FTIR analysis and potentiometric titration in identification of functional groups on the surface of new biosorbents – strawberry and raspberry seeds

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Abstract
In the present work, post-extraction residues of strawberry and raspberry seeds were used as biosorbents of micronutrients. Taking into account physical form of strawberry and raspberry seeds and relative homogeneity, they are interesting biosorbents. Their sorption properties are unknown so far.

In this study, the surface chemical functional groups of new biosorbents were identified. The biomass of post-extraction residues of strawberry and raspberry seeds was enriched with Cu(II), Mn(II) and Zn(II) ions via biosorption in stirred tank reactor (40 L) at 25 °C, pH=5. About 40 g of each micronutrient-enriched product was prepared. The role of the surface chemistry of the biomass in biosorption process was investigated by ICP–OES, FTIR and potentiometric titration. The comparison of the obtained results was performed in order to investigate the functional groups that participate in biosorption. Results obtained by ICP-OES analysis showed exchange of naturally bound alkali cations with micronutrients. Important functional groups present on the biomass of residues of berries seeds were carboxyl, amino and hydroxyl groups. There was found a correlation between cation exchange capacity assessed with potentiometric titration and sorption capacity of copper ions by ICP-OES. The use of FTIR analysis, potentiometric titration and ICP-OES is a useful approach in the elaboration of biosorption phenomenon.

Keywords: biosorption, berries seeds, functional groups, FTIR, ICP-OES

1. Introduction
Waste management became more important due to the availability and lower cost of using waste materials (Schaub and Leonard 1996). An example are strawberry and raspberry seeds which are particularly interesting waste in fruits industry. About 200 000 Mg of strawberries and 120 000 Mg (GUS 2014) of raspberries are produced annually in Poland with the total global production of 1 600 000 and 460 000
Mg (Novagrim 2010; Freshplaza 2014). Constituents of berries: anthocyanins and polyphenols such as ellagic acid, are beneficial in protecting cells from various health injuries, such as ageing and different forms of cancer, as it was shown in in vitro and in vivo tests (Juranic et al., 2005).

In the processing of raspberry and strawberry fruits, the seeds become a by-product. Raspberry and strawberry seeds constitute up to 50% of the waste (Samoraj et al., 2014). Seeds of raspberries and strawberries may be subjected to supercritical extraction (Nakonieczna et al., 2014). The obtained oil is rich in tocopherols, vitamin E, lipids, phospholipids, free fatty acids (Oomah et al., 2000). Seed oil has superior antioxidant potential as compared to vitamins C, E, and \( \beta \)-carotene (Zafra-Stone et al., 2007). Natural antioxidants (including phenolic compounds) may prevent lipid peroxidation in edible oils as in the case of synthetic antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene (Parry and Yu 2004). It is very important because oxidation of lipids has been gaining a great concern because of its influence on a quality and nutritive value decrease of fat and fat-containing food, as well as the diverse biological activity of the products formed (Malecka et al., 2003).

Utilization of berries seeds by the supercritical extraction of oils generates post-extraction residues. In the last time, special attention is paid to the use of process wastes from biomass processing which provides an opportunity to substitute chemical fertilizers. There are many advantages of this type of residues for example: slow release of micronutrients, growth stimulants, improvement of soil structure (Hue and Silva 2000; Tuhy et al., 2014). The new idea involves the use of supercritical post-extraction residues of berries seeds as components of fertilizers (Chojnacka et al., 2014).

Taking into account physical form of strawberry and raspberry seeds, post-extraction residues and relative homogeneity, they are interesting biosorbents. The quality of biosorbents depends on strong molecular interactions (i.e. electrostatic attractions), chemical state and composition of surface functional groups. The state - ionization or protonation of the functional groups present on the surface depends directly on medium acidity (pH) and dissociation constant. Usually, value of this constant for chemical
groups presented by biomolecules closely corresponds to $pK_a$ value of appropriate inorganic acid or base, because of the presence of different types of functional groups (commonly): amine, carboxyl, hydroxyl, phosphoryl and sulfone groups on the biomass surfaces with amphoteric properties (Dmytryk et al., 2014). In general, surface chemical reactions between mentioned functional groups allow (mainly by complexation) for selective binding of micronutrients (Pagnanelli et al., 2008).

On the surface of the strawberry and raspberry seeds polysaccharides, fatty acids, proteins, amino acids are found (Parry and Yu 2004). These molecules contain a variety of functional groups (e.g. carboxyl, hydroxyl etc.) which can participate in the biosorption of microelement ions (Michalak et al., 2013). Their sorption properties are unknown, so far. Due to biosorption, there is a possibility to increase the content of essential micronutrients such as zinc, manganese and copper in the biomass. As it was reported previously, micronutrients after biosorption are bound to the biomass matrices in bioavailable form (Tuhy et al., 2014; Chojnacka et al., 2014).

In present work, post-extraction residues of strawberry and raspberry seeds were examined as a new biosorbent. The surface chemical functional groups were identified. The role of the surface chemistry of the biomass on biosorption was investigated. The biomass of strawberry and raspberry post-extraction seeds residues was enriched with Zn(II), Cu(II) and Mn(II) which are important in plant feeding. In order to explain the mechanism of the biosorption, different analytical techniques were applied. The surface functional groups of the biomass were identified by Fourier Transform Infrared (FTIR) analytical technique and potentiometric titration. These techniques are reported as useful methods in the determination of the presence of functional groups on the biosorbents surface (Yun et al., 2001). Surface characterization by titration modeling have been applied also by other researchers to different heterogeneous natural materials (soils, agricultural wastes, bacterial biomasses) (Pagnanelli et al., 2000, 2003, 2004, 2006). The content of elements in metal loaded biomass was examined by Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP–OES).
2. Material and methods

2.1. Biosorbent

The biomass of post-extraction residues of raspberry and strawberry seeds was obtained from the New Chemical Syntheses Institute – Supercritical Extraction Department in Puławy.

2.2. Biosorption process

The biomass was enriched with Zn(II), Cu(II) and Mn(II) ions via biosorption in stirred tank reactor (40 L) at 25 °C, pH=5 (Tuhy et al., 2014). Aqueous solution of microelement ions (300 mg/L) was prepared by dissolving ZnSO$_4$·7H$_2$O, CuSO$_4$·5H$_2$O, MnSO$_4$·H$_2$O in deionized water.

2.3. ICP-OES analysis

For the ICP-OES multielemental analysis of samples known mass of each material was digested in microwave system Milestone Start D (USA) with spectrally pure nitric acid – 69% m/m (Suprapur, Merck, USA) in teflon bombs. All mineralization parameters were matched to assure complete digestion of samples. After mineralization, samples were analyzed with ICP–OES (Varian–Vista MPX, Australia). Samples were introduced with ultrasonic nebulizer CETAC U5000AT+. The analyses were carried out in Laboratory Accredited by Polish Centre of Accreditation (PCA) according to PN-EN ISO/IEC 17025:2005. Quality assurance of the test results was achieved by using Combined Quality Control Standard from ULTRA SCIENTIFIC, USA. All samples were analyzed in three repeats (the relative standard deviation was <5%).

2.4. FTIR analysis

Functional groups presented on the surface of the biomass were identified by FTIR spectroscopy. FTIR absorption spectra were performed in the wave number range 4000-400 cm$^{-1}$. Approximately 2 mg of
biological material sample was prepared and compressed with 200 mg of dry KBr. Analysis was performed with Spectrum System 2000 FT–IR (Perkin Elmer). Studies were carried out the Central Laboratory of Instrumental Analysis at Wroclaw University of Technology.

2.5. Potentiometric titration

Second method used for the identification of functional groups on the biomass was potentiometric titration. For the analysis, 1.0 g of the biomass was suspended in 100 mL of ultrapure deionized water. Titration was performed in two steps with automatic titrator (T50, Mettler Toledo). In first step, pH was lowered to 2.5. After that the biomass was titrated with NaOH (0.1 M). All probable pKₐ points (acidic dissociation constants) on titration curve were assigned using graphical method with Origin Pro 2015 software. From 1ˢᵗ derivative curve, all peaks were determined. From 2ⁿᵈ derivative curve, all crossing points with x-axis were chosen from population of Xₐd Addition values (molar ratio of added titrant – mmol/g of biosorbent), determined in first step. These points are probable Xₐd values for all measured pH at pKₐ points.

3. Results and discussion

Biosorption is a complex process. Biomass can bind metal ions by (I)chemical exchange of cations (ion exchange) or protons (proton displacement), chelation or complexation and (II) physical (van der Waals forces and electrostatic interaction) (Davis et al., 2003; Michalak et al., 2013). The identification of biosorption mechanism might be evaluated by: potentiometric titration, XRF, FTIR, SEM-EDX (Michalak et al., 2013). Main role in the biosorption process play functional groups (especially carboxyl) which are present on the biomass surface.

In the present work, qualitative determination of functional groups on the surface of the biomass was conducted with FTIR analytical technique and potentiometric titration (Yun et al., 2001). FTIR allows
identification of different functional groups on the cell wall structure and provides important information related to the nature of the bonds (Michalak et al., 2013; Murphy et al., 2007). The potentiometric titration is also useful in description of aqueous biomass suspension. However, post-extraction residues of strawberry and raspberry seeds have not been studied in this respect, so far. Prepared materials were also subjected to ICP-OES analysis in order to determine which metal ions were exchanged during the process.

3.1. Multielemental analysis

In the present study, both tested biosorbents had the highest biosorption capacity for copper ions, then zinc and finally for manganese. This phenomenon can be explained by characteristics of the metal ionic properties. Taking into account several parameters (Can and Jianlong 2007) listed in Table 1. It may be assumed that AR, IP and Xm are responsible for the highest biosorption capacity of the examined biomasses for Cu(II) ions.

Table 1. Metal ionic properties

<table>
<thead>
<tr>
<th>Micronutrient</th>
<th>Biosorption capacity (mg/g)</th>
<th>Oxidation number (OX)</th>
<th>Atomic weight (AW) (u)</th>
<th>Atomic radius (AR) (Å)</th>
<th>Ionization potential (IP) (eV)</th>
<th>Electronegativity(Xm) (Pauling Units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>12.6</td>
<td>9.58</td>
<td>63.5</td>
<td>1.35</td>
<td>20.3</td>
<td>1.90</td>
</tr>
<tr>
<td>Zn</td>
<td>5.13</td>
<td>4.78</td>
<td>2</td>
<td>65.4</td>
<td>1.31</td>
<td>18.0</td>
</tr>
<tr>
<td>Mn</td>
<td>5.03</td>
<td>2.50</td>
<td>54.9</td>
<td>1.12</td>
<td>15.6</td>
<td>1.65</td>
</tr>
</tbody>
</table>

Basing upon data from Table 2, the content of K(I) in the enriched with Zn(II), Cu(II) and Mn(II) ions biomass of strawberry seeds decreased by 80.0%, 83.4% and 48.9%, respectively, when compared to
the natural biomass. Similarly for raspberry seeds decreases were 89.5%, 94.4%, 89.3% for Zn(II), Cu(II) and Mn(II) enriched biomass, respectively. In case of Mg, decrease was 62.9% (81.2%), 67.9% (90.6%), 79.5% (80.4%) for Zn(II), Cu(II) and Mn(II) enriched biomass, respectively, for strawberry seeds (and raspberry). For Ca decrease after biosorption was smaller – 8.42% (55.1%), 12.4% (76.6%), 3.0% (39.2%) for strawberry seeds (and raspberry). Content of S was 32.6% (22.5%), 34.1% (2.63%), 38.2% (28.3%) lower for Zn(II), Cu(II) and Mn(II) enriched biomass, for strawberry seeds (and raspberry), respectively.

During biosorption process, mainly light metal cations (K(I), Mg(II), Ca(II)) which are bound with functional groups (presented on the surface), were exchanged with micronutrient cations (Davis et al., 2003). In conducted biosorption process of Zn(II), Cu(II) and Mn(II) on strawberry and raspberry seeds, mainly K, Mg, Ca and S ions took part in ion exchange.
Table 2. Multielemental composition of natural and enriched witch micronutrients strawberry and raspberry seeds biomass

<table>
<thead>
<tr>
<th>Element</th>
<th>S - natural</th>
<th>S + Zn</th>
<th>S + Cu</th>
<th>S + Mn</th>
<th>R - natural</th>
<th>R + Zn</th>
<th>R + Cu</th>
<th>R + Mn</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>43.6</td>
<td></td>
<td>5134</td>
<td></td>
<td>151</td>
<td>59.1</td>
<td>34.6</td>
<td>4776</td>
</tr>
<tr>
<td>Cu</td>
<td>13.5</td>
<td>12.9</td>
<td><strong>12611</strong></td>
<td>87.3</td>
<td>8.96</td>
<td>9.09</td>
<td><strong>9578</strong></td>
<td>71.5</td>
</tr>
<tr>
<td>Mn</td>
<td>85.8</td>
<td>32.8</td>
<td>25.6</td>
<td><strong>5027</strong></td>
<td>75.9</td>
<td>18.8</td>
<td>14.0</td>
<td><strong>2484</strong></td>
</tr>
<tr>
<td>Fe</td>
<td>152</td>
<td>131</td>
<td>118</td>
<td>117</td>
<td>122</td>
<td>101</td>
<td>184</td>
<td>113</td>
</tr>
<tr>
<td>Mo</td>
<td>2.34</td>
<td>1.57</td>
<td>2.11</td>
<td>2.37</td>
<td>6.54</td>
<td>2.14</td>
<td>4.41</td>
<td>3.37</td>
</tr>
<tr>
<td>Ni</td>
<td>1.63</td>
<td>2.34</td>
<td>&lt;0.03</td>
<td>0.04</td>
<td>&lt;0.03</td>
<td>0.46</td>
<td>&lt;0.03</td>
<td>1.23</td>
</tr>
<tr>
<td>P</td>
<td>2780</td>
<td>1434</td>
<td>1298</td>
<td>1784</td>
<td>1551</td>
<td>757</td>
<td>797</td>
<td>1056</td>
</tr>
<tr>
<td>K</td>
<td>3353</td>
<td>672</td>
<td>555</td>
<td>1715</td>
<td>2767</td>
<td>290</td>
<td>156</td>
<td>295</td>
</tr>
<tr>
<td>S</td>
<td>2199</td>
<td>1482</td>
<td>1450</td>
<td>1360</td>
<td>1407</td>
<td>1090</td>
<td>1370</td>
<td>1009</td>
</tr>
<tr>
<td>Ca</td>
<td>5594</td>
<td>5123</td>
<td>4899</td>
<td>5424</td>
<td>2502</td>
<td>1123</td>
<td>585</td>
<td>1521</td>
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<tr>
<td>Mg</td>
<td>2464</td>
<td>915</td>
<td>791</td>
<td>505</td>
<td>1802</td>
<td>339</td>
<td>170</td>
<td>354</td>
</tr>
<tr>
<td>Na</td>
<td>615</td>
<td>674</td>
<td>333</td>
<td>349</td>
<td>472</td>
<td>622</td>
<td>630</td>
<td>787</td>
</tr>
<tr>
<td>Al</td>
<td>40.0</td>
<td>50.4</td>
<td>51.0</td>
<td>61.8</td>
<td>57.8</td>
<td>28.4</td>
<td>44.2</td>
<td>35.9</td>
</tr>
<tr>
<td>Se</td>
<td>19.4</td>
<td>8.85</td>
<td>10.0</td>
<td>6.08</td>
<td>7.40</td>
<td>&lt;0.5</td>
<td>1.14</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Si</td>
<td>253</td>
<td>289</td>
<td>365</td>
<td>364</td>
<td>130</td>
<td>153</td>
<td>170</td>
<td>175</td>
</tr>
<tr>
<td>Ti</td>
<td>2.26</td>
<td>2.60</td>
<td>3.66</td>
<td>2.88</td>
<td>8.59</td>
<td>9.63</td>
<td>3.60</td>
<td>3.35</td>
</tr>
<tr>
<td>V</td>
<td>3.28</td>
<td>1.69</td>
<td>1.54</td>
<td>2.05</td>
<td>1.50</td>
<td>1.88</td>
<td>0.894</td>
<td>2.59</td>
</tr>
<tr>
<td>Cd</td>
<td>0.574</td>
<td>0.772</td>
<td>0.799</td>
<td>0.412</td>
<td>0.830</td>
<td>0.245</td>
<td>0.532</td>
<td>0.407</td>
</tr>
<tr>
<td>Ni</td>
<td>1.63</td>
<td>2.34</td>
<td>&lt;0.03</td>
<td>0.0414</td>
<td>&lt;0.03</td>
<td>0.465</td>
<td>&lt;0.03</td>
<td>1.23</td>
</tr>
<tr>
<td>As</td>
<td>&lt;0.3</td>
<td>&lt;0.3</td>
<td>&lt;0.3</td>
<td>8.87</td>
<td>&lt;0.3</td>
<td>&lt;0.3</td>
<td>&lt;0.3</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td>Pb</td>
<td>4.50</td>
<td>8.59</td>
<td>4.14</td>
<td>7.63</td>
<td>&lt;0.5</td>
<td>0.629</td>
<td>19.0</td>
<td>2.50</td>
</tr>
<tr>
<td>Cr</td>
<td>1.16</td>
<td>5.63</td>
<td>0.423</td>
<td>1.82</td>
<td>0.117</td>
<td>1.87</td>
<td>0.616</td>
<td>0.596</td>
</tr>
</tbody>
</table>

S- post-extraction residues of strawberry seeds
R- post-extraction residues of raspberry seeds
3.2. FTIR analysis

FTIR spectra for natural and enriched with Zn(II), Mn(II) and Cu(II) ions post-extraction residues of strawberry and raspberry seeds are presented in Figure 1 and 3, respectively. Since FTIR curves have similar shape, we assumed that the same functional groups were involved in the biosorption process. In Figure 2 and 3, FTIR spectra for each biomass were presented, separately. Nature of the surface of analyzed biosorbents is very complex because number of absorption peaks was observed.

In the literature, different wavenumbers are assigned to cellular molecules, for example: lipid 1780–1708 cm\(^{-1}\), amide I: 1705–1575 cm\(^{-1}\), phosphoryl 1350–1190 cm\(^{-1}\), amide II 1575–1480 cm\(^{-1}\), carboxyl 1064–880 cm\(^{-1}\) (Stehfest et al., 2005). The biomass of raspberry and strawberry seeds has a number of functional groups from macromolecules such as: polysaccharides, fatty acid, amino acids, phenols etc. (Parry and Yu 2004; Oomach et al., 2000).

The comparison of FTIR peaks for natural and Mn, Cu and Zn loaded biomass with literature data is presented in Table 3. In comparison between natural and metal loaded strawberry and raspberry seeds, there are visible spectra transitions, which allow for identification of functional groups (Figure 2 and Figure 4). In this study, minor changes in the wavenumber for both investigated materials were detected for amine group. Smaller shifts were observed for carboxyl group, amide I band, phosphoryl and phosphine. This shows which functional groups were actually involved in the binding of microelement ions from the aqueous solution. Most of the peaks in FTIR spectra of Cu, Zn and Mn enriched biomass appeared at the similar wavelength.
Table 3. Comparison of FTIR peaks for natural, Mn, Cu and Zn loaded biomass

<table>
<thead>
<tr>
<th>Strawberry seeds</th>
<th>Raspberry seeds</th>
<th>Assigned group</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak at</td>
<td>Peak at</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak at 3339</td>
<td>Peak at 3390</td>
<td>Amine</td>
<td>3200-3500 (Lambert et al., 1987; Feride et al., 2012)</td>
</tr>
<tr>
<td>341</td>
<td>3390</td>
<td></td>
<td></td>
</tr>
<tr>
<td>314</td>
<td>3390</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-*</td>
<td>-*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn 9</td>
<td>Zn 342</td>
<td>Amine</td>
<td></td>
</tr>
<tr>
<td>Cu 2</td>
<td>Cu 341</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mn 8</td>
<td>Mn 341</td>
<td></td>
<td></td>
</tr>
<tr>
<td>321</td>
<td>341</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3307</td>
<td>341</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn 342</td>
<td>Zn 342</td>
<td>Carboxylic</td>
<td>2900-3300 (Michalak et al., 2013)</td>
</tr>
<tr>
<td>Cu 0</td>
<td>Cu 341</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mn 6</td>
<td>Mn 341</td>
<td></td>
<td></td>
</tr>
<tr>
<td>321</td>
<td>341</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3307</td>
<td>341</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak at 2928</td>
<td>Peak at 2928</td>
<td>CH₃ of saturated CH primarily from lipids</td>
<td>3000-2800 (Stehfest et al., 2005)</td>
</tr>
<tr>
<td>292</td>
<td>292</td>
<td>P-H phosphines</td>
<td>2280-2410 (Lambert et al., 1987)</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak at 2856</td>
<td>Peak at 2856</td>
<td>C=O strech of COOH, C=O of fatty acids</td>
<td>1740 (Fourest and Volesky 1996), 1780-1708 (Stehfest et al., 2005)</td>
</tr>
<tr>
<td>285</td>
<td>285</td>
<td>Amide I band (with proteins)</td>
<td>1705-1575 (Stehfest et al., 2005; Lambert et al., 1987; Feride et al., 2012)</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak at 2341</td>
<td>Peak at 2341</td>
<td>CH₃ of proteins</td>
<td>1455 (Stehfest et al., 2005)</td>
</tr>
<tr>
<td>236</td>
<td>236</td>
<td>N-H of amides associated with proteins amide II band</td>
<td>1575-1480 (Stehfest et al., 2005; Feride et al., 2012)</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak at 1650</td>
<td>Peak at 1650</td>
<td>C-O of COO⁻ carboxylic group</td>
<td>~1400 (Dmytryk et al., 2014; Chojnacka et al., 2005)</td>
</tr>
<tr>
<td>151</td>
<td>151</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak at 1318</td>
<td>Peak at 1318</td>
<td>P=O of phosphoryl</td>
<td>1350-1188 (Stehfest et al., 2005; Feride et al., 2012)</td>
</tr>
<tr>
<td>131</td>
<td>131</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak at 13135</td>
<td>Peak at 13135</td>
<td></td>
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<tr>
<td>-</td>
<td>802</td>
<td>1</td>
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<td>1064-880 (Stehfest et al., 2005; Lambert et al., 1987)</td>
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<td></td>
<td>809</td>
<td>-</td>
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<td>803</td>
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* natural biomass
Fig. 1. Superposition of FTIR spectra for natural (black), enriched with Zn(II) (green), Cu(II) (pink) and Mn(II) ions (blue) strawberry seeds

Fig. 2. Peak designation for strawberry seeds FTIR spectra (natural – black, enriched with Zn(II) – green, Cu(II) ions – pink and Mn(II) ions – blue)
Fig. 3. Superposition of FTIR spectra for natural (black), enriched with Zn(II) (green), Cu(II) (pink) and Mn(II) ions (blue) raspberry seeds.

Fig. 4. Peak designation for strawberry seeds FTIR spectra (natural – black, enriched with Zn(II) – green, Cu(II) ions – pink and Mn(II) ions – blue)
3.3. Potentiometric titration

Biomass, after protonation, was titrated with 0.1 M NaOH. Titration curves were plotted for strawberry (Figure 5) and raspberry (Figure 8) seeds to identify the functional groups capable of cation exchange. 1st and 2nd derivative curves were analyzed – Fig. 6, 9 and 7, 10, respectively. The first derivative plots (Figures 6 and 9) consist of the midpoint of successive amounts of NaOH added (X_{add}) versus ΔpH/ΔX_{add}. Reading the location of each peak on the x-axis gave the number of acidic groups on the biomass surface (Murphy et al., 2007; Yalçın et al., 2012). From 2nd derivative curve, all crossing points with x-axis were chosen from the population of X_{add} values determined from 1st derivative. These points are probable X_{add} values for all measured pH at pK_a points. The titration curve with probable pK_a alignment, assumed from 1st and 2nd derivative, is presented in Figure 5 and 8 for strawberry and raspberry seeds, respectively. The pK_a value can directly give the approximate charged state of that functional group at a specific solution pH (Satyanarayana et al., 2012). Functional groups present on cell surface determined by potentiometric titration are presented in Table 4. Different functional groups participated in metal ions binding, depending on pH: 2–5 carboxyl group, pH 5–9 carboxyl and phosphate group, pH 9–12 phosphate and hydroxyl (or amine) group (Chojnacka et al., 2005). In this study, post-extraction residue of strawberry and raspberry seeds possessed several groups with distinguishable pK_a. Dominating groups were carboxyl, amine and hydroxyl. The biosorption process of Cu(II), Mn(II) and Zn(II) ions was carried out at pH 5. Under these conditions carboxyl groups were deprotonated and therefore available for metal ions. Hydroxyl and amino groups can play a significant role in metal binding at higher pH values (Yalçın et al., 2012). Concentration of available groups at pH 5 was 0.397 and 0.265 meq/g for raspberry and strawberry seeds, respectively. These values stays in correlation with biosorption capacity (q) of tested biomass for Zn(II)(R^2=0.923), Cu(II)(R^2=0.989) and Mn(II)(R^2=0.958) ions – Table 5.
### Table 4. Functional group assigned to assumed pK<sub>a</sub> from 1<sup>st</sup> and 2<sup>nd</sup> derivative

<table>
<thead>
<tr>
<th>Possible pK&lt;sub&gt;a&lt;/sub&gt; (raspberry seeds)</th>
<th>Possible pK&lt;sub&gt;a&lt;/sub&gt; (strawberry seeds)</th>
<th>Literature</th>
<th>Functional group</th>
</tr>
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<tr>
<td>3.481</td>
<td>3.337</td>
<td>2–4</td>
<td>Sulphate, nitrate</td>
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<td>-</td>
<td>3.152</td>
<td></td>
<td>Terminal carboxyl</td>
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<td>4.056</td>
<td>4.232</td>
<td>3.1</td>
<td>Carboxyl</td>
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<td>4.601</td>
<td>4.454</td>
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<td>5.697</td>
<td>5.834</td>
<td>6–7</td>
<td>Thiol</td>
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<td>7.279</td>
<td>7.764</td>
<td>7</td>
<td>Phosphoryl</td>
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<td>9.187</td>
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<td>10.144</td>
<td>10.382</td>
<td>9–12</td>
<td>Amine and hydroxyl</td>
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<td>10.865</td>
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<td>11.351</td>
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### Table 5. Comparison of biosorption capacity and X<sub>ad</sub,d at pH 5

<table>
<thead>
<tr>
<th>Parameter</th>
<th>X&lt;sub&gt;ad&lt;/sub,d at pH 5</th>
<th>q based on Zn(II)</th>
<th>q based on Mn(II)</th>
<th>q based on Cu(II)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>(meq/g)</td>
<td>(meq/g)</td>
<td>(meq/g)</td>
<td>(meq/g)</td>
</tr>
<tr>
<td>Strawberry</td>
<td>0.397</td>
<td>0.157</td>
<td>0.183</td>
<td>0.397</td>
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<tr>
<td>Raspberry</td>
<td>0.265</td>
<td>0.146</td>
<td>0.090</td>
<td>0.302</td>
</tr>
<tr>
<td>R&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.923</td>
<td>0.958</td>
<td>0.989</td>
<td></td>
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</table>
Fig. 5. Titration curve of protonated strawberry seeds with probable $pK_a$ alignment, assumed from 1\textsuperscript{st} and 2\textsuperscript{nd} derivative of results.

Fig. 6. First derivative potentiometric titration curve of protonated strawberry seeds.
Fig. 7. Second derivative potentiometric titration curve of protonated strawberry seeds

Fig. 8. Titration curve of protonated raspberry seeds with probable pK<sub>a</sub> alignment, assumed from 1<sup>st</sup> and 2<sup>nd</sup> derivative of results
Fig. 9. First derivative potentiometric titration curve of protonated raspberry seeds

Fig. 10. Second derivative potentiometric titration curve of protonated raspberry seeds
4. Conclusion

In this study, the surface chemical functional groups of new biosorbent (post-extraction residues of strawberry and raspberry seed) were identified. This by-product of supercritical extraction of berries seeds has a potential to be applied as a component of fertilizers. This approach fits the sustainable waste management that encourages the recycling and recovery of waste that is produced. The role of the surface chemistry of the biomass in biosorption process was investigated using FTIR and potentiometric titrations.

It was shown that both types of biomass had good biosorption capacity, especially for Cu(II) ions. The content of copper in the enriched biomass was 2 times higher than zinc and manganese in the case of strawberry seeds, 5 times higher for manganese and more than two times higher for zinc in the case of raspberry seeds. Potentiometric titration and FTIR technique showed that carboxyl, hydroxyl and amino groups were dominating on the surface of the biosorbent. While the biosorption was carried out in pH 5, carboxyl groups participated mainly in the biosorption process. ICP-OES analysis of the biomass confirmed that the main mechanism of the biosorption was ion exchange of micronutrients with K(I), Ca(II), Mg(II) ions.

Acknowledgements

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References


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