Identification and Quantification of Phenolic Compounds from Red Currant (Ribes rubrum L.) and Raspberries (Rubus idaeus L.)

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Abstract. The extracts obtained from two types of berries: red currant and raspberries, were evaluated for their phenolic content. They were identified and quantified by using an optimized HPLC method. During the analysis several phenolic compounds were found, like: gallic acid, (+)-catechin, syringic acid, cinnamic acid, chlorogenic acid, ferulic acid, rutin, and quercetin. The total amount of phenolic compounds analyzed found in red currant was greater than the one found in raspberries, due to the low variety of phenolic compounds extracted. The greatest amount of gallic acid, (+)-catechin, syringic acid, cinnamic acid, chlorogenic acid, ferulic acid and rutin was determined from the extraction of red currant berries and the raspberries extract contained the greatest source of quercetin. This study shows that red currant can provide the highest and most varied content of phenolic compounds from the analyzed berries.

Introduction

Nowadays the exploitation of bioactive compounds from vegetable products is of great concern all over the world. These compounds have found many applications in different fields of interest.

Raspberries (Rubus idaeus L.) (Fig. 1) and red currant (Ribes rubrum L.) (Fig. 2) are berries widely spread all over Europe [1] and they are known for their food and flavoring qualities alongside with their involvement in preserving human health [2, 3], due to their important content of phenolic compounds [4].

Red currant has beneficial effects when consumed regularly. It has been shown that these berries reduce the incidence of several diseases as hypertension and other cardiovascular diseases, osteoporosis, diabetes, cancer and inflammatory diseases [5].

Raspberries are known for their antitumoral, antibacterial, antiinflammatory [6,7] and antioxidant activities [8].

It has been shown that the most health benefits of these berries are related to the content in phenolic compounds [4].

Phenolic compounds protect cells against damage due to their antioxidative, anti-inflammatory and anticarcinogenic activities. [9, 10].

The phenolic compounds are divided in different classes based on their chemical structures. Phenolic acids include hydroxybenzoic acids (gallic acid, syringic acid) and hydroxycinnamic acids (caffeic acid, cinnamic acid, chlorogenic acid, ferulic acid). Flavonoids include flavonols (quercetin, rutin), flavanones, flavanols (catechin), flavonones, anthocyanins and isoflavones. The most representative stilbene is resveratrol. Plants rich in these active principles have antioxidant effects and are widely used nowadays for their health beneficial properties [11].

Scientists are most interested in the detailed chemical composition and the potential health benefits of these berries [5, 12].
The analyzed phenolic compounds have different applications concerning the preservation of human health. For example, the gallic acid has antihyperglycaemic, anti-tumoral, anti-microbial, cardio protective and neuroprotective activities [13-17]. (+)-Catechin has implications in cardiovascular diseases, diabetes and it protects the skin against UVB radiation [18-20]. The syringic acid has antibacterial, anti-steatotic, anti-inflammatory and neuroprotective properties [21-23], the cinnamic acid has anti-tumoral properties and it has been proven that it improves the vascular function [24-25]. The chlorogenic acid has antihypertensive effects alongside with the improvement of the vessel function and the protection of the endothelial cells against oxidative damage and neuroprotective effects [26-28]. The ferulic acid prevents Alzheimer's disease, has antitumoral properties as well as antimicrobial and anti-inflammatory properties [29-31]. Quercetin has protective effects against retinal degeneration, anti-tumoral and antihyperglycaemic properties alongside with the neuroprotective and cardio protective effects [32-35] and rutin has the ability to decrease glucose concentrations in plasma, presents anti-inflammatory, anti-tumoral and neuroprotective effects [36-38].

All these phenolic compounds have great beneficial effect concerning human health. Their identification and quantification in raspberries and red currant is a matter of great interest due to the fact that these berries are widely consumed all around the world.

**Materials and methods**

**Sample preparation**

The berries were harvested from Sibiu County, Romania. They were frozen and stored at -20°C. Before the analysis they were defrosted, dried at 40°C and grounded on a domestic mill [7].

The extraction was performed by adding 10 mL of solvent methanol and purified water 70:30 (V/V) to 500 mg of berries. Covered and put in an ultrasound bath at 40°C for 30 minutes. The supernatant layer was centrifuged at 5000 rpm for 10 minutes, and the resulting supernatant layer was brought to dryness. The residue was dissolved in 10 mL of solvent, filtered a brought to volume to 10 mL using the same solvent [39].

**Phenolic profile**

The quantitative and qualitative analysis of phenolic compounds was carried out on a Agilent Technologies 1200 series HPLC system, equipped with degasser, quaternary pump, diode array detector, thermostated autosampler and thermostated column compartment. The column used was Zorbax Eclipse Plus C18 (250 mm x 4.6 mm i.d. x 5µm), at controlled temperature of 25°C. The elution was performed using purified water (mobile phase A), methanol (mobile phase B) and purified water and glacial acetic acid 96:4 (V/V) (mobile phase C). The gradient program was used as follows: 0 min: 15% B and 85% C, 15 min: 75% A and 25% B, 20 min: 15% A and 85% B, 40 min: 40% A and 60% B, 45 min: 5% A and 95% B, 55 min: 5% A and 95% B, 60 min: 85% A and 15% B and 70 min: 85% A and 15% B. The flow rate program was used as follows: 0 min: 0.5 mL/min and 15 → 70 min: 0.8 mL/min. The injection volume was 5 µL and the detection was
performed at 280, 303, 330 and 360 nm [39]. The standards of gallic acid, ferulic acid, syringic acid, cinnamic acid, chlorogenic acid, caffeic acid, (+)-catechin, resveratrol, quercitin, rutin were purchased from Sigma Aldrich, at HPLC purity.

Results and discussions

The detection of the phenolic compounds was performed at different wavelengths because of the absorption maxima determined for each analyzed compound. Four wavelengths were determined to be suitable for the ten compounds analyzed: 280 nm for gallic acid, syringic acid, (+)-catechin and cinnamic acid, 303 nm for resveratrol, 330 nm for ferulic acid, caffeic acid and chlorogenic acid, and 360 nm for rutin and quercitin [39] (Fig. 3, 4).

The highest amount of phenolic compounds was found in red currant, the total amount reached was 94.43 mg / 100 g dry weight (d.w.) and for raspberries 5.79 mg / 100 g d.w. (Tables 1,2). The amount of phenolic compounds found in red currant shows a possible increase of effects in comparison with the raspberries due to the higher quantity of phenolic compounds [40].

Red currant had the highest amount of different compounds like rutin (8.50 mg/100g d.w.), cinnamic acid (0.32 mg/100g d.w.), ferulic acid (1.00 mg/100g d.w.), chlorogenic acid (3.63 mg/100g d.w.) syringic acid (31.65 mg/100g d.w.), (+)-catechin (42.43 mg/100g d.w.) and gallic acid (6.90 mg/100g d.w.). The highest amount of quercetin (1.52 mg/100g d.w.) was found in raspberries (Tables 1, 2).

**Table 1. Phenolic compounds from red currant**

<table>
<thead>
<tr>
<th>Standard</th>
<th>Sample area (mAU*s)</th>
<th>Standard area (mAU*s)</th>
<th>Sample mass (mg)</th>
<th>Standard mass (mg)</th>
<th>Standard concentration %</th>
<th>mg phenolic compounds/100 g d.w.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercetin</td>
<td>0</td>
<td>371.7557</td>
<td></td>
<td>5.19</td>
<td>95</td>
<td>0.00</td>
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<tr>
<td>Rutin</td>
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<td>225.8515</td>
<td></td>
<td>6.34</td>
<td>94</td>
<td>8.50</td>
</tr>
<tr>
<td>Gallic acid</td>
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<td>5.79</td>
<td>98.5</td>
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<tr>
<td>Syringic acid</td>
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<td>5.45</td>
<td>95</td>
<td>31.65</td>
</tr>
<tr>
<td>Caffeic acid</td>
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<td></td>
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<td>98</td>
<td>0.00</td>
</tr>
<tr>
<td>Cinnamic acid</td>
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<td></td>
<td>5.68</td>
<td>99</td>
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<tr>
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<td></td>
<td>5.05</td>
<td>99</td>
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<tr>
<td>(+)-Catechin</td>
<td>109.55875</td>
<td>100.7379</td>
<td></td>
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<td>98</td>
<td>42.43</td>
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<tr>
<td>Resveratrol</td>
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<td>1058.129</td>
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<td>0.00</td>
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<tr>
<td>Chlorogenic acid</td>
<td>34.52472</td>
<td>356.8267</td>
<td></td>
<td>5.15</td>
<td>95</td>
<td>3.63</td>
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<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td>521.47</td>
<td></td>
<td></td>
<td><strong>94.43</strong></td>
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Figure 3. Chromatogram of red currant sample: A-280nm, B-303nm, C-330nm, D-360nm
1-gallic acid, 2-(+)-catechin, 3-syringic acid, 4-cinnamic acid, 5-chlorogenic acid, 6-ferulic acid, 7-rutin

Table 2. Phenolic compounds from raspberries

<table>
<thead>
<tr>
<th>Standard</th>
<th>Sample area (mAU*s)</th>
<th>Standard area (mAU*s)</th>
<th>Sample mass (mg)</th>
<th>Standard mass (mg)</th>
<th>Standard concentration %</th>
<th>mg phenolic compounds/100 g d.w.</th>
</tr>
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<tbody>
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<td>5.19</td>
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<td>5.79</td>
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<td>1.23</td>
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<td>5.21</td>
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</tr>
<tr>
<td>Cinnamic acid</td>
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<td>1117.151</td>
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<td>5.68</td>
<td>99</td>
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<td>5.05</td>
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<td>0.27</td>
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<tr>
<td>(+)-Catechin</td>
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<td>100.7379</td>
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<td>5.19</td>
<td>98</td>
<td>0.00</td>
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<tr>
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<td>Chlorogenic acid</td>
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<td></td>
<td>5.15</td>
<td>95</td>
<td>0.00</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>5.79</strong></td>
</tr>
</tbody>
</table>
Figure 4. Chromatogram of raspberry sample: A-280nm, B-303nm, C-330nm, D-360nm

1-gallic acid, 3-syringic acid, 6-ferulic acid, 8-quercetin

Resveratrol and caffeic acid were not detected neither in red currant or raspberries. Quercetin was not detected in red currant and rutin, cinnamic acid, (+)-catechin, and chlorogenic acid were not detected in raspberries.

Due to the fact that raspberries have a lower quantity and diversity of phenolic compounds than the red currant, the beneficial effects of raspberries for human health are diminished in comparison to red currant.

Conclusions

This study was conducted in order to identify and quantify phenolic compounds extracted from red currant and raspberries using ultrasounds, raised temperature, and methanol and purified water as solvents.

The highest quantity of total phenolic compounds analyzed was determined from red currant. Raspberries had a low quantity of phenolic compounds due to the many compounds that were not detected.

Red currant can provide the highest and most varied content of phenolic compounds from the analyzed berries.
References


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