Extraction and separation of polyphenol classes in chokeberry and elderberry extracts

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Abstract

Chokeberry (Aronia melanocarpa) and elderberry (Sambucus nigra L.) fruits are one of the richest dietary sources of polyphenols, a natural bioactive compounds. Anthocyanins are the most prevalent polyphenols in these fruits although other classes such as phenolic acids, flavonols, and flavanols are present as well. Those phenolic subgroups can show different bioactivities. That is why it can often be appropriate to examine bioactivities of these subgroups separately. The aim of this study was to to extract and determine polyphenols from chokeberry and elderberry and to separate polyphenol subgroups present in the extracts. The extraction was conducted using 80 % methanol as a solvent with the help of ultrasonic bath, and repeating the extraction step four times. Polyphenol subgroups found in extracts were separated using gel chromatography fractionation with Sephadex LH-20 as a stationary phase and different percentages of methanol in water as a mobile phase. Polyphenols in extracts and fractions were analysed using reversed-phase high performance liquid chromatography (RP-HPLC). The amount of polyphenols extracted from chokeberries and elderberries was 15557 and 10009 mg/kg of fresh fruit weight, respectively. Four fractions were separated for each fruit. Flavanols were eluted in fraction 1 (100% and 100%) and phenolic acids in fraction 2 (100% and 100%), anthocyanins were dominant in fraction 3 (90 % and 49 %), while flavonols were prevalent in fraction 4 (57 % and 91 % ) for chokeberry and elderberry, respectively. Using gel-chromatography fractionation, four fractions containing different polyphenol subgroups were separeated which could be useful in further research.

Introduction

Chokeberry (Aronia melanocarpa) and elderberry (Sambucus nigra L.) fruits are one of the richest dietary sources of polyphenols, a natural bioactive compounds. Anthocyanins are the most prevalent polyphenols in these fruits although other classes such as phenolic acids, flavonols, and flavanols are present as well. Those phenolic subgroups can show different bioactivities. That is why it can often be appropriate to examine bioactivities of these subgroups separately. The aim of this study was to extract and determine polyphenols from chokeberry and elderberry and to separate polyphenol subgroups present in the extracts. The extraction was conducted using 80 % methanol as a solvent with the help of ultrasonic bath, and repeating the extraction step four times. Polyphenol subgroups found in extracts were separated using gel chromatography fractionation with Sephadex LH-20 as a stationary phase and different percentages of methanol in water as a mobile phase. Polyphenols in extracts and fractions were analysed using reversed-phase high performance liquid chromatography (RP-HPLC). The amount of polyphenols extracted from chokeberries and elderberries was 15557 and 10009 mg/kg of fresh fruit weight, respectively. Four fractions were separated for each fruit. Flavanols were eluted in fraction 1 (100% and 100%) and phenolic acids in fraction 2 (100% and 100%), anthocyanins were dominant in fraction 3 (90 % and 49 %), while flavonols were prevalent in fraction 4 (57 % and 91 % ) for chokeberry and elderberry, respectively. Using gel-chromatography fractionation, four fractions containing different polyphenol subgroups were separated which could be useful in further research.

Methodology

Ultrasonic-assisted extraction

Separation of polyphenol classes with Sephadex LH-20

RP-HPLC analysis

Recording UV-Vis Spectrum

Results

Amount of polyphenols (mg/kg of fresh fruit weight)

<table>
<thead>
<tr>
<th></th>
<th>Chokeberry</th>
<th>Elderberry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavanols</td>
<td>100 %</td>
<td>100 %</td>
</tr>
<tr>
<td>Phenolic acids</td>
<td>100 %</td>
<td>100 %</td>
</tr>
<tr>
<td>Anthocyanins</td>
<td>90 %</td>
<td>57 %</td>
</tr>
<tr>
<td>Flavonols</td>
<td>49 %</td>
<td>49 %</td>
</tr>
</tbody>
</table>

Conclusion

- Four fractions were separated for each fruit
- Flavanols were eluted in fraction 1
- Phenolic acids were eluted in fraction 2
- Anthocyanins were dominant in fraction 3
- Flavonols were prevalent in fraction 4

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