Comprehensive two-dimensional gas chromatography and three-dimensional fluorometry for detection of volatile and bioactive substances in some berries

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ABSTRACT

The volatile fractions of Cape gooseberry and blueberry 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). The highest amount of alcohol (51.8%), ester (32.8%) and carboxylic acid (6.9%) was in blueberry in comparison with gooseberry and oppositely ketones (14.7%), aldehydes (9.9%) and terpenes (8%) were found in gooseberry. The bioactive compounds and antioxidant capacities were higher in blueberries than in gooseberries. Three dimensional fluorescence emission spectrometry (3D-FL) was applied to determine and to compare experimentally found binding parameters of berries extracts with human serum albumin (HSA). The fluorescence quenching of HSA by polyphenols from berries was a result of the formation of a polyphenol–HSA complex. The binding abilities of berries were highly correlated with the bioactivity of polyphenols and volatile substances. The cluster analysis (CA) and linear discriminant analysis (LDA) was applied to differentiate the berries samples according to their type.

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1. Introduction

Berries are consumed because of their attractive color, special taste and aroma, and are considered one of the richest sources of natural antioxidants [1,2]. Aroma is one of the most important attributes that affects the flavor of a fruit and thus its acceptability and consumption [3]. Comprehensive two-dimensional gas-chromatography (GC × GC) is a great tool to explore complex essential oils. Rosemary and oregano were studied and then compared by the amount of terpenes in their aroma fractions [4]. The compositions of the essential oils obtained by hydrodistillation of the ripe fruits, flowering aerial parts and roots of Elaeoselinum asclepium (L.) Bertol subsp. meoides (Desf.) Fiori (Apiaceae) were determined by GC–MS analysis. All the analyzed parts of the fruit were very rich in α-pinene and other terpenes (77.1%, 92.2% and 60.8%, respectively) [5]. Amount of monoterpene alcohol and its glycose precursors in grapes was determined in different types of grapes. Aglycone analysis was conducted on the basis of fast acid hydrolysis and solid phase extraction (SPE-C18) with subsequent GC–MS analysis [6]. There are some reports for determination of aroma active volatiles in different berries, such as four southern high bush blueberry cultivars. Solid phase microextraction (SPME) in combination with gas chromatography-offactometry (GC-O) and identification via GC-PFPD and GC–MS using retention indices of reference compounds and mass spectral data was applied [7]. The aroma differences of the pomegranate juices mixed with five different concentrations of blueberry, blackberry, or raspberry, solid phase microextraction–gas chromatography–mass spectrometry was used to determine the volatile compounds present in the mixtures [8]. Volatile components in Cape gooseberry fruit at ripe stage were collected using headspace-solid phase micro extraction, and analyzed by gas chromatography–mass spectrometry [9]. The volatile compositions of two strawberry varieties (‘Albion’ and ‘Juliette’) grown in Australia were analyzed with comprehensive two-dimensional gas chromatography (GC × GC) combined with time-of-flight mass spectrometry (TOFMS). Ninety-four compounds were tentatively or positively identified between the two varieties of which twenty have apparently not been previously reported to be components of strawberry volatiles [10]. Polyphenols are responsible for the major organoleptic characteristics of plant-derived foods, including fruits and berries, and beverages.
Chlorogenic acid (CGA) is one of the most abundant polyphenol compounds in human diet. It is also an active component in traditional Chinese medicines which are used for treatment of various diseases [14]. The consumption of berries has been linked to the prevention of some chronic and degenerative diseases. The term ‘berry fruits’ encompasses the so-called ‘soft fruits’, enhanced other antioxidant enzymes in kidney homogenate compared to cisplatin were studied [12,13]. The binding of several polyphenols to bovine binding properties of different berries with human serum albumin (Vaccinium corymbosum, (E)-linalool oxide and (E)-linalool) investigated [16,17]. α-Pinene, one of the common monoterprenoids, emitted from several aromatic plants including forest trees, is known for its growth-inhibitory activity. It is concluded that α-pinene inhibits early root growth and causes oxidative damage in root tissue through enhanced generation of ROS, as indicated by increased lipid peroxidation, disruption of membrane integrity and elevated antioxidant enzyme levels [18]. The information of combination of spectroscopic and fluorometric methods for comparison of different fruits is limited. Based on the discussed above data of the properties of different berries the object of study was to determine the binding properties and the bioactive compounds will be determined by three dimensional fluorescence with interaction of HSA and the berries extracts.

### 2. Materials and methods

#### 2.1. Reagents and materials

For volatile four analytical terpene standards were used to confirm the identity of selected compounds (Sigma-Aldrich, St. Louis, MO, USA). The standards included α-pinene, α-terpinolene, β-linalool, (−)-terpinen-4-ol, β-pinene, camphene, β-myrcene, α-phellandrene, p-cymene, (R)-(−)-limonene, γ-terpinene, α-ocimene, (E)-linalool oxide and β-cyclocitrinal. 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. For the bioactive compounds 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), human serum albumin (HSA), Tris, tris(hydroxymethyl)1 aminomethane, Folin–Ciocalteu reagent, 2, 2’-Azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt (ABTS); (ABTS*•−), lanthanum (III) chloride hexahydrate; CuCl2 × 2H2O; and 2,9-dimethyl-1,10-phenanthroline (neocuprine) and α-pinene were used.

#### 2.2. Sample preparation

All berries were purchased at the local market in Gdansk, Poland. During the studies two types of the fruit samples (V. corymbosum and P. peruviana) were washed and homogenized before each analysis. After this step 8.0 g of a blended sample was placed into the 20 mL vial and 2.0 g of oven-dried sodium chloride was also transferred. The vial was sealed with a cap with PTFE-lined silicone septum to prevent the loss of volatiles. 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 (Virtils 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

Volatiles from the fruit samples were extracted using headspace solid-phase microextraction (HS-SPME). Prior to extraction

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Compounds identified and quantified in berry samples by SPME-GC × GC-TOFMS.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>Compound</td>
</tr>
<tr>
<td>1</td>
<td>α-Pinene</td>
</tr>
<tr>
<td>2</td>
<td>Camphene</td>
</tr>
<tr>
<td>3</td>
<td>β-Pinene</td>
</tr>
<tr>
<td>4</td>
<td>β-Myrcene</td>
</tr>
<tr>
<td>5</td>
<td>α-Phellandrene</td>
</tr>
<tr>
<td>6</td>
<td>p-Cymene</td>
</tr>
<tr>
<td>7</td>
<td>(R)-(−)-limonene</td>
</tr>
<tr>
<td>8</td>
<td>α-Ocimene</td>
</tr>
<tr>
<td>9</td>
<td>γ-Terpene</td>
</tr>
<tr>
<td>10</td>
<td>(E)-linalool oxime</td>
</tr>
<tr>
<td>11</td>
<td>α-Terpine</td>
</tr>
<tr>
<td>12</td>
<td>β-Linalool</td>
</tr>
<tr>
<td>13</td>
<td>(−)-Terpinen-4-ol</td>
</tr>
<tr>
<td>14</td>
<td>β-Cyclocitrinal</td>
</tr>
</tbody>
</table>

* RTI(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 amongst the samples where a compound was detected.

* Forward similarity; value out of 1000.

* Concentration of a compound for certain type of sample; C_f for blueberry and C_M for Cape gooseberry.
process the samples were incubated at 50 °C for 10 min and agitated at 700 rpm. Extraction in 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). 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 Equity 1 stationary phase (Supelco, Bellefonte, PA, USA) and a 1.6 m × 0.10 mm × 0.10 μm secondary column (2D) with Sol–Gel–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 according to the program of 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.

2.5. Data analysis

Data processing was performed using the algorithm for peak deconvolution included in the ChromaTOF software (LECO Corp.,

Fig. 1. Comparison of total relative area (in percentage) of compounds belonging to each chemical family: (1) – terpenes, (2) – esters, (3) – carboxylic acids, (4) – ketones, (5) – aldehydes, (6) – alcohols, for blueberry (A) and Cape gooseberry (B). Relative amount of terpenes (in percentage) present in volatile fraction of blueberry (I) and Cape gooseberry (II).

Fig. 2. Upper part: Extracted ion contour plots (for masses 91, 93, 121 and 136) comparing the terpene profiles for the two types of fruits analyzed. Peak identification – (1) γ-terpinene, (2) α-terpinolene, (3) β-linalool, (4) α-terpinen-4-ol for blueberry (A) and Cape gooseberry (B). Lower part: Comparison of GC × GC-TOFMS chromatograms obtained for selected masses (91, 93, 121, 136) and illustrating the terpene profiles of blueberry (A) and Cape gooseberry (B).
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 fourteen analytes (α-pinene, α-terpinolene, β-linalool, α-terpineol, β-pinene, camphene, β-myrcene, α-phellandrene, p-cymene, (R)-(−)-limonene, α-ocimene, fenchone, (E)-linalool oxide, β-cyclocitral) was confirmed by the comparison of retention times (in1D and 2D) with authentic standards (Table 1). Furthermore a fiber blank run was done every 10 analyses of fruit samples to consider the influence of column or SPME fiber degradation.

2.6. Software for chemometric data analysis

Interpretation of obtained dataset was performed using free R software (version 3.0.2) being a part of Free Software Foundation (Boston, MA, USA). The cluster analysis (CA) with the furthest neighbor method and linear discriminant analysis (LDA) were applied as an unsupervised and supervised classifier, respectively, to differentiate the berries samples according to their type.

2.7. Determination of bioactive compounds and antioxidant activities

2.7.1. Extraction of phenolic compounds

The lyophilized samples of berries (1 g) were extracted with 40 mL of ethanol/water (20%:80%) at 40°C during 4 h. Ultrasound-assisted extraction was carried out with Ultrasonic Cleaner Delta DC-80H, operating frequency: 40 kHz, output power: 80 W, and heater: 45 W. The extracts were filtered in a Buchner funnel. These extracts were submitted for determination of bioactive compounds[19,20]. The polyphenols were determined by Folin–Ciocalteu method with measurement at 750 nm with spectrophotometer (Hewlett-Packard, model 8452A, Rockvile, USA). The results were expressed as mg of gallic acid equivalents (GAE) per g DW[21].

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

(1) By 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 NH₄Ac buffer solution, acidified and nonacidified methanol extracts of berry (or standard) solution (x, in mL) and H₂O [(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 [22].

(2) By 2, 2′-Azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt (ABTS): ABTS•⁺ was generated by the interaction of ABTS (7 mM) and K₃S₂O₈ (2.45 mM). This solution was diluted with methanol until the absorbance in the samples reached 0.7 at 734 nm [11].
2.8. Fluorometric measurements

Fluorometric measurements were used for the evaluation of binding properties of berries extracts to human serum albumin. Two dimensional (2D-FL) and three dimensional (3D-FL) fluorescence measurements were recorded on a model FP-6500, Jasco spectrofluorometer, serial N261332, Japan, equipped with 1.0 cm quartz cells and a thermostat bath and the excitation and emission slits were set at 5 nm while the scanning rate was 1200 nm min⁻¹. For the fluorescence measurement, 3.0 mL of 2.0 × 10⁻⁶ mol L⁻¹ HSA solution and various amounts of berries extracts were added to a 1.0 cm quartz cell manually using a micro-injector. The total accumulated volume of berries extracts was no greater than 130 μL. The corresponding fluorescence emission spectra were then recorded in the range of 300–500 nm upon excitation at 280 nm in each case. The three-dimensional fluorescence spectra were measured under the following conditions: the emission wavelength was recorded between 200 and 795 nm, the initial excitation wavelength was set at 200 nm with an increment of 5 nm, and the others scanning parameters were just the same as those for the fluorescence emission spectra. All solutions for protein interaction were prepared in 0.05 mol L⁻¹ Tris–HCl buffer (pH 7.4), containing 0.1 mol L⁻¹ NaCl.

3. Results and discussion

3.1. Volatile substances

The composition of volatile fractions of Cape gooseberry has not been described by the use of chromatographic techniques till now. With regard to that fact, the first approach in this issue was to compare volatile fraction composition of this fruit with other fruits which are widely studied blueberries. For these two types of samples all identified compounds were grouped according to their chemical family (Fig. 1), namely into a terpene, ester, carboxylic acid, ketone, aldehyde and alcohol group. As it can be observed, the volatile fraction of these fruits differs with regard to the specific type. The highest amount of alcohol, ester and carboxylic acid compounds were found in blueberry (Fig. 1A). Oppositely to that, ketones, aldehydes and terpenes were found in Cape gooseberry (Fig. 1B). The determined amounts of four terpenes in two berries showed that gooseberry contained 3 times more amount than blueberry (Table 1). For this last group of compounds, their contribution in aroma profile was lower than 130 μL. The corresponding fluorescence emission spectra were then recorded in the range of 300–500 nm upon excitation at 280 nm in each case. The three-dimensional fluorescence spectra were measured under the following conditions: the emission wavelength was recorded between 200 and 795 nm, the initial excitation wavelength was set at 200 nm with an increment of 5 nm, and the others scanning parameters were just the same as those for the fluorescence emission spectra. All solutions for protein interaction were prepared in 0.05 mol L⁻¹ Tris–HCl buffer (pH 7.4), containing 0.1 mol L⁻¹ NaCl.

3.2. Multivariate data analysis

Fig. 3 presents the cluster obtained diagram using complete-linkage cluster analysis (CA). Applied cluster analysis is a method of graphical presentation of the information stored in a matrix of a distance between the object set. A measure of distance between objects is the Euclidean distance. There are a few ways to create the dendrogram tree. One of a few and the most used is the furthest neighbor method, which emphasizes difference between elements of dataset. This classifier belongs to unsupervised cluster analysis classifiers (so called “without teacher”). The horizontal axis of the diagram is completely arbitrary and does not possess the character of number axis. The vertical axis is the exponent of a distance or probability, for which two objects create a cluster. Analyzing dendrogram tree it can be observed that analyzed objects (fruit samples) are divided into two main classes. The first one includes the objects from 8 to 14, the second one contains the features from 1 to 7. The complete-linkage cluster analysis method of hierarchical clustering confirms an ability to differentiate blueberry and Cape gooseberry using selected terpenes (β-pinene, camphene, β-myrcene, α- phellandrene, p-cymene, (R)-(−)-limonene, α-ocimene, fenchone, (E)-linalool oxide, β-cyclocitrall), which constitute one of the main components of volatile fraction of selected fruits. The effectiveness of this differentiation method was 100%. Considering the problem of discrimination of different type of samples the linear discriminant analysis (LDA) was also used. In this paper we have also applied the LDA supervised classifier in order to compare its effectiveness with above-mentioned CA unsupervised classifier. Classifier evaluation was averaged evaluation of ten cross-validations by variable addition method. With regard to this evaluation the best input factors chosen for LDA analysis. The total area of peaks related to the specific group of compounds was variable for LDA. The summary area of peaks belonging to all esters and ketones presents in volatile fraction of selected berries (blueberry, Cape gooseberry) was used as best LDA variables. Fig. 3 shows that this statistical method allows for full discrimination of the types of berries. In this case the efficiency of differentiation was also 100%. Using the above presented methods it can be stated that the main components of volatile fraction and separately terpene group of compounds are characteristic for selected berries and can be easily used for differentiation. Our data can be compared with some data reported in the literature. Of the 43 volatiles found to have aroma activity, 38 were identified and 13 had not been previously reported in blueberries. Although linalool and (E)-2-hexenal were common major aroma impact volatiles, dominant aroma-active volatiles were different for each cultivar [7]. When the volatile components were determined in gooseberry solid phase microextraction fiber coating DVB/CAR/PDMS showed a strong extraction capacity for volatile compounds and produced the best result in case of total peak areas. A total of 133 volatile compounds were identified in fruit pulp: among them 1-hexanol (6.86%), eucalyptol (6.66%), ethyl butanoate (6.47%), ethyl octanoate (4.01%), ethyl decanoate (3.39%), 4-terpineol (3.27%), and 2-methyl-1-butanol (3.10%) were the major components in the sample extracts [9].

3.3. Bioactive compounds

The bioactive compounds and their antioxidant capacities in two investigated berries are presented in Table 2. The lowest data were obtained for α-terpinolene in comparison with blueberry and nearly equal to gooseberry. The obtained results are comparable with our previous data. Polyphenols (mg GAE g⁻¹ DW) in water extracts of gooseberries and blueberries were 6.24±0.6 and 57.47±4.2 and the TAC (μM TE g⁻¹ DW) determined by ABTS and CUPRAC assays were 18.70±1.8 and 13.44±1.2 and 254.83±25.6 and 250.95±18.6. Polyphenols (mg GAE g⁻¹ DW) in methanol extracts were 3.77±0.1 and 57.96±0.4 and the TAC values (μM TE g⁻¹ DW) determined by ABTS and CUPRAC assays showed 19.13±2.1 and 12.71±1.1 and 265.92±25.4 and 265.76±20.5 for gooseberries and blueberries, respectively [2]. Extractions of bioactive substances with ethyl acetate showed the lowest results in comparison with water and methanol: polyphenols (mg GAE g⁻¹ DW) were 0.29±0.1 and 3.87±0.4 and TAC values by CUPRAC (μM TE g⁻¹ DW) showed 0.88±0.1 and 12.40±1.1.
for gooseberries and blueberries, respectively [12]. The results shown in Table 2 slightly differ from the results reported previously [2,12], depending on the solvent used, its ratio to the dry material and also on the time and type of extraction. The optimization of ultrasound operational parameters (solvent polarity, sample particle size), according to a specific plant matrix (leaves, flowers, stems) is also of main importance in order to achieve high extraction efficiency [23]. Sonication time and the temperature were optimized based on our previous results and the highest polyphenol concentration and antioxidant capacity [19].

3.4. Emission spectral studies

Addition of ethanolic polyphenol extract of blueberry to HSA results in the change in both the fluorescence intensity and a shift (blue and red) in the emission maximum of HSA (Fig. 4a). Fluorescence quenching was observed up to $13.52 \times 10^{-2} $ mg GAE g$^{-1}$ DW extract of blueberry and above this concentration there was no change in the fluorescence intensity of HSA. 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. These results are supported by others that the fluorescence studies indicate that the binding site of the additive involves modifications of environment around Trp214 at the level of subdomain IIA [24]. The fluorescence intensity of HSA in the absence of berry extracts at the emission maximum is about 916.89 (Fig. 4a, line 1), then with the addition of $13.52 \times 10^{-2} $ mg GAE g$^{-1}$ DW of blueberry polyphenol extract the fluorescence intensity dropped to 174.58 (Fig. 4a, line 14). Decrease in the fluorescence intensity was about 80.95%. The change in the fluorescence intensity of HSA during addition of blueberry (Fig. 4a) was lower than for gooseberry (Fig. 4b). The shifts in the emission maximum of HSA and the variations in the fluorescence intensity on the addition of polyphenol ethanol extract of gooseberry and blueberry were from...
353 nm to 352 nm and from 353 nm to 359 nm. From the emission spectral studies it is understandable that berry extracts influence the fluorescence quenching. At the same added volume of ethanol extracts (40 μL), which contained different amounts of soluble polyphenols, in gooseberry (0.88 × 10⁻² mg GAE g⁻¹ DW), α-terpinolene (1.09 × 10⁻² mg GAE g⁻¹ DW) and blueberry, (4.16 × 10⁻² mg GAE g⁻¹ DW), the decrease in the fluorescence intensity was 14.35% (Fig. 4c, line 2 from the top), 15.71% (Fig. 4c, line 3 from the top), and 48.71% (Fig. 4c, line 4 from the top). In the case of gooseberry and α-terpinolene the change was smaller as much as three times in comparison with blueberry. With addition to HSA of polyphenols in gooseberry (1.10 × 10⁻² mg GAE g⁻¹ DW) and blueberry (5.20 × 10⁻² mg GAE g⁻¹ DW) fluorescence intensity was 16.84% and 53.08% (Fig. 4d, lines 3 and 5 from the top), respectively. After incubation of these reactions during 24 h at 37 °C the change was 22.93% and 75.49% (Fig. 4d, lines 4 and 6 from the top), respectively. The incubation decreased the change in the intensity in gooseberries of 36.2% and blueberries of 42.2% in comparison with the reactions without incubation. The 3D-spectrum of HSA in the absence and presence of berry extracts is provided in Fig. 5. In the 3D-contour spectra of HSA with berry extracts (Figs. 5b, 5c), a blue color in the center of each figure represents the maximum intensity which corresponds to the emission maximum resulting from tryptophan amino acid. A single contour is obtained for HSA (Fig. 5a) which corresponds to 280 nm and 300 nm as the excitation and emission wavelengths, respectively. The quenching properties of these berries are directly correlated with their antioxidant properties and the amount of polyphenols (Table 2). The binding of antioxidants to HSA was also studied with the combination of berry extract and α-terpinolene. One antioxidant such as polyphenol extract of gooseberry decreased the intensity of HSA and by addition of α-terpinolene (Fig. 4c, line 5 from the top) by increasing the concentration of free polyphenols at the action sites increased the decrease in comparison with the gooseberry extract. Our results are in line with Liu et al. [25], who found that a combination of two drugs decreased the binding affinity of a drug with BSA, which released another drug and increased the concentration of available free drug in the blood. As it was mentioned before, HSA is the most abundant protein in blood plasma and an important carrier for many drugs. Drug interactions are very important in multi-drug therapy. The efficacy of the individual antioxidants and the efficacy of a combination of two antioxidants were measured using fluorescence spectroscopy and showed a synergistic effect. Our very recent results [1,12,13] showed that the fluorescence is significantly quenched, because of the conformation of proteins, phenolic acids and flavonoids. Our result is in agreement with others that quercetin, as an aglycon, is more hydrophobic and demonstrates strong affinity toward HSA. Influence of glycation of plasma proteins in diabetes on the binding interaction with polyphenols was shown in [26]. The non-enzymatic glycation of HSA leads to a conformational change in HSA, which in turn influences the ligand binding properties. HSA glycation is believed to reduce the binding affinities for acidic drugs.
such as dietary polyphenols and phenolic acids. From the results obtained, besides the main binding analysis performed, we conclude that this technique is more sensitive than thought because we can detect several interactions that have not been proven by other methods. 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. Among these polyphenols, (−)-epicatechin-3-gallate showed the highest Stern–Volmer modified quenching constant, followed by (−)-epigallocatechin-3-gallate. Similarly, (−)-epicatechin-3-gallate had the highest effect on the molecular docking predicted high binding energies for (−)-epicatechin-3-gallate and (−)-epigallocatechin-3-gallate for the binding site on BSA near Trp213 [16]. The binding constants ranked in the order quercetin > rutin > calycosin > calycosin-7-O-(3p)-o-glucoside [formononetin-7-O-(3p)-o-glucoside] [17]. It was the best correlation between the obtained results of polyphenols [27] in ethanol/water extracts with the data of fluorescence intensity measurements, shown in our previous results [1,2] and in the present. The combination of two-dimensional chromatography with the fluorescence studies are in agreement with others for characterization of main compounds in the fruits [28–32].

4. Concluding remarks

The compositions of volatile fractions of Cape gooseberry and blueberry 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). The highest amount of alcohol (51.8%), ester (32.8%) and carboxylic acid (6.9%) was in blueberry. Oppositely, ketones (14.7%), aldehydes (9.9%) and terpenes (8%) were in gooseberry. The bioactive compounds and antioxidant capacities were higher in blueberries than in gooseberries. Three dimensional fluorescence emission spectrometry was applied to compare experimentally determined binding parameters of berries extracts with HSA. The fluorescence quenching of HSA by berries polyphenols was a result of the formation of a polyphenol–HSA complex. The binding abilities of berries were directly linked with the bioactivity of polyphenols and volatile substances. The cluster analysis (CA) and linear discriminant analysis (LDA) were applied to differentiate the berries samples according to their type.

References