Comparative assessment of two extraction procedures for
determination of bioactive compounds in some berries used for
daily food consumption

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Summary
Two extractions with methanol and water were used to determine the antioxidant and binding properties of some berries as a supplement to food. Fluorometry, FTIR spectra and radical scavenging assays were used for characterisation of bioactive compounds (polyphenols, flavonoids, flavanols and tannins) and the levels of their antioxidant activities (AAs). The contents of bioactive compounds and AAs in water and methanol polyphenol extracts in gooseberries, blueberries and cranberries differed, but not always significantly. Water extracts of gooseberries showed the lowest amounts of polyphenols (mg GAE g⁻¹), 6.24 ± 0.6, and flavonoids (mg CE g⁻¹), 0.29 ± 0.01, and AAs (μMTE g⁻¹) determined by DPPH, FRAP, ABTS and CUPRAC assays such as 6.05 ± 0.6, 8.07 ± 0.9, 18.70 ± 1.8 and 13.44 ± 1.2, respectively, in comparison with blueberries and cranberries. Polyphenol content highly correlated with antioxidant activity (R² from 0.94 to 0.81). The quenching properties of berries were studied by the interaction of water and methanol polyphenol extracts with HSA by 3D fluorescence. In conclusion, the bioactivity of gooseberries was lower than in blueberries and cranberries. Gooseberries can be used as a new source for food consumption and supplementation based on their antioxidant and binding properties. 3D fluorescence spectroscopy and FTIR spectroscopy can be applied as additional analytical tools for rapid estimation of the quality of different food products.

Keywords
Antioxidant activity, berries, bioactive compounds, food consumption.

Introduction
Consumption of berries has become popular among health-conscious consumers due to the high levels of valuable antioxidants, such as phenolics, which include flavonoids, tannins, flavanols and phenolic acids (Wolfe et al., 2008 Battino et al., 2009; El Gharras, 2009; Paredes-Lopez et al., 2010; You et al., 2011; Kang et al., 2012). Recent studies in vitro and in vivo have improved the scientific understanding of how berries promote human health and prevent chronic illnesses such as some cancers, heart and neurodegenerative diseases (Seeram, 2010). Administration of a freeze-dried powder of mulberry (Morus alba L.) fruit to rats on a high-fat diet resulted in a significant decline in levels of serum and liver triglyceride, total cholesterol and serum low-density lipoprotein cholesterol, and a decrease in the atherogenic index (Yang et al., 2010). Oxidative stress and hypogonadism are linked to the increased incidence of cardiovascular disease (Deyhim et al., 2007). Cranberry was investigated as a chemotherapeutic agent (Elberry et al., 2010). The effect of particle size,
use of infrared radiation and type of freeze-drying (vacuum or atmospheric) on some nutritional properties of blueberries was investigated (Reyes et al., 2011). The purpose of some studies was to investigate and compare the composition, stability and antioxidant properties of berry extracts from selected cultivars using some extraction methods (Chanda & Kaneria, 2012; Khoo et al., 2012). Physalis peruviana, commonly known as cape gooseberry, is an Andean Solanaceae fruit with high nutritional value and interesting medicinal properties. Physalis peruviana has been used in folk medicine for its medicinal properties including anticancer, antimycobacterial, antiplatelet, antiinflammatory properties (Franco et al., 2007). Three species of Physalis fruit (Physalis ixocarpa Brot, Physalis pruinosa L. and Physalis peruviana L.) from Colombia, Egypt, Uganda and Madagascar were analysed by multivariate analysis (El Sheikh et al., 2012). In our recent research, the methanol extracts from different berries were investigated and compared (Arancibia-Avila et al., 2011). Bioactive compounds (polyphenols, flavonoids, flavanols, tannins, anthocyanins and ascorbic acid) and the level of antioxidant activity (AA) estimated by ABTS, DPPH, FRAP and CUPRAC assays of water, acetone and hexane extracts of Chilean ‘Murtilla’ and ‘Myrteola’ berries, Chilean and Polish blueberries, Chilean raspberries and Polish black chokeberry were determined and compared (Arancibia-Avila et al., 2012). We were interested to investigate water and methanol extracts of relatively less known gooseberry and to compare its composition with the widely consumed berries. The water extracts of berries are important from the point of view of tea consumption. To meet this aim, the contents of bioactive compounds (polyphenols, flavonoids, flavanols and tannins) and the level of antioxidant activities (AAs) were determined and compared. To receive reliable data, the AA was determined by four assays: CUPRAC, ABTS, DPPH and FRAP (Brand-Williams et al., 1995; Benzie & Strain, 1996; Re et al., 1999; Apak et al., 2004). To determine the fluorescence properties of the extracted bioactive compounds, in vitro studies were performed by interaction of proteins with flavonoids. Human serum albumin is the drug carrier protein and serves to greatly amplify the capacity of plasma to transport drugs. It was interesting to investigate in vitro how this protein interacts with flavonoids extracted from berry samples in order to obtain useful information about the properties of flavonoid–protein complex. Therefore, the functional properties of a new kind of berry were studied by the interaction of water and methanol polyphenol extracts with a small protein such as HSA (Zhang et al., 2009). Therefore, the aim of this research was to evaluate the bioactivity of gooseberries in comparison with more consumed ones such as blueberries and cranberries. FTIR spectra and fluorescence spectroscopy were used for characterisation of the phytochemicals in berries, extracted with water and methanol. As far as we know, no results of such investigations were published.

Material and methods

Reagents

6-Hydroxy-2,5,7,8-tetramethylethroman-2-carboxylic acid (Trolox), 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-pierylyhazyl (DPPH), Folin–Ciocalteu reagent (FCR), CuCl2 × 2H2O, 2,9-dimethyl-1,10-phenanthroline (neocuproine), lanthanum (III) chloride heptahydrate and FeCl3 × 6H2O were purchased from Sigma Chemical Co., St Louis, MO, USA. 2, 4, 6-Tripyridyl-2-triazine (TPTZ) was purchased from Fluka Chemie, Buchs, Switzerland. All reagents used were of analytical grade. Deionised and distilled water was used throughout the experiment.

Samples

Cape gooseberries (Physalis peruviana), blueberries (Vaccinium corymbosum) and cranberries (Vaccinium macrocarpon) were investigated. The fruits were harvested at their mature stage. All berries were purchased at the local market in Gdansk and Warsaw, Poland. For the investigation, five replicates of five berries each were used. Their edible parts were separated manually without using steel knives. The separated berries were weighed, chopped and homogenised under liquid nitrogen in a high-speed blender (Hamilton Beach Silex professional model) for 1 min. A weighed portion (50–100 g) was then lyophilised 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 ºC until the bioactive substances were analysed.

Determination of bioactive compounds and antioxidant activity

The contents of polyphenols, tannins, flavonoids and flavanols in the extracts of the studied berries were determined as previously described (Gorinstein et al., 2009, 2010). The lyophilised samples of berries (1 g) were extracted with 100 mL of methanol and water (1:1) at room temperature and in darkness for 24 h. The extracts were filtered in a Buchner funnel. The polyphenols were determined by the of Folin–Ciocalteu method with measurement at 750 nm using spectrophotometer (model 8452A; Hewlett-Packard, Rockville, MD, USA). The results were expressed as mg of gallic acid equivalents (GAE) per g DW (Singleton et al., 1999). Condensed tannins (procyanidins)
were extracted with 4% methanol vanillin solution, and the extracts were measured at 500 nm. Flavonoids, extracted with 5% NaNO₂, 10% AlCl₃ × 6H₂O and 1 M NaOH, were measured at 510 nm. The amount of total flavanols was estimated using the p-dimethylaminocinnamaldehyde (DMACA) method, and then the absorbance was read at 640 nm (Feucht & Polster, 2001). (+)-Catechin served as a standard for spectrophotometric determination of flavonoids, flavanols and tannins, and the results were expressed as catechin equivalents (CEs).

The antioxidant activity was determined by four assays

1. 2, 2-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS⁺) radical cation was prepared by the addition of ABTS (7 mM) and K₂S₂O₈ (2.45 mM). This solution was diluted with methanol until the absorbance of the samples reached 0.7 at 734 nm (Re et al., 1999).

2. Ferric-reducing antioxidant power (FRAP) reagent (2.5 mL of a 10 mmol ferric-tripryidyltriazine solution in 40 mmol HCl plus 2.5 mL of 20 mmol FeCl₃ × H₂O and 25 mL of 0.3 M acetate buffer, pH 3.6) (900 μL) was mixed with 90 μL of distilled water and 30 μL of berry samples as the appropriate reagent blank. The absorbance was measured at 595 nm (Benzie & Strain, 1996).

3. Cupric reducing antioxidant capacity (CUPRAC) was determined based on the utilisation of the copper (II)–neocuproine [Cu (II)-Nc] reagent as the chromogenic oxidising agent. The absorbance at 450 nm was recorded against a reagent blank (Apak et al., 2004).

4. 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) has an absorption band at 515 nm, which disappears upon reduction by an antiradical compound. DPPH solution (3.9 mL, 25 mg L⁻¹) in methanol was mixed with the sample extracts (0.1 mL) and then the reaction progress was monitored at 515 nm until the absorbance was stable (Brand-Williams et al., 1995).

Fluorometry and fourier transform infrared (FT-IR) spectra studies

Two-dimensional (2D FL) fluorescence spectra measurements for all berry extracts at a concentration of 0.01 mg mL⁻¹ were recorded on a model FP-6500, Jasco spectrofluorometer, serial N261332, Japan, equipped with 1.0-cm quartz cells and a thermostat bath. The 2D FL spectroscopy measurement was taken at emission wavelengths from 310 to 500 nm and at excitation of 295 nm (Arancibia-Avila et al., 2011, 2012). Quercetin was used as a standard. All solutions for protein interaction were prepared in 0.05 M Tris-HCl buffer (pH 7.4), containing 0.1 M NaCl. The final concentration of HSA was 2.0 × 10⁻⁶ M. The HSA was mixed with quercetin in the proportions of HSA–extract = 1:1 (Wulf et al., 2005; Xiao et al., 2011a,b).

The presence of polyphenols in the investigated berry samples was studied by Fourier transform infrared (FT-IR) spectroscopy. A Nicolet iS 10 FT-IR Spectrometer (Thermo Scientific Instruments LLC, Madison, WI, USA), with the smart iTR ™ ATR (Attenuated Total Reflectance) accessory, was used to record IR spectra (Sinelli et al., 2008).

Statistical analyses

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.

Results

Bioactive compounds

The results of the determination of the contents of the bioactive compounds in all studied samples are summarised in Table 1. Water and methanol extracts of gooseberries showed lower amounts of polyphenols, flavonoids, flavanols and tannins (6.24–3.77 mg GAE g⁻¹; 0.29–0.45 mg CE g⁻¹; 6–8 µg CE g⁻¹; and 1.01–1.24 mg CE g⁻¹, respectively, Table 1) than blueberries and cranberries.

Antioxidant activity

As can be seen from Table 2, the AA (µM TE g⁻¹) for gooseberries by DPPH, FRAP, ABTS and CUPRAC assays was 6.05–4.61; 8.07–7.61; 18.70–19.13; and 13.44–12.71, respectively. The antioxidant activity of blueberries was higher than that of gooseberries and cranberries. As was calculated, a very good correlation was found between the antioxidant activity and the contents of total polyphenols in water and methanol extracts. The correlation between the antioxidant activity and polyphenols was between 0.87 and 0.78.

Fluorometry spectra studies and FTIR spectra

The quenching properties of the berry samples are shown in two-dimensional fluorescence spectra (2D FL) and also their comparison with quercetin (Q). One of the main peaks for HSA was found at λex/em of 220/360 nm. The second main peak appeared for...
these samples at λ ex/em of 280/350 nm (Fig. 1). Water phenolic extracts showed slightly higher antioxidant properties than the methanol ones, but the differences were not always significant in all extracts. The interaction between HSA and the water extracts (WE) of berries, HSA, WE and Q (Fig. 1a), showed slight change in the position of the main peak at the wavelength of 360 nm and the decrease in the relative fluorescence intensity (RFI). The following changes appeared when the water extracts of berries were added to HSA [initially the main peak was at emission of 360 nm and FI of 890.21 (Fig. 1a, the upper line is HSA)]. The reaction of blueberry water extracts (BLUEBWE) with HSA (second line from the top) and with BLUEBWE, HSA and Q (fifth line from the top) decreased the RFI of HSA by 28.1% and 41.7%, respectively. The reaction of cranberry water extracts (CRANBWE) with HSA (third line from the top) and with CRANWE, HSA and Q (sixth line from the top) decreased the RFI of HSA by 13% and 29.9%, respectively. The reaction of gooseberry water extracts (GOOSEBWE) with HSA (fourth line from the top) and with GOOSEBWE, HSA and Q (seventh line from the top) decreased the RFI of HSA by 3.9% and 27.8%, respectively. These results showed that the binding properties of gooseberries were 7.2 and 1.5 times and 3.3 and 1.1 times lower than that of blueberries and cranberries, respectively. The following changes appeared when the methanol extracts of berries were added to HSA (initially the main peak was at emission of 360 nm and FI of 890.21, Fig. 1b, the upper line is HSA). The reaction of blueberry methanol extracts (BLUEBMeOH) with HSA (second line from the top) and with BLUEBMeOH, HSA and Q

Table 1 Bioactive compounds in water (H2O) and methanol (MeOH) polyphenol extracts of gooseberries (GOOSEB, Physalis peruviana), cranberries (CRAN, Vaccinium macrocarpon) and blueberries (BLUEB, Vaccinium corymbosum)‡,†

<table>
<thead>
<tr>
<th>Extracts of berries</th>
<th>POLYPHEN, mg GAE</th>
<th>FLAVON, mg CE</th>
<th>FLAVAN, µg CE</th>
<th>TANNINS, mg CE</th>
</tr>
</thead>
<tbody>
<tr>
<td>GOOSEB, H2O</td>
<td>6.24 ± 0.6e</td>
<td>0.29 ± 0.01f</td>
<td>6 ± 0.8d</td>
<td>1.01 ± 0.2c</td>
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<tr>
<td>CRAN, H2O</td>
<td>15.32 ± 2.5h</td>
<td>3.06 ± 0.4b</td>
<td>249 ± 14.5c</td>
<td>2.30 ± 0.7f</td>
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<tr>
<td>BLUEB, H2O</td>
<td>57.47 ± 4.2a</td>
<td>6.68 ± 0.6a</td>
<td>1762 ± 25.6a</td>
<td>5.00 ± 0.6p</td>
</tr>
<tr>
<td>GOOSEB, MeOH</td>
<td>3.77 ± 0.1i</td>
<td>0.45 ± 0.01f</td>
<td>8 ± 1.1d</td>
<td>1.24 ± 0.1c</td>
</tr>
<tr>
<td>CRAN, MeOH</td>
<td>20.25 ± 0.4g</td>
<td>2.20 ± 0.1b</td>
<td>393 ± 20.3c</td>
<td>1.76 ± 0.1c</td>
</tr>
<tr>
<td>BLUEB, MeOH</td>
<td>57.96 ± 0.4h</td>
<td>6.68 ± 0.7a</td>
<td>3210 ± 40.4e</td>
<td>24.80 ± 2.5g</td>
</tr>
</tbody>
</table>

POLYPHEN, polyphenols; CE, catechin equivalent; GAE, gallic acid equivalent; FLAVON, flavonoids; FLAVAN, flavonols; nd, not determined; DPPH, 2,2-diphenyl-1-picrylhydrazyl; CUPRAC, cupric reducing antioxidant capacity; ABTS, 2, 2-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt; FRAP, ferric reducing antioxidant power.

*Values are means ± SD of five measurements.
†Values in columns for every bioactive compound bearing different superscript letters are significantly different (P < 0.05).
‡Per gram dry weight.

Table 2 Antioxidant activities in water (H2O) and methanol (MeOH) extracts of gooseberries (GOOSEB, Physalis peruviana) and blueberries (BLUEB, Vaccinium corymbosum)‡,†

<table>
<thead>
<tr>
<th>Extracts of berries</th>
<th>DPPH, µM TE g⁻¹ DW</th>
<th>FRAP, µM TE g⁻¹ DW</th>
<th>ABTS, µM TE g⁻¹ DW</th>
<th>CUPRAC, µM TE g⁻¹ DW</th>
</tr>
</thead>
<tbody>
<tr>
<td>GOOSEB, H2O</td>
<td>6.05 ± 0.6e</td>
<td>8.07 ± 0.9e</td>
<td>18.70 ± 1.8d</td>
<td>13.44 ± 1.2e</td>
</tr>
<tr>
<td>CRAN, H2O</td>
<td>44.23 ± 4.5e</td>
<td>22.45 ± 2.4b</td>
<td>64.83 ± 6.5c</td>
<td>28.45 ± 2.7b</td>
</tr>
<tr>
<td>BLUEB, H2O</td>
<td>75.09 ± 6.2b</td>
<td>177.25 ± 14.6a</td>
<td>254.83 ± 25.6a</td>
<td>250.95 ± 18.6*</td>
</tr>
<tr>
<td>GOOSEB, MeOH</td>
<td>4.61 ± 0.4a</td>
<td>7.61 ± 0.9e</td>
<td>19.13 ± 2.1d</td>
<td>12.71 ± 1.1f</td>
</tr>
<tr>
<td>CRAN, MeOH</td>
<td>23.25 ± 2.6a</td>
<td>26.11 ± 2.1b</td>
<td>68.40 ± 6.3c</td>
<td>32.67 ± 3.1b</td>
</tr>
<tr>
<td>BLUEB, MeOH</td>
<td>142.03 ± 11.4a</td>
<td>149.00 ± 11.7a</td>
<td>265.92 ± 25.4*</td>
<td>265.76 ± 20.5*</td>
</tr>
</tbody>
</table>

POLYPHEN, polyphenols; CE, catechin equivalent; GAE, gallic acid equivalent; FLAVON, flavonoids; FLAVAN, flavonols; nd, not determined; DPPH, 2,2-diphenyl-1-picrylhydrazyl; CUPRAC, cupric reducing antioxidant capacity; ABTS, 2, 2-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt; FRAP, ferric reducing antioxidant power.

*Values are means ± SD of five measurements.
†Values in columns for every bioactive compound bearing different superscript letters are significantly different (P < 0.05).
‡Per gram dry weight.
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The reaction of cranberry methanol extracts (CRANBMeOHE) with HSA (fourth line from the top) and with CRANBMeOHE, HSA and Q (seventh line from the top) decreased the RFI of HSA by 13.9% and 18.9%, respectively. The lowest decrease was with GOOSEBMeOHE without quercetin, but the synergism of quercetin with cranberries and gooseberries showed similar results. The water extracts showed higher binding properties to berries than the methanol, and the difference was significant in all berries. Our most recent results showed that the fluorescence is significantly quenched, because of the conformation of the HSA changes in the presence of quercetin and berry extracts. This interaction between quercetin and HSA was investigated using tryptophan fluorescence quenching. Other results (Xiao et al., 2011a,b; Zhang et al., 2009) differ from that reported by us, probably because of the variety of antioxidant abilities of pure flavonoids and different ranges of fluorometry scanning used in a similar study. Our in vitro results of interaction between HSA and quercetin can be compared with other reports (Zhang et al., 2009). There are not too many applications of 3D fluorescence spectra; therefore, our present conclusions—that 3D fluorescence can be used as an additional tool for the characterisation of the polyphenol extracts of berries cultivars—correspond with the previous data (Gorinstein et al., 2010) and can be applied to any food analysis.

FTIR spectra of water (a) and methanol (b) extracts of gooseberries, blueberries and cranberries are presented in Fig. 2 (lines from the bottom to the top). The FTIR wave numbers in polyphenol water extracts showed a broad band at 3273 cm⁻¹ for gooseberries and blueberries, but for cranberries, there was a shift to 3332 cm⁻¹ (phenolic OH band). Other bands were detected at 2342, 2349 and 2345 cm⁻¹ for gooseberry, blueberry and cranberry, respectively. At 1642 cm⁻¹ (C=O stretching phenyl ring amino acid-1), this band was detected for gooseberry and blueberry and at 1636 cm⁻¹ only for cranberry (Fig. 2a). The methanol polyphenol extracts (Fig. 2b) showed similar bands at 3313, 2943 and 2834 cm⁻¹ for three berries. At 1652 cm⁻¹ (characteristic CO stretching), bands appeared for gooseberry and blueberry and at 1715 cm⁻¹ for cranberry. In the range of 1445 cm⁻¹, a band was found for gooseberry. At 1410 cm⁻¹, a band was found for blueberry and at 1391 cm⁻¹ (–OH phenolic bending) for cranberry. The common bands at 1115 cm⁻¹ (aromatic bending and stretching) and at 821 cm⁻¹ were estimated for all berries. The comparison between the berries, their extracts and some standards in the range of common peaks is shown in Tables 3–4. The best matching in the common range of the peaks was in water extracts of the berries between 3200 and 3000 cm⁻¹ (Table 3) of 87% with tannic acid, 78% with hesperidin and 64% with gallic acid. Caffeic and tannic acids showed the matching in the range of 2500–2000 cm⁻¹ (Table 3) of 40%. In phenolic extracts with methanol, similar matching of the peaks was found in comparison with tannic acid (84%) and hesperidin (70%). Quercetin in the range of 3500–3100 cm⁻¹ (Table 4) showed similarity with the same bands of 70%, which was three times higher than that in water phenolic extract. In the range of 3000–1600 cm⁻¹ (Table 4), caffeic, gallic, tannic and ferulic acids showed matching with the investigated berries from 30 to 12%. These matching results for the first time show that FTIR spectra can be used for the rapid estimation of extracted bioactive compounds. Quercetin exhibited the highest matching in the investigated fruit extracts in comparison with fisetin, and caffeic and gallic acids in methanol extracts of investigated berries. Difference between the standards and the investigated samples can be explained by the extraction procedures of the main polyphenols.

Discussion

A number of reviewed articles showed that the main bioactive compounds determining the nutritional quality of berries are polyphenols, anthocyanins and flavonoids (Battino et al., 2009; Dai et al., 2009; Bowen-Forbes et al., 2010). Seeram (2010) discussed also that phytonutrients ranged from fat-soluble/lipophilic to water-soluble/hydrophilic compounds. The health benefits of blueberries and cranberries have long been recognised, but less is known about gooseberries. It was of great interest to compare gooseberry with blueberry and to find out whether the bioactivity of gooseberry is on the same level as these berries in order to use it as a novel additional food source. As was declared in the Results, the contents of bioactive compounds (polyphenols, flavonoids, flavanols and tannins) and AA in water and methanol extracts were the lowest in gooseberries. Our results, connected with the bioactive compounds and AAs, are in correspondence with others, showing that water extracts of blackberries contain high amounts of bioactive compounds (Dai et al., 2009). Our results correspond also with the research of Wu et al. (2006), where concentrations of total anthocyanins varied considerably from 0.7 to 1480 mg per 100 g FW in gooseberry (‘Careless’ variety) and chokeberry, respectively. DPPH radical scavenging activity of currant varied from 12.67 to 31.18 mmol TE kg⁻¹ (Wojdyo et al., 2013), and it was
similar to the results obtained in this research. Total phenolic content of four berry fruits (strawberry, saskatoon berry, raspberry and wild blueberry), chokecherry and seabuckthorn ranged from 22.83 to 131.88 g kg\(^{-1}\), which corresponds with our results as well. Conclusions made in the report of Elberry et al. (2010) are in line with our results about the high antioxidant activity of berries. Our results are in accordance with You et al. (2011), where four rabbiteye blueberry cultivars (Powderblue, Climax, Tifblue and Woodward) grown organically and conventionally were compared regarding their chemical profiles and antioxidant capacity in terms of total phenolic content and antioxidant values determined by ABTS, DPPH, FRAP and CUPRAC assays. The comparison of the results of different solvents in dabai fruit parts (methanol, ethanol, ethyl acetate, acetone and water) and total phenolics, total flavonoids and antioxidant capacity (ABTS\(^+\) and FRAP assays) were in accordance with our data (Khoo et al., 2012). The acetone extract had maximum phenol and flavonoid content and showed the best DPPH free radical scavenging activity and reducing capacity assessment. Ethyl acetate extract showed best superoxide radical scavenging activity, while aqueous extract showed best hydroxyl radical scavenging activity (Chanda & Kaneria, 2012).

Table 3 Matching of the peaks (%) in the FTIR spectrum of extracted polyphenols in water from gooseberries (GB, *Physalis peruviana*), cranberries (CB, *Vaccinium macrocarpon*) and blueberries (BB, *Vaccinium corymbosum*) and standards

<table>
<thead>
<tr>
<th>Range of bands</th>
<th>Standards</th>
<th>GB (%)</th>
<th>BB (%)</th>
<th>CB (%)</th>
<th>GB (%)</th>
<th>BB (%)</th>
<th>CB (%)</th>
<th>GB (%)</th>
<th>BB (%)</th>
<th>CB (%)</th>
</tr>
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<tbody>
<tr>
<td>3200–3000 cm(^{-1})</td>
<td>Gallic acid</td>
<td>64</td>
<td>64</td>
<td>64</td>
<td>37</td>
<td>33</td>
<td>36</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2500–2000 cm(^{-1})</td>
<td>Ferulic acid</td>
<td>23</td>
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<td>23</td>
<td>26</td>
<td>19</td>
<td>25</td>
<td>2</td>
<td>3</td>
<td>3</td>
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<tr>
<td>1800–1500 cm(^{-1})</td>
<td>Fisetin</td>
<td>20</td>
<td>19</td>
<td>16</td>
<td>35</td>
<td>34</td>
<td>35</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>3500–3100 cm(^{-1})</td>
<td>Hesperidin</td>
<td>78</td>
<td>77</td>
<td>78</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>28</td>
<td>28</td>
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<tr>
<td>3000–2800 cm(^{-1})</td>
<td>Tannic acid</td>
<td>87</td>
<td>87</td>
<td>87</td>
<td>41</td>
<td>40</td>
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<td>1800–1600 cm(^{-1})</td>
<td>Caffeic acid</td>
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<td>1500–700 cm(^{-1})</td>
<td>Quercetin</td>
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<td>26</td>
<td>1</td>
<td>1</td>
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</tr>
</tbody>
</table>

Table 4 Matching of the peaks (%) in the FTIR spectrum of extracted polyphenols in methanol from gooseberries (GB, *Physalis peruviana*), cranberries (CB, *Vaccinium macrocarpon*) and blueberries (BB, *Vaccinium corymbosum*) and standards

<table>
<thead>
<tr>
<th>Range of bands</th>
<th>Standards</th>
<th>GB (%)</th>
<th>BB (%)</th>
<th>CB (%)</th>
<th>GB (%)</th>
<th>BB (%)</th>
<th>CB (%)</th>
<th>GB (%)</th>
<th>BB (%)</th>
<th>CB (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3500–3100 cm(^{-1})</td>
<td>Quercetin</td>
<td>70</td>
<td>71</td>
<td>70</td>
<td>1</td>
<td>5</td>
<td>6</td>
<td>1</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>3000–2800 cm(^{-1})</td>
<td>Ferulic acid</td>
<td>17</td>
<td>17</td>
<td>18</td>
<td>14</td>
<td>12</td>
<td>11</td>
<td>18</td>
<td>8</td>
<td>18</td>
</tr>
<tr>
<td>1800–1600 cm(^{-1})</td>
<td>Fisetin</td>
<td>10</td>
<td>17</td>
<td>17</td>
<td>10</td>
<td>15</td>
<td>12</td>
<td>3</td>
<td>22</td>
<td>3</td>
</tr>
<tr>
<td>1500–700 cm(^{-1})</td>
<td>Hesperidin</td>
<td>69</td>
<td>69</td>
<td>70</td>
<td>34</td>
<td>35</td>
<td>40</td>
<td>20</td>
<td>26</td>
<td>9</td>
</tr>
<tr>
<td>3500–3100 cm(^{-1})</td>
<td>Tannic acid</td>
<td>84</td>
<td>84</td>
<td>84</td>
<td>14</td>
<td>16</td>
<td>14</td>
<td>9</td>
<td>35</td>
<td>2</td>
</tr>
<tr>
<td>3000–2800 cm(^{-1})</td>
<td>Caffeic acid</td>
<td>34</td>
<td>38</td>
<td>35</td>
<td>27</td>
<td>30</td>
<td>24</td>
<td>16</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td>1800–1600 cm(^{-1})</td>
<td>Gallic acid</td>
<td>59</td>
<td>57</td>
<td>57</td>
<td>11</td>
<td>13</td>
<td>11</td>
<td>19</td>
<td>28</td>
<td>16</td>
</tr>
<tr>
<td>1500–700 cm(^{-1})</td>
<td></td>
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<td></td>
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</table>
et al. (2012) showed that gooseberry (*Physalis peruviana*) fruit is one of the less used raw materials of plant origin, which can be used for human nutrition and can be promoted as a food additive in fresh and processed food, as an extract from fresh or frozen fruits. The methanolic extracts of three cultivars expressed high antioxidant activity and correlated with the amount of polyphenols. We have investigated the binding properties of quercetin in aqueous and methanol media, using UV/vis and fluorometry, which is one of the

Figure 2 FTIR spectra of: (a) water extracts of gooseberries, blueberries and cranberries; (b) methanol extracts of gooseberries, blueberries and cranberries from the bottom to the top. The elliptical symbols showed the similar range of the spectra in two extracts.
major phenolic compounds found in berries. Our results were in accordance with Guo et al. (2007), who demonstrated that quercetin and other phenolic compounds can effectively modulate iron biochemistry under physiologically relevant conditions, providing insight into the mechanism of action of bioactive phenolics (Guo et al., 2007). Our results are in agreement with Xiao et al. (2011a) as well that dietary flavonoids are important polyphenols in berries as they are of great interest for their bioactivities, which are related to the antioxidative property. The binding affinities with HSA were strongly influenced by the structural differences of dietary polyphenols from berries. The HSA–polyphenol interaction weakened with the free radical scavenging potential of polyphenols. The structural difference of flavonoids strongly affects the binding process with plasma proteins. Flavonoids played as a hydrogen bond acceptor when bound to HSA (Xiao et al., 2011a,b). The relatively high binding properties of gooseberries are important from the point of view of their incorporation in food products as an important ingredient. Our in vitro fluorometry studies are in agreement with others, who investigated the properties of berries in vivo. So, the drinking of cranberry juice for 4 months affected antioxidant capacity and lipid profile in orchidectomised rats. Orchidectomy depressed plasma antioxidant capacity of plasma and increased triglyceride and cholesterol values of liver and plasma (Deyhim et al., 2007). Rats fed with goldenberry (Physalis peruviana) juice showed lower levels of total cholesterol, total triacylglycerol and total low-density lipoprotein cholesterol, as well as higher levels of high-density lipoprotein cholesterol in comparison with animals fed with HCD and cholesterol-free diet (Ramadan, 2012). It is possible to supplement food products with the extracts of the studied berries, as it was shown in the study by Lastaw ska (2010). The selected products were in the form of hard gelatin capsules. They contained the extracts from chokeberry, cranberry and blueberry. All studied preparations showed antioxidant properties and may provide substantial antioxidant protection. The in vitro antioxidant capacity varied considerably and was associated with the content of polyphenols in the capsule. The studied gooseberry can be used as dry or fresh material or as water extracts. The most important aspect is the prevention of antioxidant properties during the food processing. Our present results are in correspondence with the previous results, where only aqueous extracts were used, with other kinds of berries. In our previous report, it was shown that aqueous extracts of investigated berries were subjected to different times of thermal processing. Only thermal treatment of studied berries influences their quality: berries after 10 and 20 min of thermal processing preserved their bioactivity (Arancibia-Avila et al., 2012). This is in accordance with Reyes et al. (2011), who showed that ascorbic acid content was decreased in freeze-dried blueberries compared with fresh fruit, while polyphenols were decreased in atmospheric freeze-drying unlike in vacuum freeze-drying, where this nutritional property was increased. The results show promising perspectives for the exploitation of berry species with considerable levels of nutrients and antioxidant capacity in foods. Our data add valuable information to current knowledge of the nutritional properties of berries, such as the considerable antioxidant and binding capacities that were found. In conclusion, the bioactivity of gooseberries is lower and comparable with blueberries and cranberries. Gooseberries are a promising exotic fruit that could be made into many novel dishes. 3D fluorescence spectroscopy and FTIR spectroscopy were used as additional tools for the characterisation of the polyphenol extracts in different berry cultivars. The analytical methods used in this study can be applied for any food analysis.

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