Genistein isoflavone glycoconjugates in sour cherry 
(*Prunus cerasus* L.) cultivars

László Abrankó\textsuperscript{a,b}, Ádám Nagy\textsuperscript{a}, Blanka Szilvássy\textsuperscript{a}, Éva Stefanovits-Bányai\textsuperscript{a}, Attila Hegedűs\textsuperscript{c}

\textsuperscript{a}Corvinus University of Budapest, Faculty of Food Science, Department of Applied Chemistry, 1118 Budapest, Villányi út 29-33.
\textsuperscript{b}Hungarian Academy of Sciences, Research Centre for Natural Sciences, Institute of Organic Chemistry, 1117 Budapest, Magyar tudósok körútja 2.
\textsuperscript{c}Corvinus University of Budapest, Faculty of Horticultural Science, Department of Genetics and Plant Breeding, 1118 Budapest Ménesi út 44.
Polyphenols: plant secondary metabolites

Flavonoids:
- Apigenin (flavone)
- Cyanin (anthocyanidin)
- Genistein (isoflavone)
- Trans-resveratrol (stilbene)
- Quercetin (flavonol)

Polyphenols:
- Caffeic acid (hydroxycinnamic acid)
- Gallic acid (benzoic acids)
Polyphenols: plant secondary metabolites

In plants:

- Pigments
- Stress defence system
  - UV irradiation,
  - wounding,
  - fungal infection

When consumed by human:

- Flavouring potential (astringency)
- **Health promoting effects:**
  - Anticarcinogenic effects
  - Cardiovascular protective effects
  - Anti-inflammatory effects
Importance of genistein

Genistein compounds show various health effects through multiple mechanisms of action

- Can improve lipid profile → lower blood pressure → cardiovascular protection (1)

- Potent *in vitro* growth inhibitor of various cancer cells → cancer prevention (2)

- Promising therapeutic agent in diabetes and obesity (3)

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Genistein in food

Genistein have been found almost exclusively in leguminous plants.

Highest levels in soy (*Glycine max*) and soy-based products.

~5.6 - 276 mg/100 g in raw mature soybeans (4)

~0.2 - 0.6 mg genistein and daidzein combined per 100 g (5)

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Background of the current work
Need for profiling of "colourless" antioxidants

- Flavonoids
- Phenolic acids
Methodology for the comprehensive profiling of polyphenols in fruit samples

High performance liquid chromatography (HPLC) – Time-of-flight mass spectrometry (TOFMS)

Main features of the technique:

- Elemental formulae ($C_xH_yO_z$) of unknown compounds.
- Structural information on intact polyphenol compounds.


Conjugated forms are predominant in plants
Conjugated forms are predominant in plants

Saccharides
• glucose
• rhamnose
• rutinose

Aglycone
Conjugated forms are predominant in plants

**Acids**
- phenolic acids
  - caffeic acid
  - ferulic acid
- aliphatic acids
  - acetic acid
  - malonic acid

**Saccharides**
- glucose
- rhamnose
- rutinose
Profiling the intact forms is essential in nutrition studies.

Quercetin-glucoside

Different bioavailability

Quercetin-glucosyl-rhamnoside (rutin)

LPH

Hydrolyzed to aglycone in small intestine

Microbial enzymes

Hydrolyzed to aglycone and smaller metabolites in colon

Structure elucidation of intact polyphenols based on the detection of their typical building blocks.

MS fragmentation

m/z 611.1607 → m/z 465.1028 → m/z 303.0500

neutral loss

146.0579 u

162.0528 u

neutral loss
TOF chromatogram of genistein profiling

Extracted ion chromatogram of m/z \textbf{271.0601} \pm 5\text{mDa}

Genistein aglycone
TOF chromatogram of genistein profiling

Extracted ion chromatogram of 433.1129 ± 5mDa

m/z 433.1129 genistein aglycone + hexoside

m/z 271.0601 Genistein aglycone
TOF chromatogram of genistein profiling

Extracted ion chromatogram of 433.1129 ± 5mDa

m/z 433.1129 genistein aglycone + hexoside

m/z 271.0601 Genistein aglycone

genistein ✅
TOF chromatogram of genistein profiling (zoom)

- m/z 271.0601
- m/z 433.1129
- m/z 595.1658

Gen + Hex + Hex

m/z 271.0601

m/z 433.1129

m/z 595.1658
TOF chromatogram of genistein profiling (zoom)

m/z 433.1129

Genistein-7-O-β-glucoside (genistin) ✅
## Concentrations of genistein compounds I.

(mg/100 g fresh weight)

<table>
<thead>
<tr>
<th>Variety</th>
<th>Year</th>
<th>Mass spectrometry (MS/MS)</th>
<th>UV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Genistin</td>
<td>Genistein</td>
</tr>
<tr>
<td>Oblachiskha</td>
<td>2009</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
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<tr>
<td>VN-7</td>
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<td>Érdi bőtermő</td>
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<td>Csengődi</td>
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<tr>
<td>Cigány404</td>
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<td>Korai pipacs</td>
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<tr>
<td>VN-4</td>
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<tr>
<td>Sárdy SF</td>
<td>2010</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
</tr>
</tbody>
</table>

*limit of quantification, LOQ, n.d. = not detected*
Concentrations of genistein compounds II.

(mg/100 g fresh weight)

<table>
<thead>
<tr>
<th>Variety</th>
<th>Year</th>
<th>Mass spectrometry (MS/MS)</th>
<th>UV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Genistin</td>
<td>Genistein</td>
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<tr>
<td>Pipacs1</td>
<td>2009</td>
<td>4.49±0.62</td>
<td>1.39±0.05</td>
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<tr>
<td>Kántorjánosi</td>
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<td>0.54±0.01</td>
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<td>Debreceni bőtermő</td>
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<td>0.52±0.01</td>
<td>0.06±0.001</td>
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<tr>
<td>Éva</td>
<td>2010</td>
<td>0.79±0.01</td>
<td>0.05±0.002</td>
</tr>
</tbody>
</table>

1.94<sup>a</sup>  
43.0<sup>b</sup>  
0.64<sup>a</sup>  
1.29<sup>c</sup>  
2.30<sup>d</sup>

<sup>a</sup> green soybean  

<sup>b</sup>  

<sup>c</sup> raw soybean sprout  

<sup>d</sup>  
Conclusions

- **Genistein** and **genistin (genistein-7-O-β-glucoside)** along with another **genistein-hexoside** and a **genistein-dihexoside** could be identified some sour cherry varieties.

- Quantification of **genistein**, **genistin (genistein-7-O-β-glucoside)** and **genistein-hexoside** could be also carried out.

- Considerable natural variation in the concentration of genistein compounds was observed among sour cherry cultivars.

- The highest levels (4.5 mg/100g f.w., genistin and 1.4 mg/100g f.w. genistein) were observed in **Pipacs1**.
Thank you for your attention!