Conventional and non-conventional extraction of anthocyanins from *Rubus idaeus* L. grown in Romania

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Abstract: Red raspberry – the fruit of *Rubus idaeus* L. represents an important plant food abundant in anthocyanins known for their pharmacological effects and strong antioxidant properties. The aim of the present paper was to investigate the optimum extraction conditions through conventional and ultrasound-assisted extraction UAE processes in order to recover high anthocyanins content in the hydroethanolic crude extract from red raspberries grown in Romania. Concentration of total anthocyanins was performed by the spectrophotometric pH differential method. The total anthocyanins content in red raspberry crude extracts obtained under UAE conditions was found similar to that obtained by conventional extraction, but the extraction time was significantly shorter. Total anthocyanins content was 40.84 mg 100g⁻¹ FM by conventional extraction taking 24 hours at low temperature, while a content of 36.56 mg 100g⁻¹ FM was obtained through the UAE process at an ultrasonic power of 200 W taking 20 minutes at room temperature. Reduced time of extraction through UAE is particularly favorable for the extraction of these thermolabile compounds.

Key-Words: raspberry, *Rubus idaeus* L., total anthocyanins, extraction, ultrasound-assisted extraction, pH differential

1 Introduction

Phytochemicals represent an important class of chemical compounds such as phenolics, carotenoids, alkaloids, vitamins, nitrogen and organosulfur compounds, which are largely distributed in plants and which provide human health-promoting and disease-preventing benefits.

Within the Rosaceae family, *Rubus* genus includes plants producing fruits (strawberries, raspberries, cherries, plums, rowans, hawthorns) known for their high content of bioactives showing a variety of biological and pharmacological effects supported by epidemiological studies [1-2]. Raspberry fruits are rich in flavonoids, in particular anthocyanins. Anthocyanins are water-soluble pigments which display beneficial properties based on their free-radical scavenging and antioxidant capacities [3].

Selection of the appropriate technique of extraction and analysis of anthocyanins from different samples becomes crucial for the recovery of a high content and for the preservation of the biological activity.

Several conventional and modern (non-conventional) extraction procedures have been described for phytochemicals, in particular anthocyanins, each of them being subjected to improved optimization by varying parameters such as solvent type, solvent concentration, liquid/solid ratio, time and temperature of extraction. Conventional extraction of anthocyanins is carried out commonly in acetone or acidified methanolic solutions in order to obtain the stable flavylium cation of red colour [4-5], but excess acid and/or methanol evaporation may cause partial hydrolysis of acylated anthocyanins [6]. Ultrasound-assisted extraction (UAE) has been applied to different phytochemicals as an economically efficient extraction method [7-8]. Anthocyanins, in particular those from grapes have been subjected to other modern extraction techniques, such as pressurized liquid extraction (PLE) and supercritical fluid extraction (SPE) [9-10] but with modest success, because these biomolecules are heat-sensitive and watersoluble.
The aim of the present paper was to compare conventional and UAE extraction of anthocyanins from red raspberry (*Rubus idaeus* L.) in terms of resulting recovery of total anthocyanins in the crude hydroethanolic extract. In order to investigate the optimal conditions for obtaining high total anthocyanins content from red raspberry varieties, various UAE conditions (extraction time, solvent/sample ratio) were experimented. Quantitative analysis of total anthocyanins in the selected samples was performed by UV-Vis spectroscopy.

2 Materials and Methods

2.1. Plant samples

Summer-bearing red raspberry varieties from spontaneous flora of Sibiu/Romania and red raspberry (*Rubus idaeus* L., cv. The Latham) from Dragomiresti/Romania cultivated field were used in the present investigation. Fresh samples were kept at – 18 °C until analyzed. Reducing sample size of plant material by grounding was performed before extraction.

2.2. Determination of moisture, refractive index and total soluble solids

Moisture content of fruit samples was determined at 105 °C using the A&D ML-50 moisture analyzer. Refractive index and total soluble solids (TSS) content was measured with an Abbe refractometer (Krüss AR2008) at a standardized temperature (21 °C) in fruit juices obtained by manually pressing. Values are expressed as refractometric TSS (°Brix).

2.3. Anthocyanins extraction

2.3.1. Conventional extraction

Crushed fruits of *Rubus idaeus* L., obtained by using a mortar and pestle were mixed with 70 % ethanol (v/v) at 4 °C overnight. Extraction was facilitated by occasional shaking. The obtained extracts were filtered and centrifuged at 8000 rpm, at 4 °C for 10 minutes. The NF800R refrigerated centrifuge (HT) was used.

2.3.2. Ultrasound-assisted extraction (UAE)

The ultrasound-assisted extraction (UAE) was carried out in an ultrasonic device (Elmasonic S60H) with an ultrasonic power of 200 W and frequency of 37 kHz, equipped with a digital timer and a temperature controller. Accurately weighed grounded samples of red raspberries were mixed with an appropriate amount of 70 % ethanol (v/v). The vials containing the samples in the selected solvent were immersed into water in the ultrasonic device, and irradiated for the predetermined extraction time and at various solvent/sample ratios, at 20 °C and 30 °C. The temperature was controlled and maintained at desired value by periodical ice addition in the water bath.

2.4. Total anthocyanins assay

The content of total anthocyanins in the obtained crude extracts was determined spectrophotometrically by the pH differential method [5]. Measurements were made in duplicate. The Specord 200Plus UV-Vis spectrophotometer (Analytik Jena) was used. Content of total anthocyanins was expressed as cyanidin-3-O-glucoside (Cyn-3-O-G) according to its molar extinction.

3 Results and Discussion

Conventional extraction

Anthocyanins occur naturally as glycosides, so that polar solvents are essential for the achievement of a good extraction yield. Despite the fact that non-acidified and acidified methanol has been the most efficient reported solvent for anthocyanin extraction, in the present investigation on red raspberry we have substituted it with the more eco-friendly solvent – 70 % (v/v) ethanol, in all extraction runs. The content of total anthocyanins were spectrophotometrically evaluated in two varieties of red raspberry from spontaneous flora and one cultivated (cv. The Latham).

The crude anthocyanin extracts were characterized for several physical chemical parameters as presented in Table 1.

High content of total anthocyanins was found in red raspberry samples collected from spontaneous flora compared to the cultivated one.
Table 1. Physical chemical attributes of the studied red raspberry samples/extracts.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Moisture (%)</th>
<th>Total soluble solids (°Brix)</th>
<th>Refractive index (n)</th>
<th>Total anthocyanins (mg 100g⁻¹ FM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red raspberry cv. The Latham (Rubus idaeus L.)</td>
<td>84.4</td>
<td>10.8</td>
<td>1.3483</td>
<td>27.03</td>
</tr>
<tr>
<td>Red raspberry 1 (spontaneous flora)</td>
<td>87.6</td>
<td>10.9</td>
<td>1.3495</td>
<td>41.52</td>
</tr>
<tr>
<td>Red raspberry 2 (spontaneous flora)</td>
<td>88.2</td>
<td>7.9</td>
<td>1.3447</td>
<td>40.84</td>
</tr>
</tbody>
</table>

Ultrasound-assisted extraction (UAE)

In this investigation, sample 2 of red raspberry fruits from the spontaneous flora was used for extraction of anthocyanins with 70 % ethanol (v/v). All UAE experiments were performed at 20 °C.

As solvent volumes highly influence the content of anthocyanins in crude extracts, the appropriate solvent/sample ratio was evaluated. The obtained results are presented in Figure 1.

![Fig. 1. Total anthocyanins (TA) content in red raspberry (Rubus idaeus L.) according to different solvent/sample ratio of ultrasound-assisted extraction, at 20 °C.](image)

The results indicate that 10/1 (v/w) and 15/1 (v/w) should be the appropriate solvent/sample ratios for achieving good anthocyanins extraction yield for short time (15 min).

As extraction time affects the extraction yield as well, we evaluated the appropriate UAE time in order to obtain an increase concentration of total anthocyanins in the crude extract prepared for a 10/1 (v/w) solvent/sample ratio. The obtained results are presented in Figure 2.

The obtained results indicate that there is about 15 % improvement in the total anthocyanins content due to the use of longer extraction time (20 min) compared to the other fast extractions through UAE, but a significant decrease in extraction time when compared to conventional extraction.

The increase of temperature to 30 °C in a longer time four point UAE experimental run (15 – 45 min) for a solvent/sample ratio of 20/1 (v/w) lead to a decrease of the extraction time in order to obtain high concentration of anthocyanins, as shown in Figure 3.
Fig. 2. Total anthocyanins (TA) content in red raspberry (*Rubus idaeus* L.) according to different times of ultrasound-assisted extraction, at 20 °C.

Fig. 3. Total anthocyanins (TA) content in red raspberry (*Rubus idaeus* L.) according to different times of ultrasound-assisted extraction, at 30 °C for a solvent/sample ratio of 20/1 (v/w).

The total anthocyanins content in red raspberry crude extracts obtained under UAE experiments was found similar to that obtained by conventional extraction, but extraction time was significantly reduced by approximately seventy-fold. Total anthocyanins in red raspberry were 40.84 mg 100g⁻¹ FM by conventional extraction taking 24 hours at low temperature, while through the UAE process at an ultrasonic power of 200 W it took 20 min to obtain 36.56 mg 100g⁻¹ FM (for 10/1 solvent/solid ratio). Reduced time of extraction through UAE is particularly favorable for the extraction of these thermolabile compounds.

As conventional extraction procedures of anthocyanins require longer extraction time which may lead to their decomposition in particular when elevated temperatures are employed, UAE technique proved to be a useful rapid tool for efficient extraction of these biomolecules.

Other reported studies focused on the UAE of red raspberry cv. Heritage and found optimized conditions to obtain a finally anthocyanins content in the purified extract of 34.5 mg 100g⁻¹ FM for much shorter time (about 3 minutes) but greater
plant material amounts (solvent/sample ratio of 4/1) at a higher power of 400 W [11]. The solvent used for the extraction in the reported study was acidified 95% ethanol.

However, comparison of levels of total anthocyanins from different reported data should be conducted with caution as reported values highly depend not only on the extraction strategies, but also on the applied quantitative analysis methods (spectrophotometric/HPLC, TA expression, etc.).

The obtained crude anthocyanin extracts from red raspberry may find further application in food supplemental or pharmaceutical products.

4 Conclusion
Extraction yields of total anthocyanins from red raspberry were investigated in the present research through conventional and UAE processes. We have substituted the most frequently used solvents such as methanol and acetone, to the more eco-friendly ethanol solution, as toxicity may interfere with the final purpose of the obtained crude extract (pharmaceutical and food applications).

The findings of this work show that increased anthocyanin recovery from red raspberry (Rubus idaeus L.) from spontaneous flora may be obtained under the following conditions: (1) discontinuous conventional extraction process at 4 °C, extraction time 24 hours, and protection against light, and more efficiently through (2) UAE process at an ultrasonic power of 200 W, at 20 °C, extraction time 20 min and a solvent/sample ratio of 10/1 (v/w). The used solvent in both type of experiments was 70% ethanol (v/v).

The overall conclusion is that UAE may find efficient application for extraction of total anthocyanins from Rubus idaeus L. fruits, as it resulted in similar yields as conventional extraction, but with great reduction of extraction time and at room temperature.

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References: