, linalool, α-terpineol, and nerol were reported (Croteau & Fagerson, 2006). Our results differ from the cited ones (Yilmaztekin, 2014a, 2014b), where alcohols consisted of 43.8% (35%, Fig. 2D1, C) with the most abundant being benzyl alcohol (peak 15, Table 1, Fig. 1C); esters 11.7% [29%, Fig. 2C with ethyl dodecanoate (peak 1, Fig. 1C), ethyl caprate (peak 5, Fig. 1C), butyl 3-hydroxybutyrate (peak 11, Fig. 1C), and methyl benzoate (peak 13, Fig. 2C)]; terpenes 11.6% [8%, Fig. 2D1, C, with α-pinene, γ-terpinene, α-terpinolene, linalool, terpinen-4-ol and α-terpineol (Table 2)]; acids 5.1% [3%, Fig. 2D1, C, with octanoic acid, 3-methylbutyl ester (peak 3, Fig. 1C)], aldehydes 1.6% [10%, Fig. 2D1, C, with benzaldehyde (peak 17, Fig. 1C), and hexanal (peak 22, Fig. 1C)]; and ketones 1.6% (15%, Fig. 2D1, C). In our research only 6 classes of the compounds were determined. The chemical classes that contribute aroma profile have a great significance not only for organoleptic profile differences, but also for their bioactive properties. As it was mentioned above the highest amount of terpenes was found in cape gooseberry. This berry can have the highest pro-health value from all three selected fruits. It is well known that terpene compounds have an antioxidant activity (Singh, Batish, Kaur, Arora, & Kohli, 2006). Essential oils are a complex of natural mixtures where the main constituents are terpenes and phenylpropanoids, which are responsible for biological properties (Astani, Reichling, & Schnitzler, 2010). Essential oils from eucalyptus, tea tree and thyme and their major monoterpene compounds α-terpinene, γ-terpinene, α-pinene, terpinen-4-ol and α-terpineol are able to inhibit herpes simplex virus type 1 *in vitro* by about >80%. Essential oils from citrus peels (Tundis et al., 2012) contained limonene, pinene, terpinene, and linalyl acetate. *C. aurantifolia* oil showed the highest radical scavenging activity on ABTS assay (IC_{50} value of 19.6 1/4 g/mL), while *C. bergamia* exhibited a good antioxidant activity by β-carotene bleaching test (IC_{50} = 42.6 1/4 g/mL after 60 min of incubation). Aydin, Turkez, and Tasdemir (2013) showed that terpinolene is a potent antiproliferative agent for brain tumor cells and may have potential as an anticancer agent. Wang et al. (2008) showed that some essential oils are effective in reducing fruit decay and increasing antioxidant levels and activities in blueberries. The most effective compound for mold retardation was *p*-cymene, followed by linalool, carvacrol, anethole, and perillaldehyde. As it was mentioned above the highest amount of terpenes can be observed after comparison of the total relative area of terpenes for each fruit (without rest of chemical classes present in volatile fraction). Our results showed that the amount of terpene for cape gooseberry was about 82% (Fig. 2D1, C, Table 2). As it is apparent from the pie charts shown in Fig. 2D1, the terpene profile of cape gooseberry is the most abundant from all three berries.

3.2. Multivariate data analysis

Despite the fact that the principal component analysis (PCA) is not even a classifier (it is a method for reduction of the dimensionality of the dataset), it allows full discrimination of selected berry species (Figs. 2D2 and 2D3). Excellent grouping of all repeats of three types of berries was achieved. Dispersion of the points within a particular group is relatively low and distances inside the groups are relatively small in comparison to the distances between the points belonging to different classes. Separation of all samples was done by the first two PCs, which represented 87.50% of the variance in the dataset (PC1, 63.74%; PC2, 23.76%). The variable in this method was a summary area of peaks belonging to the specific chemical class of compounds. Terpene, ester, carboxylic acid, ketone, aldehyde and alcohol groups of compounds were analyzed using PCA. As it was mentioned previously, the main constituents of volatile fractions of these three selected types of berries were characterized for specific species. The PCA results confirm this fact. Using only one specific chemical class, namely the terpene class of compounds, it was possible to identify the compounds responsible for excellent separation of three groups of objects on the PC1PC2 plane. The PCA-biplot, presented in Fig. 2D3, provides an overview of the correlations of compounds with certain group of objects (samples). For example, β-myrcene, α-ocimene, terpinen-4-ol, fenchone, β-cyclocitral, eucalyptol, α-ionone, α-phellandrene, γ-terpinene, camphene, *p*-cymene, and α-pinene were positively correlated with cape gooseberry. These compounds were responsible for the

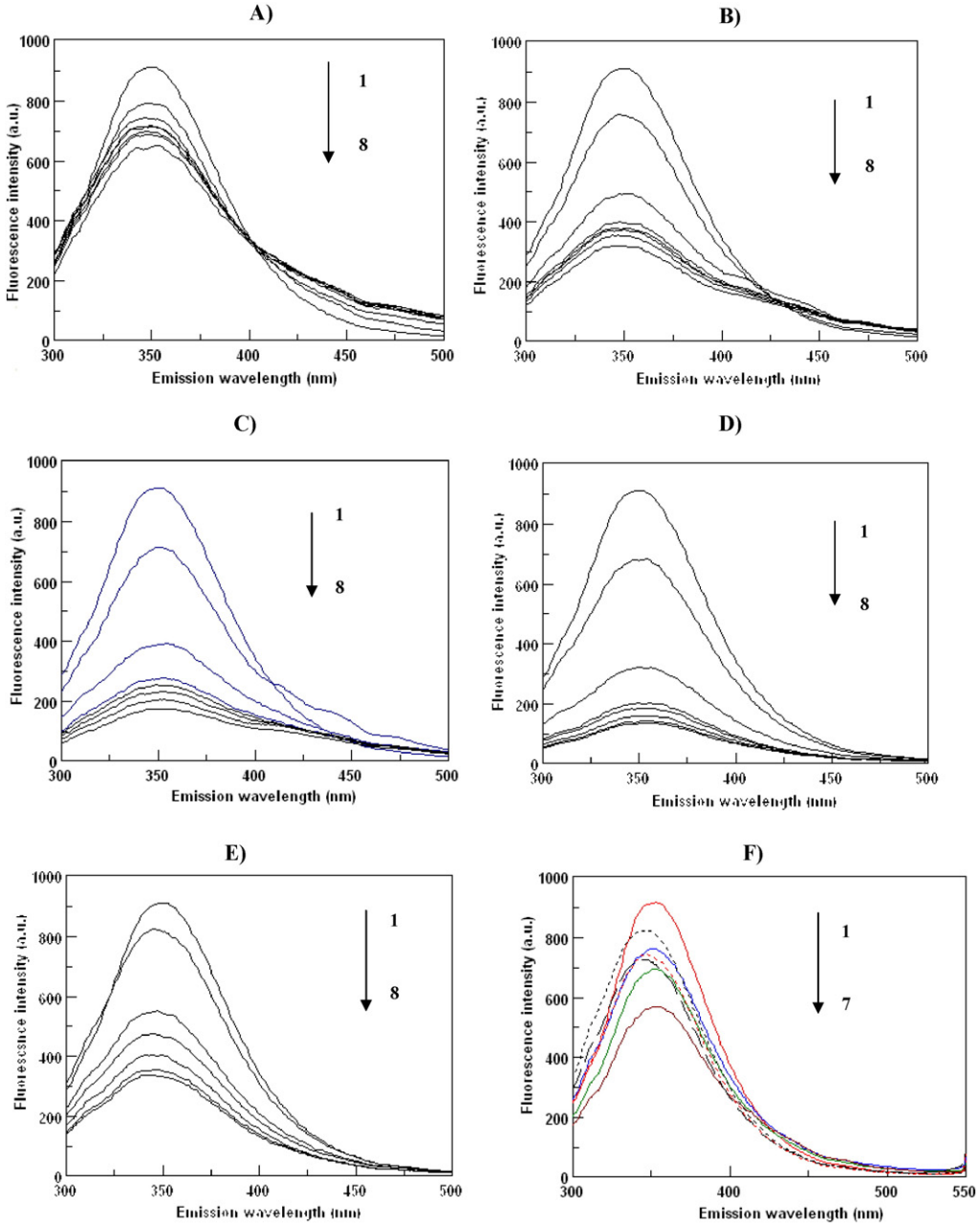


Fig. 3. Emission spectra of HSA in the absence and presence of polyphenol extracts of berries in ethanol/water (50:50) and standards at λ_{ex} 280 nm, and λ_{em} 300 nm: (A) (1) HSA (2.0×10^{-6} mol/L), (2–8) — polyphenol extracts of gooseberries ($\times 10^{-2}$ mg GAE/g DW): (2) HSA + 0.32; (3) HSA + 1.60; (4) HSA + 2.56; (5) HSA + 2.88; (6) HSA + 3.20; (7) HSA + 3.52; (8) HSA + 3.84. (B) (1) HSA (2.0×10^{-6} mol/L), (2–8) — polyphenol extracts of cranberries ($\times 10^{-2}$ mg GAE/g DW): (2) HSA + 0.62; (3) HSA + 3.08; (4) HSA + 4.94; (5) HSA + 5.55; (6) HSA + 6.17; (7) HSA + 6.79; (8) HSA + 7.40. (C) (1) HSA (2.0×10^{-6} mol/L), (2–8) — polyphenol extracts of blueberries ($\times 10^{-2}$ mg GAE/g DW): (2) HSA + 1.13; (3) HSA + 5.69; (4) HSA + 9.10; (5) HSA + 10.24; (6) HSA + 11.38; (7) HSA + 12.52; (8) HSA + 13.66. (D) (1) HSA (2.0×10^{-6} mol/L), (2–8) — α-pinene ($\times 10^{-2}$ mg GAE/g DW): (2) HSA + 0.55; (3) HSA + 2.75; (4) HSA + 4.40; (5) HSA + 4.95; (6) HSA + 5.50; (7) HSA + 6.05; (8) HSA + 6.60. (E) (1) HSA (2.0×10^{-6} mol/L), (2–8) — terpinolene ($\times 10^{-2}$ mg GAE/g DW): (2) HSA + 0.62; (3) HSA + 3.10; (4) HSA + 3.72; (5) HSA + 4.34; (6) HSA + 4.96; (7) HSA + 5.58; (8) HSA + 6.20. (F) (1) HSA (2.0×10^{-6} mol/L), (2–7) — ($\times 10^{-2}$ mg GAE/g DW) with 20 μL berries and standards in ethanol/water (50:50) solution. (2) HSA + linalool; (3) HSA + gooseberry; (4) HSA + terpinolene; (5) HSA + α-pinene; (6) HSA + cranberry; (7) HSA + blueberry. Concentrations of berries is 0.43 mg/mL and standards 0.05 mg/mL.

differentiation of this type of samples from the remaining other ones. In turn, β-pinene, (E)-linalool oxide, camphor, and limonene were associated with blueberry samples and α-terpineol and terpinolene were related to cranberry. One terpene, namely linalool, can be treated as a differentiation factor of cape gooseberry and blueberry from cranberry samples. It is not correlated with a specific group of samples. Looking globally on a PCA biplot, some more conclusions can be noticed. The variance between these three groups of objects was mainly obtained by PC2. PC1 explains only differences between cape gooseberry and the remaining two species of berries. Using first two PCs the 98.85% of the variance in the set of data can be explained. This result is better than the previous one. This means that the terpene group of compounds was more suitable for differentiation using the PCA method than all main groups of compounds presented in the volatile fraction of berries (terpene, ester, carboxylic acid, ketone, aldehyde and alcohol groups).

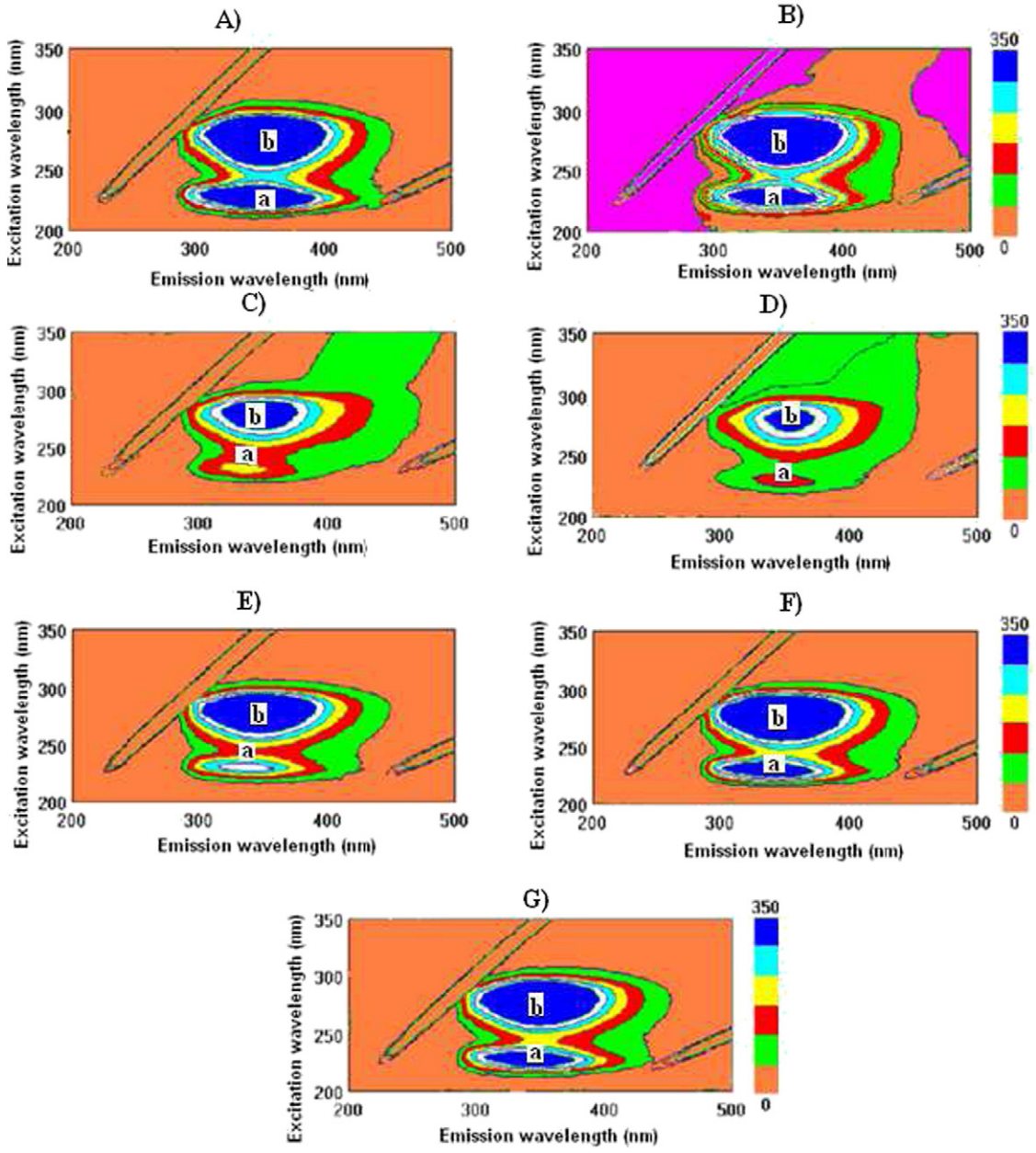


Fig. 4. 3-D contour spectral studies of HSA with 50 μL berries and standards in ethanol/water (50:50) solution. Excitation wavelength scan: 200–350 nm. Emission wavelength scan: 200–500 nm. Cross view of (A) HSA, (B) HSA + gooseberry, (C) HSA + cranberry, (D) HSA + blueberry, (E) HSA + α-pinene; (F) HSA + α-terpinolene; (G) HSA + linalool. Concentrations of berries is 0.43 mg/mL and standards 0.05 mg/mL. Spectral characteristics of peaks a and b are shown in Table 4B;

3.3. Bioactive compounds

The bioactive compounds and their antioxidant capacities in three investigated berries are presented in Table 3. The lowest data were obtained for linalool. The obtained results are comparable with our previous data. Polyphenols (mg GAE/g) in ethanol–water extracts (20:80) of gooseberries and blueberries were 9.56 ± 0.65 and 41.80 ± 3.76 and the total antioxidant capacities (μm TE/g DW) determined by ABTS and CUPRAC assays were 42.54 ± 3.76 and 304.09 ± 28.64 and 17.02 ± 1.65 and 164.87 ± 12.87 (Dymerski et al., 2015; Namiesnik et al., 2014a, 2014b). The results shown in Table 3 slightly differ depending on the solvent used. So, when 20% water and 80% ethanol were used for extracts the obtained data were lower than shown in Table 3, when water/ethanol was 50:50. Sonication time and the temperature were optimized based on our previous results and the highest polyphenol concentration and antioxidant capacity (Da Porto et al., 2013). Our results are in accordance with others, where the highest values of antioxidant capacity were observed in the 'Inka' gooseberry cultivar (9.31 g AAE (ascorbic acid equivalents)/kg FW). In this cultivar, the obtained results were corroborated also in reactive oxygen species and in the contents of total phenolics. Due to the high antioxidant capacity of this fruit species, the results presented should increase its popularity above all as a promising raw material, which can be used for human nutrition (Rop et al., 2012). The amount of total phenolics among different cranberry clones in the field collection ranged from 224.0 mg/100 g to 498.0 mg/100 g (Cesoniene et al., 2009). Viskelis et al. (2009) found the largest amount of phenolic compounds in "Black Veil" cranberry cultivar (504 mg/100 g), showing good radical scavenging activity and revealed

Table 4A
Two-dimensional fluorescence spectral characteristics of interaction between HSA and berries ethanol extracts before and after incubation.

N of lines/Fig.	Samples	λ_{em}	FI	% binding
1/Fig. 3F	HSA + ethanol BInc	353	916.89 ± 12.85^{a}	–
2/Fig. 3F	HSA + linalool BInc	344	822.92 ± 10.74^{ab}	10.25 ± 1.46^{e}
3/Fig. 3F	HSA + gooseberry BInc	350	762.56 ± 9.43^{b}	16.83 ± 1.62^{d}
4/Fig. 3F	HSA + terpinolene BInc	347	743.09 ± 8.57^{b}	18.95 ± 1.94^{cd}
5/Fig. 3F	HSA + α-pinene BInc	346	726.08 ± 9.63^{b}	20.81 ± 2.64^{c}
6/Fig. 3F	HSA + cranberry BInc	353	695.20 ± 7.22^{bc}	24.18 ± 2.32^{bc}
7/Fig. 3F	HSA + blueberry BInc	352	569.95 ± 5.52^{c}	37.84 ± 3.46^{b}
3/Fig. 5I	HSA + EtOH AInc	348	725.88 ± 12.85^{b}	20.83 ± 2.18 ^{c}
5/Fig. 5I	HSA + gooseberry AInc	336	574.27 ± 9.43^{c}	20.90 ± 1.62^{c}
7/Fig. 5I	HSA + cranberry AInc	338	399.38 ± 7.22^{d}	45.04 ± 2.32^{ab}
8/Fig. 5I	HSA + blueberry AInc	343	336.24 ± 5.52^{d}	53.71 ± 3.46^{a}

Mean ± SD (standard deviation) of 5 measurements. Average in columns marked with different letters differ significantly ($P < 0.05$). Abbreviations: HSA, human serum albumin; λ_{em}, $\lambda_{ex,}$ wavelength of emission, excitation in nm; FI, fluorescence intensity in arbitral units; BInc, before incubation; AInc, after incubation; % binding, binding capacity.

antimicrobial properties. Correlation between the volatile compounds, especially terpenes, and their antioxidant activities require more details and comparison of our present results with the cited ones in the literature in order to evaluate the multivariate methods in relation to the preprocessing of the data and their validation. The most abundant derivatives in buckthorn samples (46 volatile compounds were separated with 43 being identified) were ethyl esters of 2-methylbutanoic acid, 3-methylbutanoic acid, hexanoic acid, octanoic and butanoic acids, 3-methylbutyl 3-methylbutanoate, 3-methylbutyl 2-methylbutanoate and benzoic acid ethyl ester (over 80% of all volatile compounds). Principal component analysis showed that the first two components explain 79% of data variance, demonstrating a good discrimination between samples (Socaci et al., 2013). Our results correspond with Hanene et al. (2012), where *Hawaria* essential oil with the highest antioxidants determined by ABTS and DPPH was extracted from mature berries with 48 identified compounds, where the main ones were β-pinene, germacrene D, myrcene, abietadiene and *cis*-calamenene. Our results are in line with Pashazanousi et al. (2012), who combined in his study not only the aroma substances, but also the bioactive compounds in different extracts. In the oil of leaves were found β-pinene (20.6%), limonene (16.8%), neryl formate (14.8%) and geraniol (9.3%). In fruit peels were identified 26 compounds with limonene (60.2%), β-pinene (12.1%) and γ-terpinene (11.8%). The antibacterial test showed that the chloroformic extracts of the leaves inhibited the growth of the microorganisms. The highest total phenolic and flavonoid contents were found in methanolic extract. Messaoud and Boussaid (2011) reported that the oils from the extracts of dark blue fruits from Tunisian *Myrtus communis* morphs showed high percentages of α-pinene (11.1%), linalool (11.6%), α-terpineol (15.7%), methyl eugenol (6.2%), and geraniol (3.7%). The total phenols and antioxidant capacity were higher in dark blue berries. This is in correlation with the amount of terpenes found in these fruits. Combined results of volatile compounds and their antioxidant activities from leaf and fruit of *Melaleuca leucadendra L.* were reported by Pino, Regalado, Rodriguez, and Fernandez (2010), where the main components were viridiflorol (47.6%), globulol (5.8%), guaiol (5.3%), and α-pinene (4.5%). Dai, Zhu, Yang, and Qiu (2013) showed that the main compounds with moderate activities in the oil from *Wedelia prostrata* were limonene (11.38%) and α-pinene (10.74%). The main compounds found in the volatile oils of *Eucalyptus camaldulensis* (Ozel et al., 2008) fruit were: aromadendrene (6.45–15.02%), eucalyptol (0.17–12.61%), gamma-gurjunene (8.40–10.08%), terpinolen (1.98–8.39%), spathulenol (1.42–8.34%), α-pinene (0.85–6.81%), ledene (0.94–6.72%), and longifonene (0.07–6.22%). The chiral isomeric ratios of raspberry ketone, α-ionone, α-pinene, linalool, terpinen-4-ol, δ-octalactone, δ-decalactone, and 6-methyl-5-hepten-2-ol were found by Malowicki, Martin, and Qian (2008). From all the reported references the main monoterpenes were α-pinene (11.1%), linalool (11.6%) and α-terpineol as it was found in our studies.

3.4. Emission spectral studies

Addition of ethanolic polyphenol extract of gooseberry to HSA results in the change in both the fluorescence intensity and a shift (blue and red) in the emission maximum of HSA (Fig. 3A). Fluorescence quenching was observed starting from the polyphenol extract of 0.32×10^{-2} mg GAE/g DW (Fig. 3A, 13.59% of quenching, second line from the top) with the maximum of 29.92% (at 3.84×10^{-2} mg GAE/g

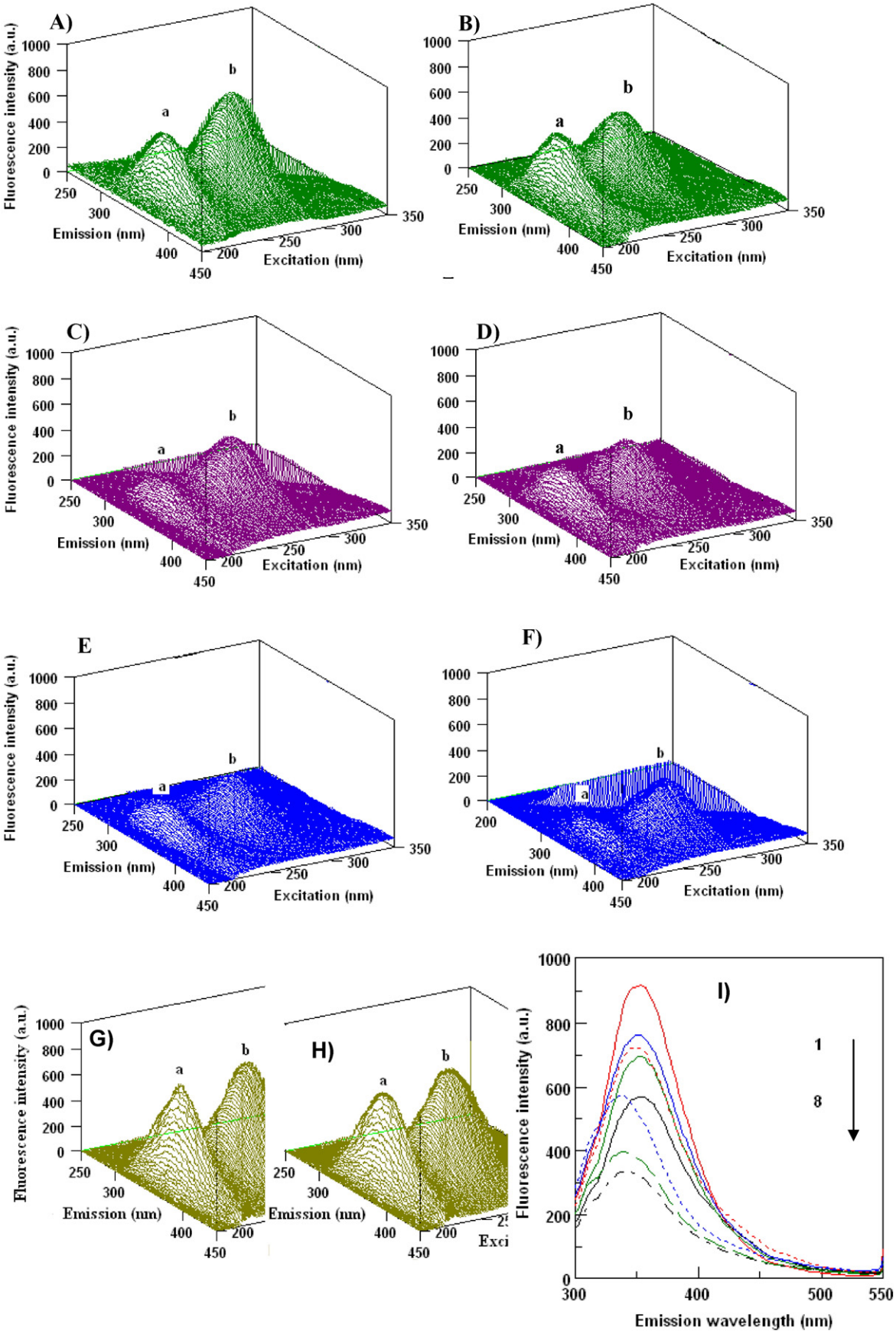
Table 4B
Three-dimensional fluorescence spectral characteristics of interaction between HSA and berries ethanol extracts before and after incubation.

Samples	Peak a		Peak b		% binding
	$\lambda_{ex}/\lambda_{em}$	FI	$\lambda_{ex}/\lambda_{em}$	FI	
HSA + EtOHBI	227/345	753.98 ± 11.5^{a}	280/352	856.74 ± 14.8^{a}	–
HSA + gooseberry BInc	228/344	674.25 ± 9.7^{b}	289/352	793.04 ± 9.1^{ab}	18.01 ± 1.5^{d}
HSA + cranberry BInc	232/335	642.88 ± 8.8^{b}	280/345	772.63 ± 9.8^{b}	25.51 ± 2.3^{bc}
HSA + blueberry BInc	228/349	563.49 ± 6.9^{c}	280/354	732.81 ± 8.4^{bc}	39.73 ± 3.7^{b}
HSA + α-pinene BInc	230/337	655.96 ± 10.5^{b}	280/347	775.63 ± 7.1^{b}	22.51 ± 2.1^{c}
HSA + linalool BInc	229/337	693.66 ± 12.5^{ab}	280/345	822.10 ± 13.1^{a}	12.09 ± 1.1^{e}
HSA + terpinolene BInc	228/344	663.52 ± 8.5^{b}	280/347	789.14 ± 7.9^{ab}	19.98 ± 2.1^{cd}
HSA + gooseberry AInc	229/332	569.44 ± 7.5^{c}	280/335	716.17 ± 7.8^{c}	23.14 ± 2.2^{bc}
HSA + cranberry AInc	230/332	483.14 ± 14.5^{cd}	280/339	645.34 ± 6.8 ^{d}	45.06 ± 4.2^{ab}
HSA + blueberry AInc	230/337	423.68 ± 12.5^{d}	280/340	638.28 ± 7.4^{d}	54.93 ± 5.1^{a}
HSA AInc	227/345	662.14 ± 13.3^{b}	280/352	787.25 ± 9.6^{ab}	20.33 ± 1.9^{cd}

Mean ± SD (standard deviation) of 5 measurements. Average in columns marked with different letters differ significantly ($P < 0.05$). Abbreviations: HSA, human serum albumin; λ_{em}, $\lambda_{ex,}$ wavelength of emission, excitation in nm; FI, fluorescence intensity in arbitral units; BInc, before incubation; AInc, after incubation; % binding, binding capacity.

Fig. 5. Three-dimensional fluorescence spectra of: A, HSA + gooseberry before incubation (BInc); B, HSA + gooseberry after incubation (AInc); C, HSA + cranberry BInc; D, HSA + cranberry AInc; E, HSA + blueberry BInc; F, HSA + blueberry AInc; G, H, HSA before and after incubation. Spectral characteristics of peaks a and b are shown in Table 4B; I, emission spectra of HSA in the absence and presence of polyphenol extracts of berries in ethanol/water (50:50) and standards at λ_{ex} 280 nm, and λ_{em} 300 nm: (1) HSA (2.0×10^{-6} mol/L) before incubation, (2–8) — polyphenol extracts of berries ($\times 10^{-2}$ mg GAE/g DW): (2) HSA + 0.64 gooseberry BInc; (3) HSA after incubation; (4) HSA + 1.24 cranberry BInc; (5) HSA + 0.64 gooseberry AInc; (6) HSA + 2.26 blueberry BInc; (7) HSA + 1.24 cranberry AInc; (8) HSA + 2.26 blueberry AInc.

DW, line 8, Fig. 3A). The changes in the intensity of HSA with cranberry were the lowest at 0.62×10^{-2} mg GAE/g DW polyphenol extract (17.29%, line 2 from the top, Fig. 3B) and the highest one at 7.40×10^{-2} mg GAE/g DW of polyphenol extract (65.02%, line 8 from the top, Fig. 3B). Slightly higher results of binding properties were evaluated with the addition of blueberry extract: with 1.13×10^{-2} mg GAE/



g DW polyphenol extract was 22.15% (line 1 from the top, Fig. 3C), and at 13.66×10^{-2} mg GAE/g DW polyphenol extract – 80.81% (line 8 from the top, Fig. 3C). The obtained data of α-pinene were similar to blueberry extract: so, at 0.55×10^{-2} mg GAE/g DW polyphenols the binding was about 26.48% (line 2 from the top, Fig. 3D) and at 6.60×10^{-2} mg GAE/g DW the results were higher than with blueberries of about 85.04%. Terpinolene showed lower results than α-pinene and these results were similar to cranberry: at 0.62×10^{-2} mg GAE/g DW polyphenols the binding was 17.29% (line 2 from the top, Fig. 3E) and at 7.40×10^{-2} mg GAE/g DW polyphenols – 65.02% (line 8 from the top, Fig.3E). The data obtained for linalool were similar to the results of gooseberry (data not shown). All investigated berry samples and three standards are presented in Fig. 3F, where the highest binding ability was in blueberry (line 7) and the lowest was with linalool (line 2). The binding properties are correlated to tryptophan amino acid. The excitation wavelength is centered around 280–285 nm and not on 275 nm, which shows that the fluorescence results from tryptophan and not from tyrosine and phenylalanine are involved. These results are supported by others that the binding site of the additive involves modifications of the environment around Trp214 at the level of subdomain IIA (Barreca, Lagana, Bruno, Magazu, & Bellocco, 2013; Paul & Guchhait, 2011; Tang et al., 2014). The binding abilities are directly related to the polyphenol content and antioxidant activity. The counter view of HSA with added polyphenol extracts of berries and the 3 standards are presented in Fig 4A–F. A blue color in the center of each figure represents the maximum intensity which corresponds to

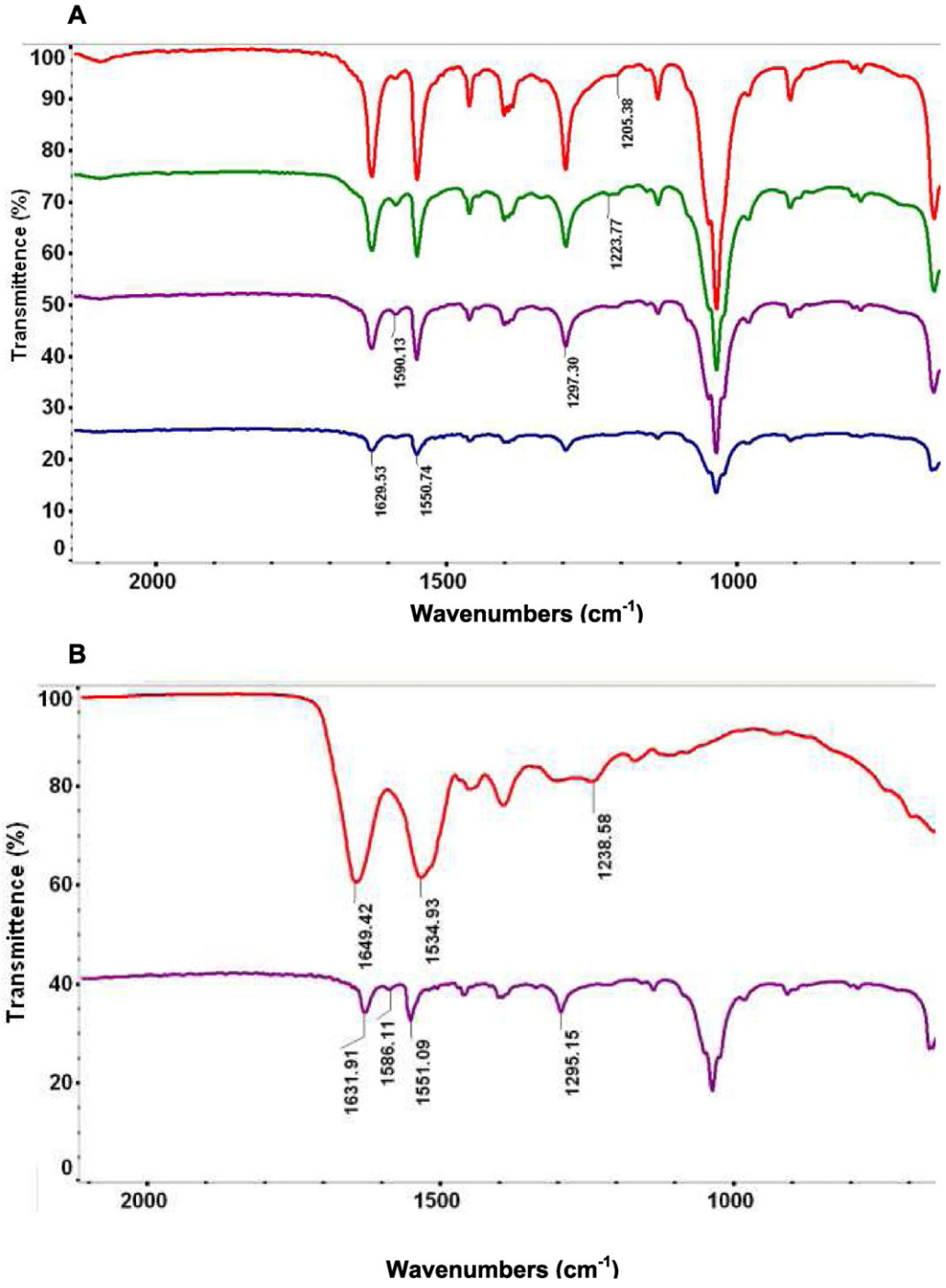


Fig. 6. FTIR spectra of A, from the top HSA + α-pinene; HSA + gooseberry; HSA + cranberry; HSA + blueberry. B, from the top: HSA, HSA + blueberry.

the emission maximum resulting from tryptophan amino acid. A single contour is obtained for HSA (Fig. 4A), which corresponds to 280 nm and 300 nm as the excitation and emission wavelengths, respectively. The highest binding ability was with the addition of blueberries to HSA (Fig. 3F, line 7; Fig. 4D, of 37.94%) and the lowest one was with linalool (Fig. 3F, line 2; Fig. 4G of 10.25%). The shifts in the emission maximum of HSA and the variations in the fluorescence intensity on the addition of polyphenol ethanol extract of berries were from 353 nm to 350 nm and from 353 nm to 344 nm for the standards (Figs. 3–4). The quenching properties of these berries are directly correlated with their antioxidant properties and the amount of polyphenols (Tables 3, 4A and 4B). As can be concluded, the binding ability for the berry extracts are in direct correlation with polyphenols and antioxidant activities; but with the standards are slightly different (Figs. 3–4). Our very recent results (Namiesnik et al., 2014a and b) showed that the fluorescence is significantly quenched, because of the conformation of proteins, phenolic acids and flavonoids. This is in the line with other reports (Middleton, Kandaswami, & Theoharides, 2000; Prabu, Gnanamani, & Sadulla, 2006) showing that the guaijaverin (Gua) is a polyphenolic substance (quercetin-3-O-α-L-arabinopyranoside) which exhibits some pharmacological activities such as antibacterial and antioxidant activities. The binding of Gua with HSA at physiological pH 7.0 was investigated by fluorescence spectroscopy. The fluorescence quenching method was used to determine the number of binding sites and binding constants. Molecular modeling calculation indicated that the Gua is located within the hydrophobic pocket of HSA subdomain IIA (Caruso et al., 2012). The binding affinities with common human plasma proteins (CHPP) were strongly influenced by the structural differences of dietary polyphenols. Polyphenols with higher affinities for purified HSA also showed stronger affinities with CHPP. The hydrophobic force played an important role in binding interaction between polyphenols and CHPP (Xiao et al., 2011). Data obtained by fluorescence spectroscopy, displacement experiments along with the docking studies suggested that the ligands bind to the residues located in site 1 (subdomains IIA), whereas epigallocatechin, that lacks the gallate moiety, binds to the other hydrophobic site 2 (subdomain IIIA) of the human serum protein (Singh et al., 2013). From the results obtained, besides the main binding analysis performed, we conclude that this technique is sensitive. Using such procedure it was possible to detect several interactions, which have not been found by other methods. The binding properties were checked when the interaction between proteins and polyphenols was under incubation during 24 h at 37 °C. The change in the fluorescence intensity before the incubation for gooseberry, cranberry and blueberry is shown in Fig. 5A, C, and E (Fig. 5G, lines 2, 3 and 5 with the percentage of binding of 16.83, 24.18 and 37.84). After the incubation (Fig. 5 B, D, and E; Fig. 5I, lines 5, 7 and 8) for gooseberries, cranberries and blueberries the binding was 20.9%, 45.0% and 53.7% (Table 4A). The spectral properties of the indicated peaks a and b (Figs. 4–5), their fluorescence intensities and peak maxima are presented in Table 4B. Incubation increased the binding abilities, but the relationship between the amount of polyphenols, their antioxidant activities and binding properties were in the same correlation. The calculated binding capacities of the investigated berries, using peaks a and b (Fig. 4, Table 4B) slightly differ from the ones where only one peak was used in 2D-FL (Table 4A). The difference was between 6 and 10% (Table 4B). The change in the binding abilities during incubation can be explained by the additional denaturation of the proteins (HSA in ethanol solution, Table 4B, Fig. 5, G, H). The interaction of HSA with the polyphenols from berries is shown in the FTIR spectra (Fig. 6). The intensities of the peaks in samples after interaction of HSA and polyphenols of investigated berries in the following ranges (1630–1632 cm^{-1}, 1551 cm^{-1} and 1297 cm^{-1}) were the lowest ones in the sample of blueberry with HSA, then increasing for cranberry, gooseberry and α-pinene (Fig. 6A). At 1590 cm^{-1} very small peaks appeared for cranberry, gooseberry and α-pinene, with a shift for blueberry at 1586 cm^{-1} (Fig. 6B). HSA showed characteristic bands for Amide I (1649 cm^{-1}), Amide II (1535 cm^{-1}), and Amide III (1239 cm^{-1}). A slight shift in Amide I of about 10 cm^{-1} appeared in the investigated samples with a characteristic band of about 1629 cm^{-1} for β-sheet. Similar shifts in the bands were fixed in Amide II and Amide III. The matching of the band intensities were compared in the ranges of Amide I, II and III. The ratios of the FTIR band intensities correlated with the ratios of fluorescence intensities (Fig. 3F). FTIR showed that the absorption bands of all investigated samples were similar, and differed only by the intensity of the peaks in the range of Amides, which is in line with others (Zhao, Zhu, Chen, & Ao, 2015). Therefore the secondary structure of the investigated samples did not change, but only the ratios between the Amide bands differ. Overall, fluorescence quenching has proven to be a very sensitive technique with many potentialities to analyze the interaction between polyphenols and proteins, which was shown in other reports as well (Barreca et al., 2013; Caruso et al., 2012). The combination of two-dimensional chromatography with the fluorescence studies are in agreement with others for the characterization of main compounds in the fruits.

4. Conclusions

The determination of the terpenes in gooseberries, blueberries and cranberries (the main group of secondary metabolites) in three berries was done by headspace solid-phase microextraction coupled with comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (HS-SPME/GC × GC-TOFMS). Between 15 and 25 main volatiles were assigned on each of the three berries' chromatograms. The compounds were organized in different groups: monoterpene hydrocarbons and monoterpene oxygen-containing compounds (oxides, alcohols, aldehydes, and ketones). The highest amount of alcohol and ester compounds (85%) is contained in blueberry; carboxylic acids, ketones and aldehydes relatively for cranberry (62%) and terpenes for cape gooseberry (8%). Comparing the total relative area of terpenes for each fruit the dominance of terpene content for cape gooseberry can be also observed (82%). Specific binding of polyphenol extracts from berries to human serum albumin (HSA) under the physiological conditions was a result of the formation of polyphenol–HSA complex. Binding parameters showed that the berries' extracts bind to HSA with different binding affinities which are related to their antioxidant properties and the changes in the secondary structure, shown as well in FTIR spectra. The terpene group of compound is more suitable for differentiation using principal component analysis (PCA) than all main groups of compounds present in the volatile fraction of berries.

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