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

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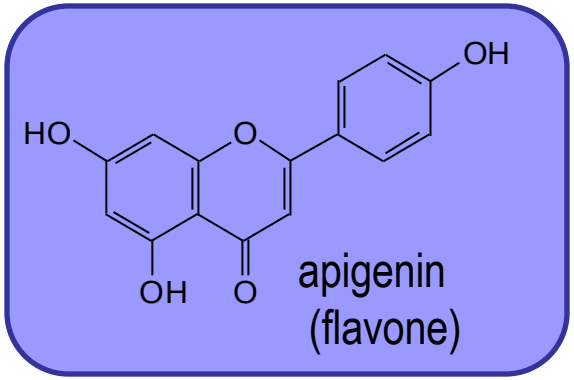
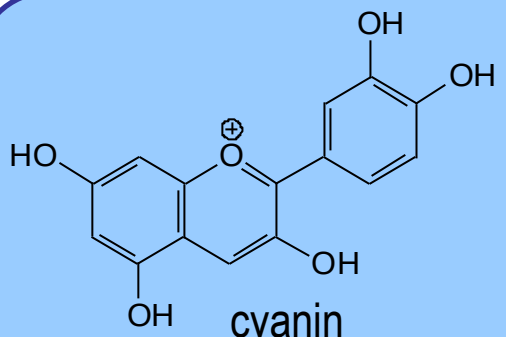
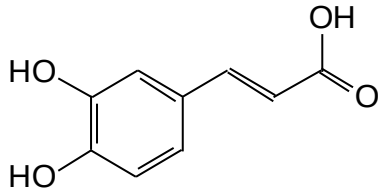
Polyphenols: plant secondary metabolites



apigenin
(flavone)

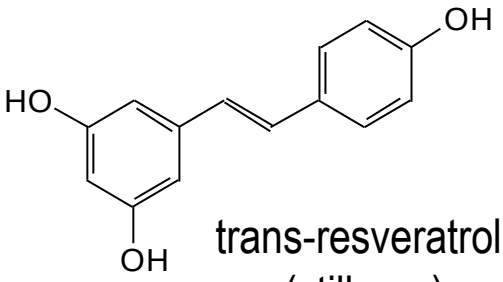


cyanin
(anthocyanidin)



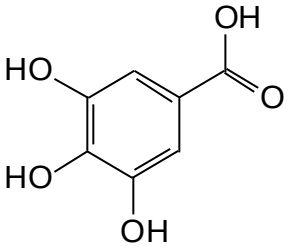
caffeic acid
(hydroxycinnamic acid)

Flavonoids

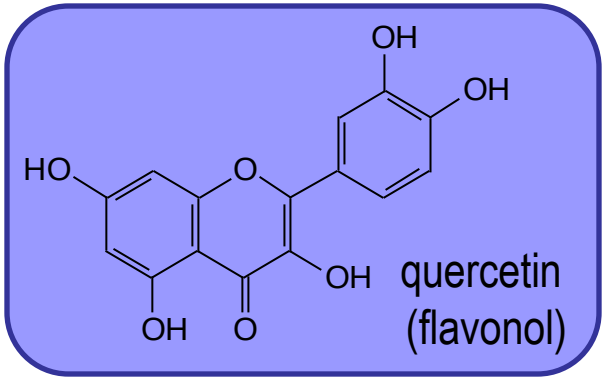


trans-resveratrol
(stilbene)

genistein
(isoflavone)



gallic acid
(benzoic acids)



quercetin
(flavonol)

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)

(1) Schwab, K., Stein, R., Scheler, C., & Theuring, F. (2012). Dietary genistein enhances phosphorylation of regulatory myosin light chain in the myocardium of ovariectomized mice. *Electrophoresis, 33(12), 1795-1803.*

(2) Li, W., Frame, L. T., Hirsch, S., & Cobos, E. (2010). Genistein and hematological malignancies. *Cancer Letters, 296(1), 1-8.*

(3) Behloul, N., & Wu, G. (2013). Genistein: A promising therapeutic agent for obesity and diabetes treatment. *European Journal of Pharmacology*, 698(1-3), 31-38.

Genistein in food

Genistein have been found almost exclusively in leguminous plants.

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

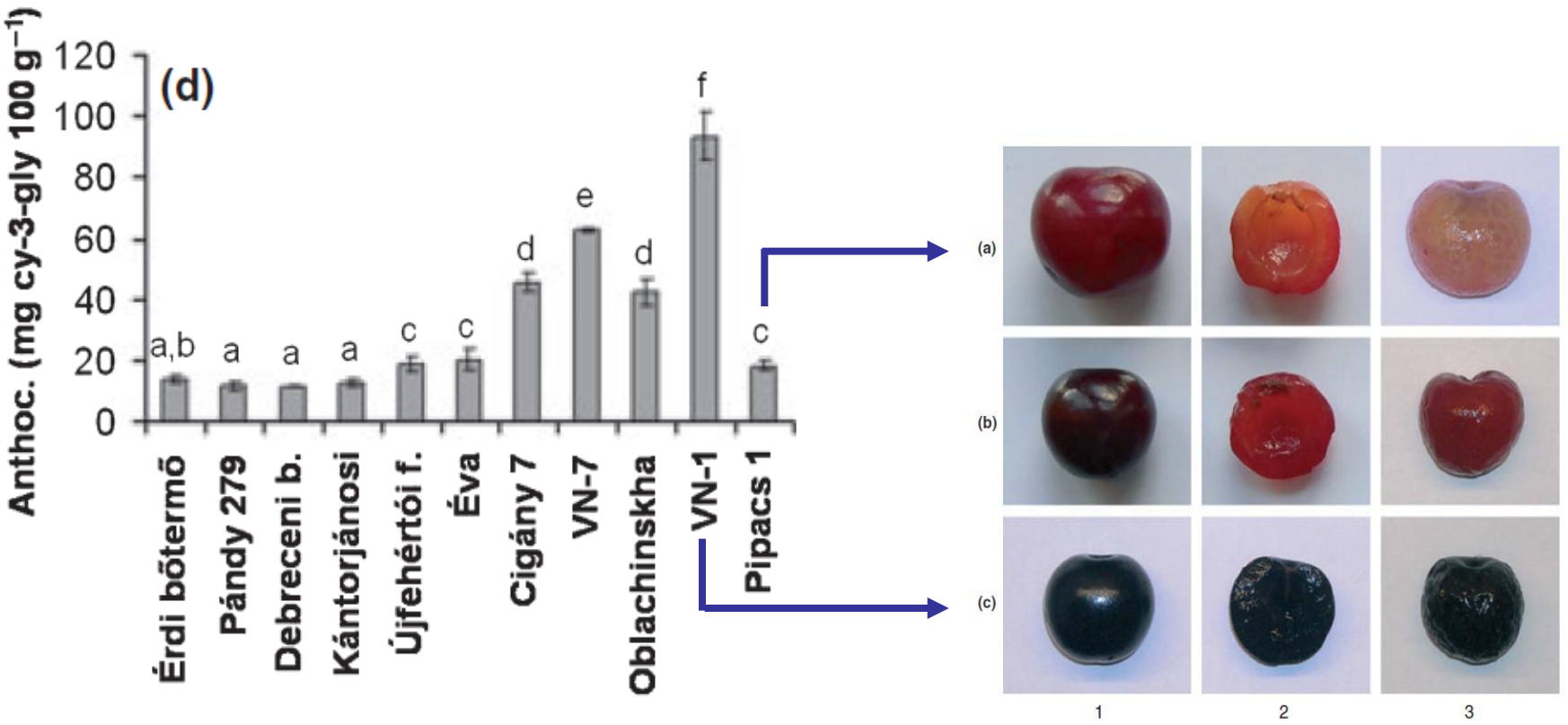
~5.6 - 276 mg/100 g in raw mature soybeans (4)

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

(4) Bhagwat, S., Haytowitz, D., & Holden, J. (2008). USDA Database for the Isoflavone Content of Selected Foods. In 2nd ed.). Beltsville, Maryland.: A. R. S. U.S. Department of Agriculture.

(5) Liggins, J., Bluck, L. J. C., Runswick, S., Atkinson, C., Coward, W. A., & Bingham, S. A. (2000). Daidzein and genistein contents of vegetables. British Journal of Nutrition, 84(5), 717-725.

Background of the current work



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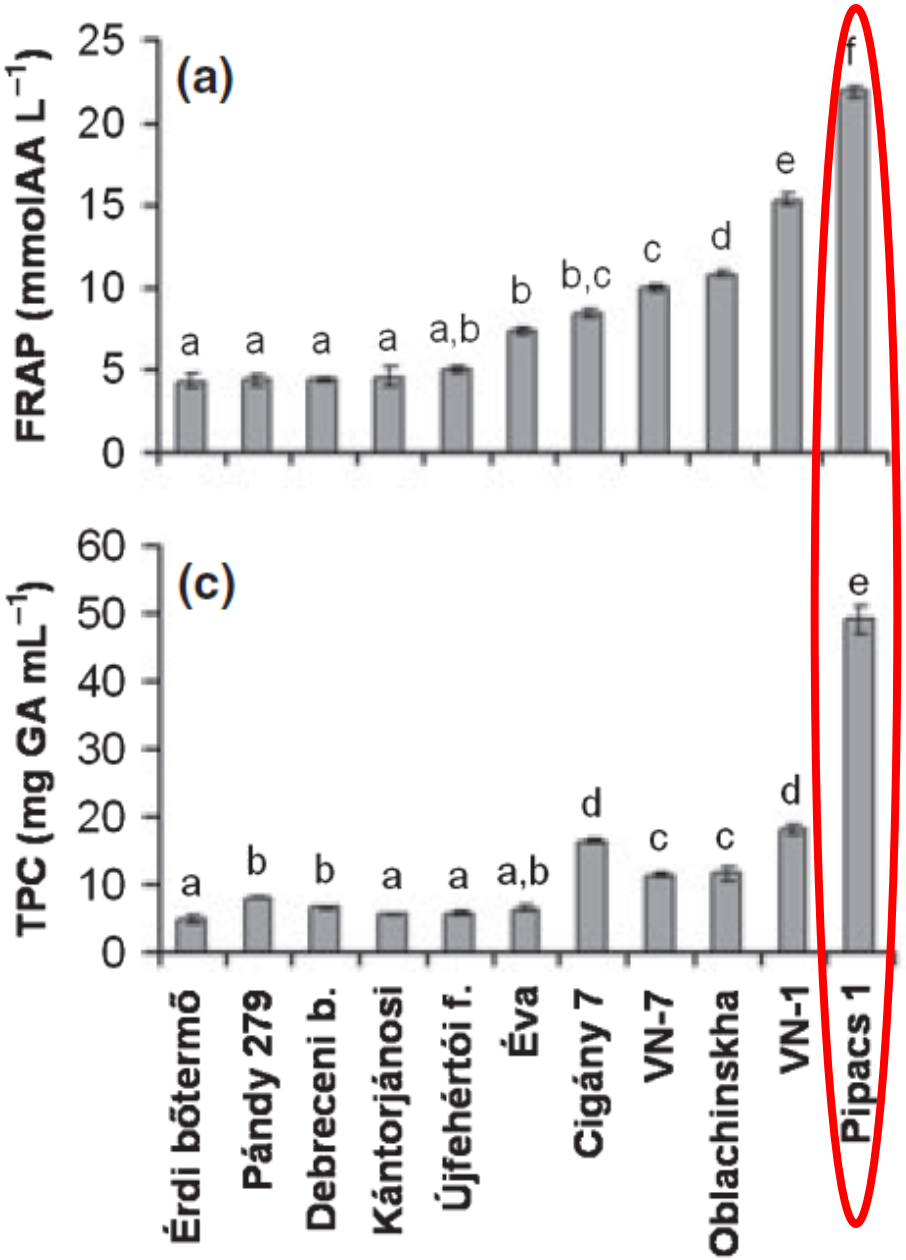


Need for profiling of „colourless" antioxidants

- **Flavonoids**
- **Phenolic acids**

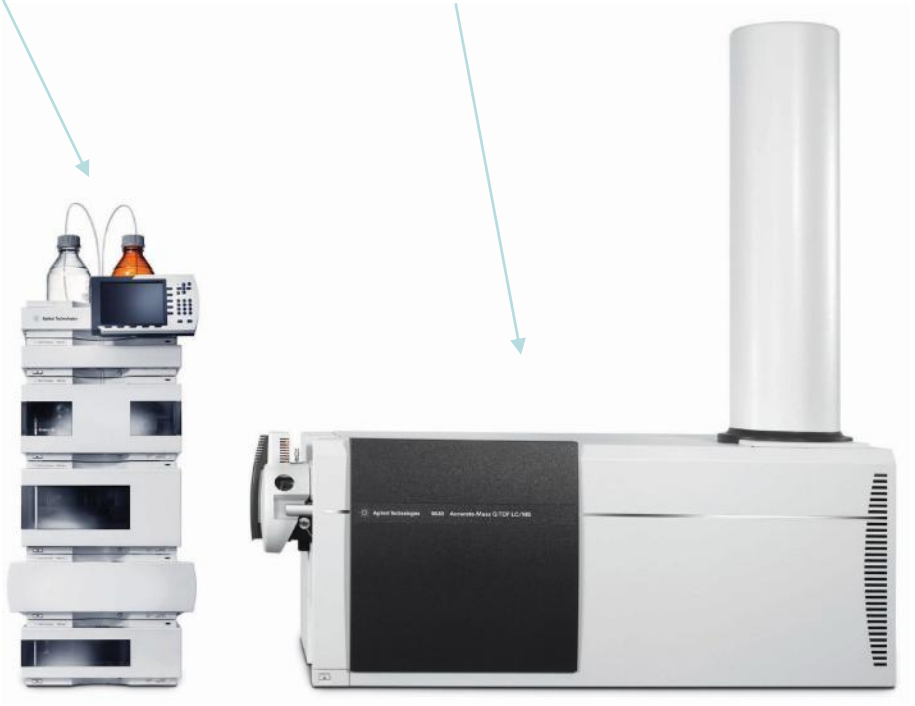
Methodology for the comprehensive profiling of polyphenols in fruit samples

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.

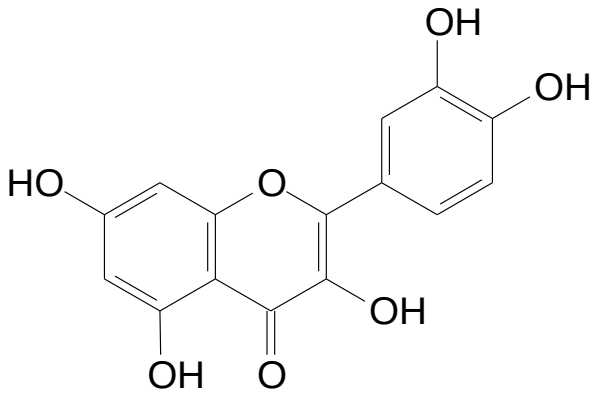


Rak, G.; Fodor, P.; Abrankó, L. *Int J Mass Spectrom*, **2010**, 290, 32-38.

Abrankó L., García-Reyes J.F., Molina-Díaz A., *J Mass Spectrom*, **2011** (5) 478-488

Conjugated forms are predominant in plants

Aglycone



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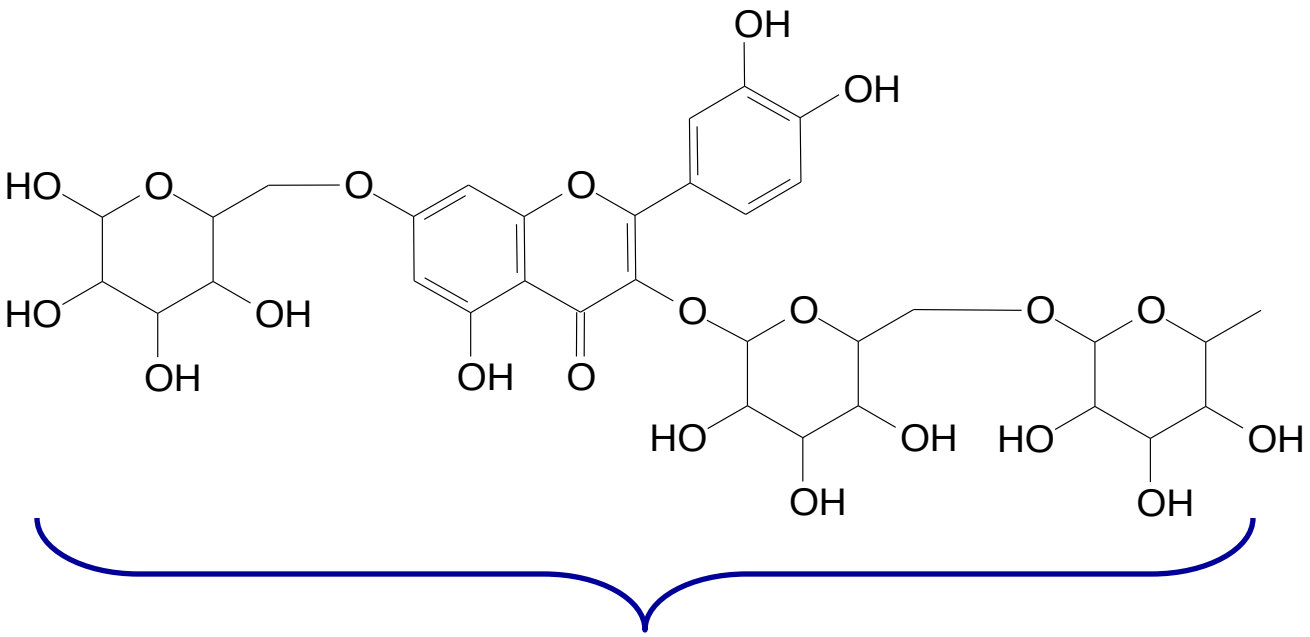
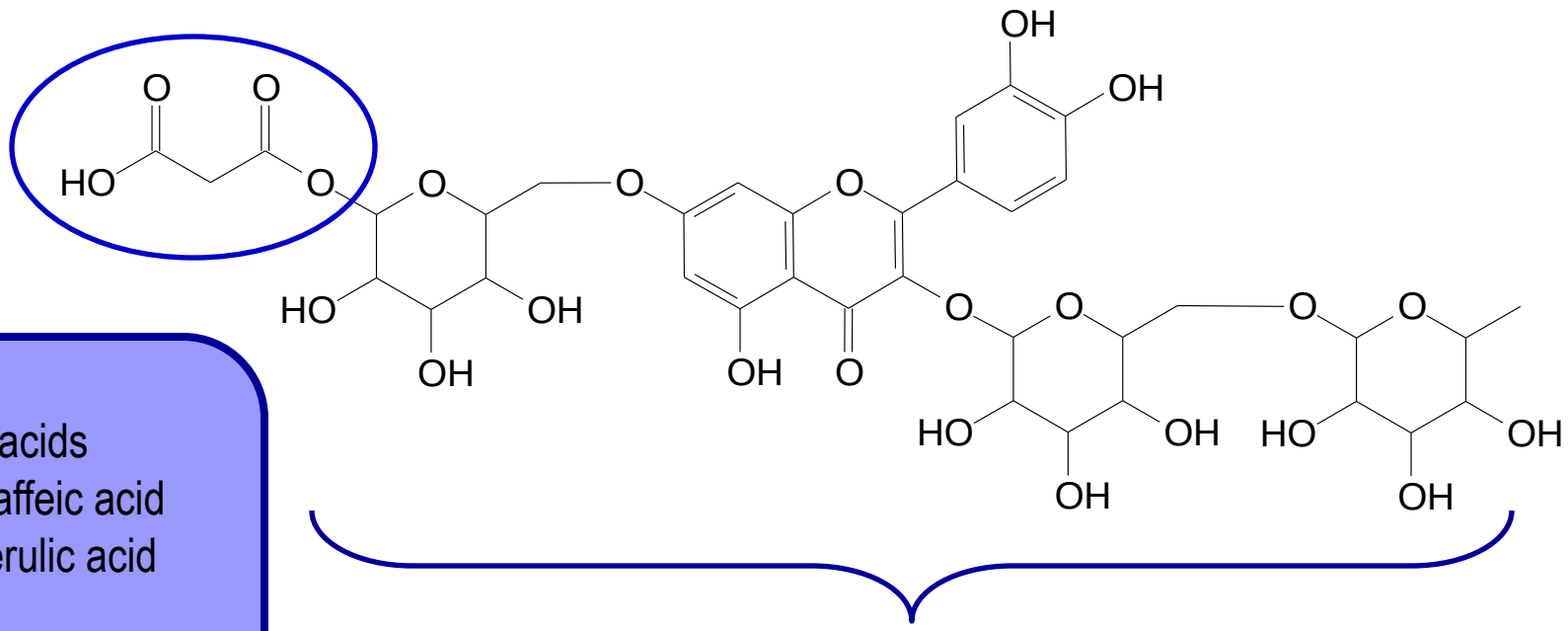


Saccharides
- glucose
- rhamnose
- rutinose

Conjugated forms are predominant in plants

Aglycone

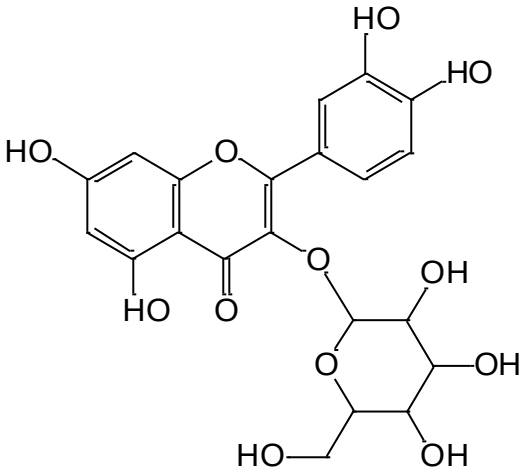


Acids
- phenolic acids
  - caffeic acid
  - ferulic acid
- aliphatic acids
  - acetic acid
  - malonic acid

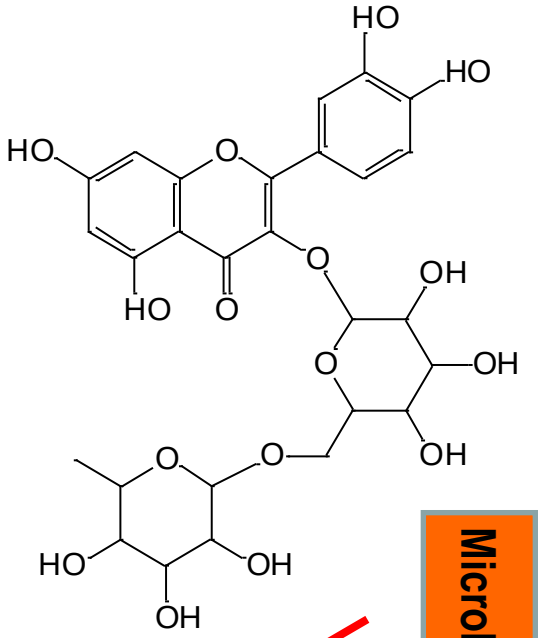
Saccharides
- glucose
- rhamnose
- rutinose

Profiling the intact forms is essential in nutrition studies

quercetin-glucoside



quercetin-glucosyl-rhamnoside (rutin)



Different bioavailability

LPH

hydrolyzed to aglycone in small intestine

LPH

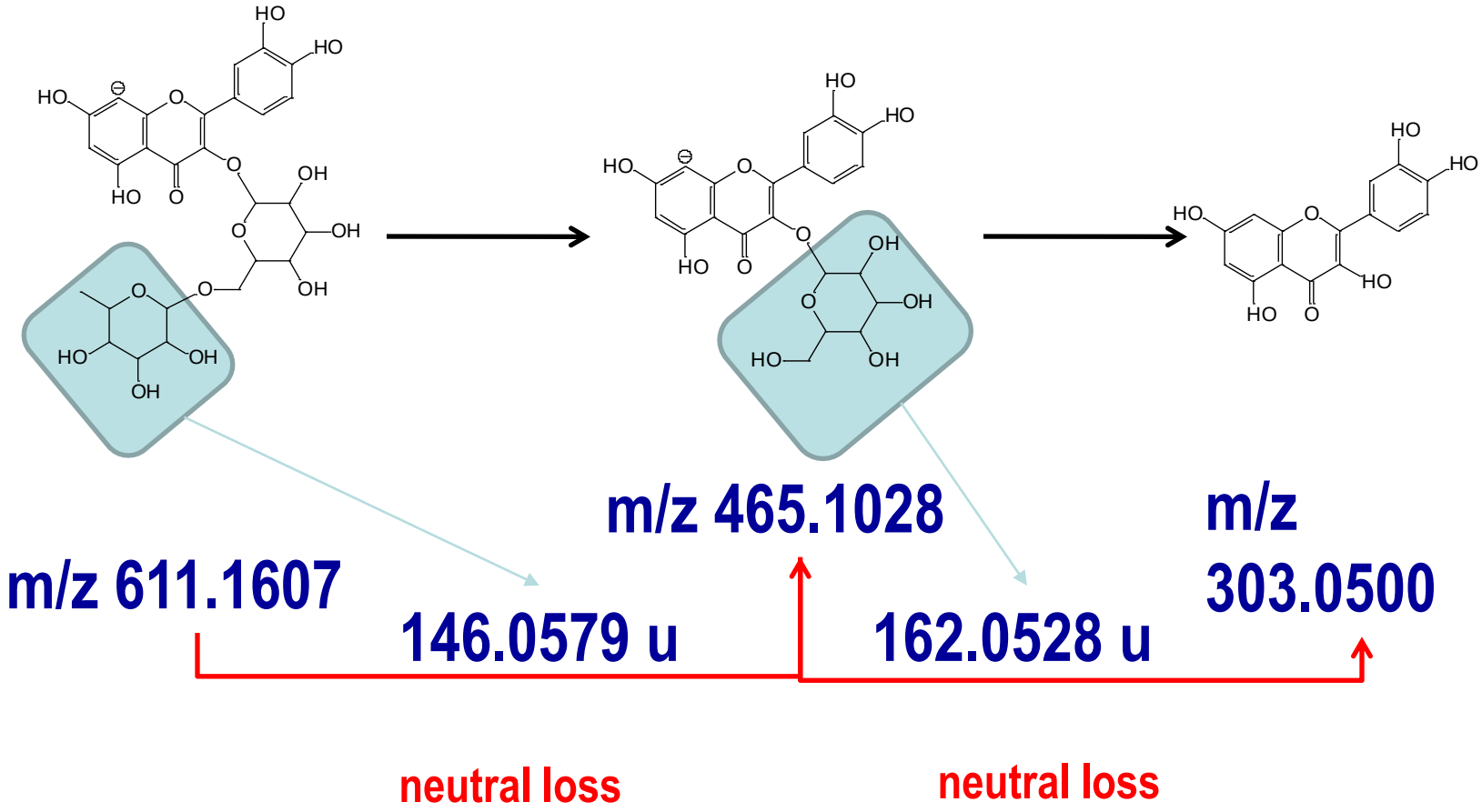
Microbial enzymes

hydrolyzed to aglycone and smaller metabolites in colon

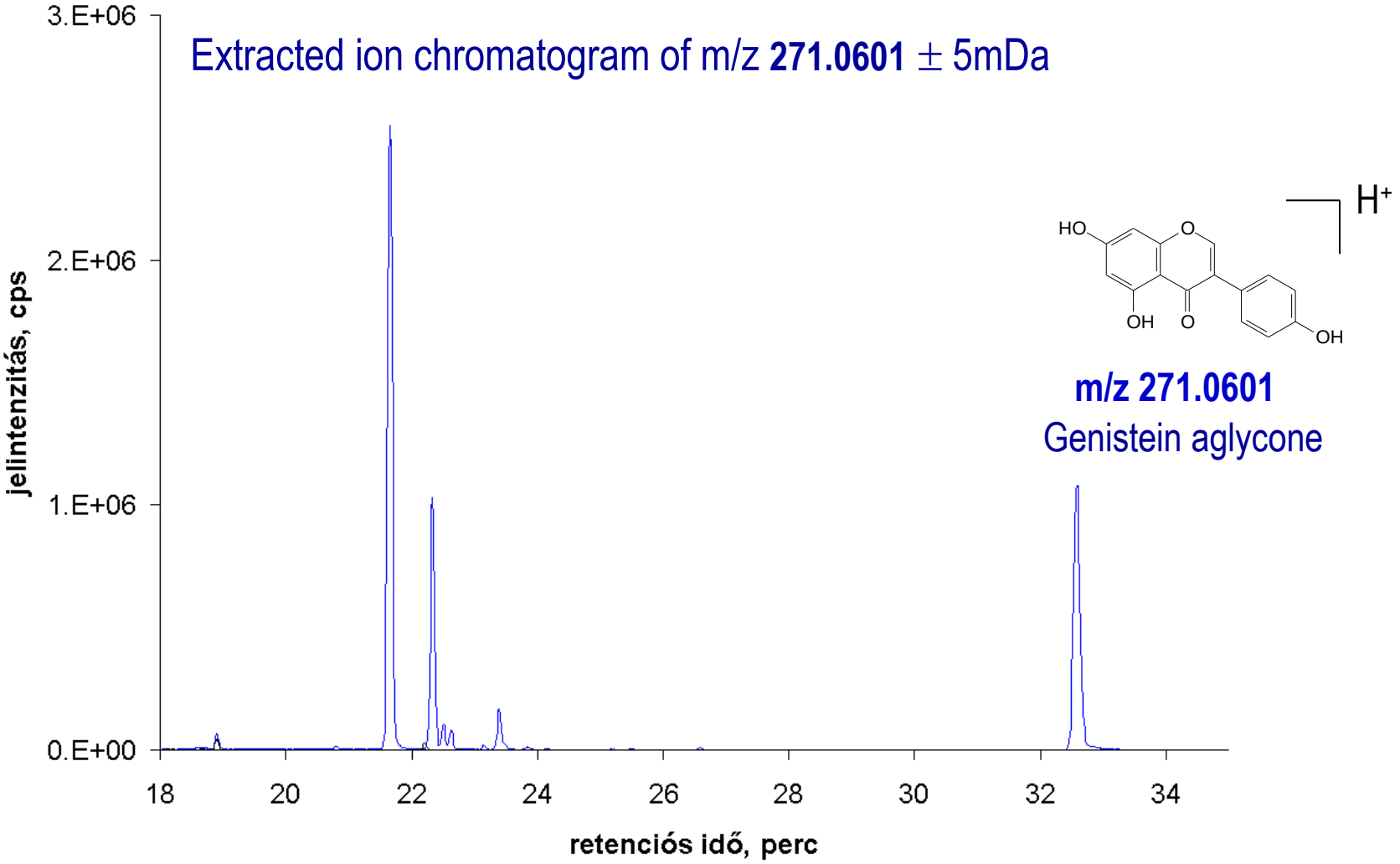
Scholz S, Williamson G., Interactions affecting the bioavailability of dietary polyphenols in vivo, *Int J Vitam Nutr Res.* **2007** 77 (3) 224-235.

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

MS fragmentation



TOF chromatogram of genistein profiling



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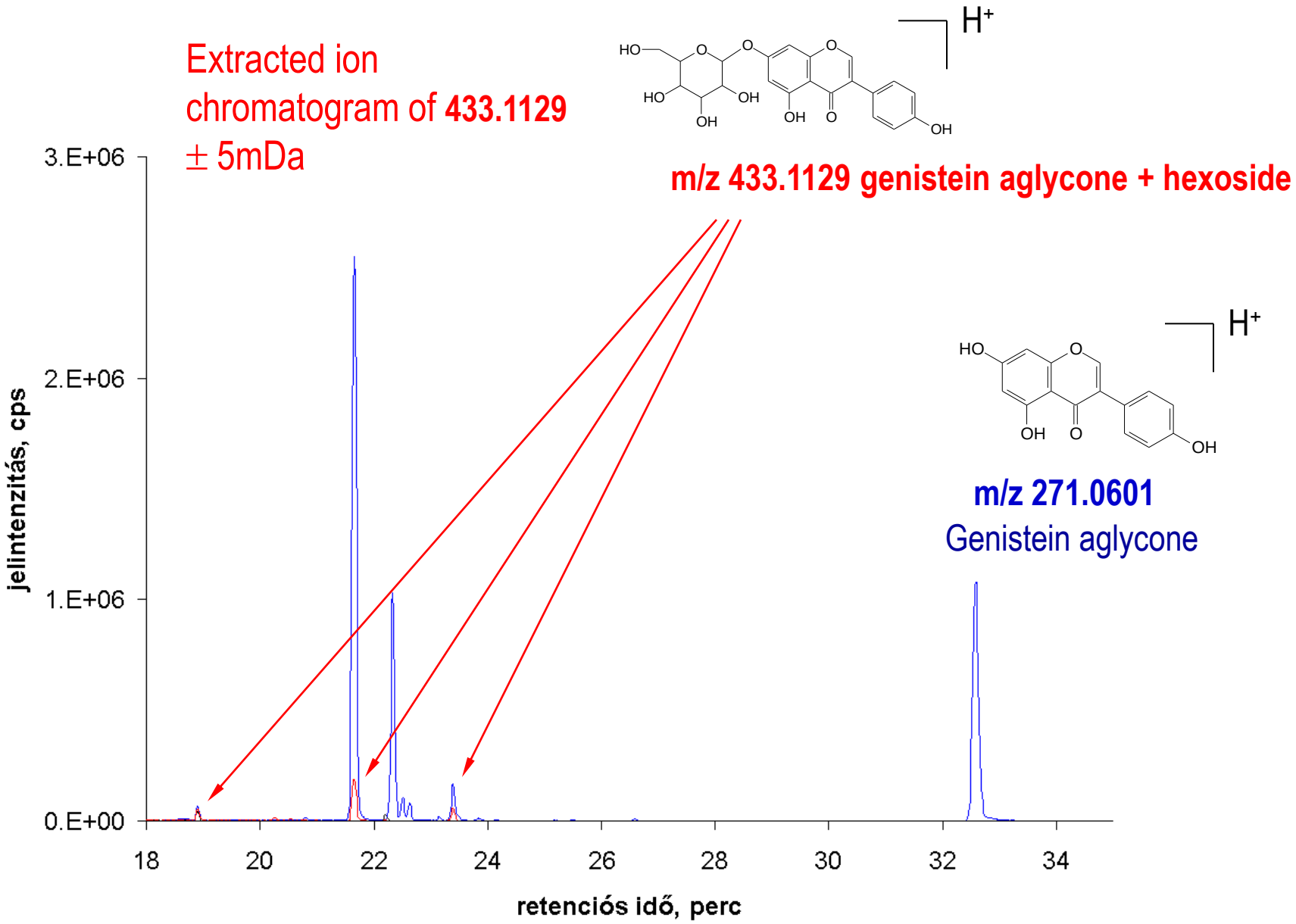

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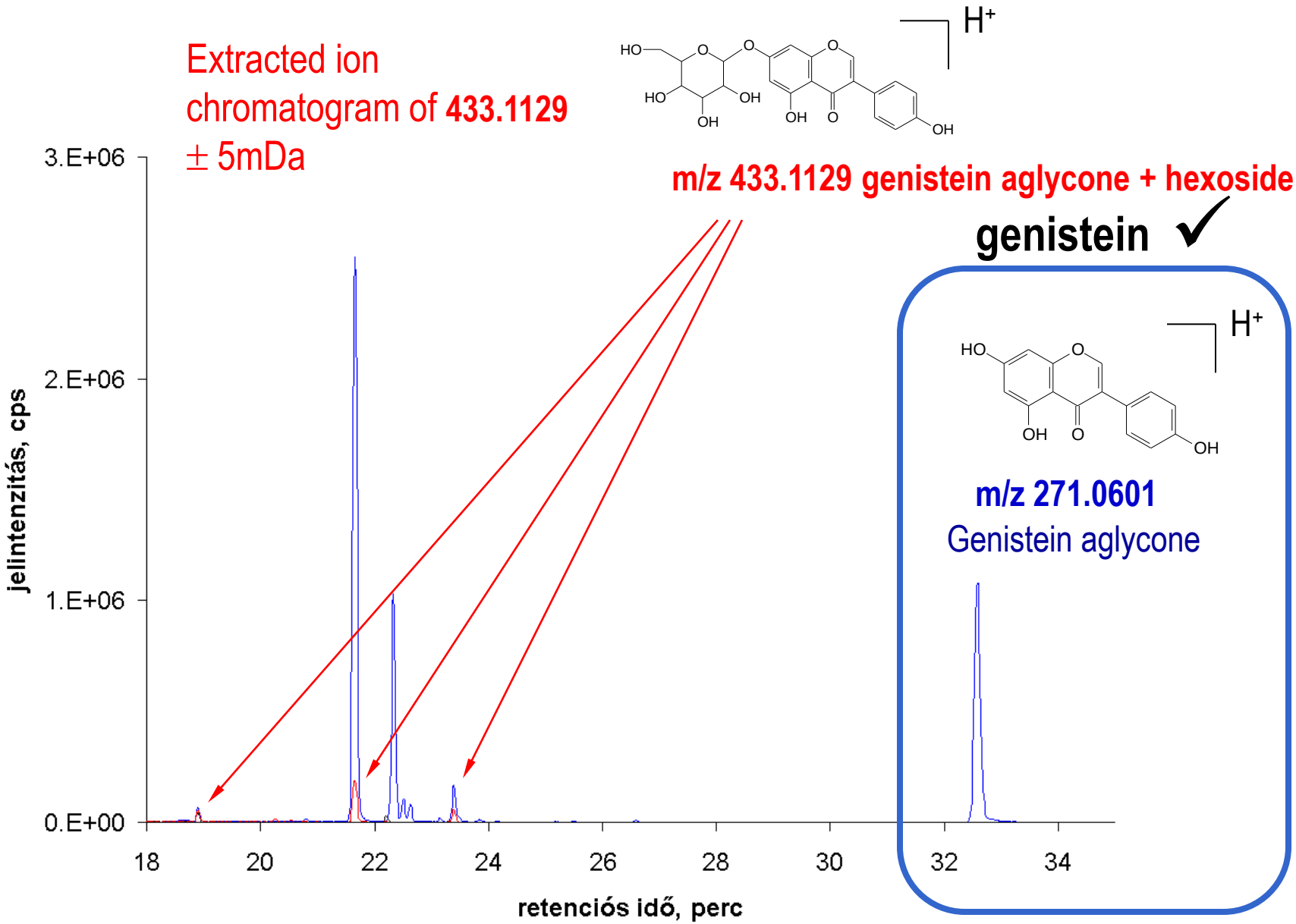
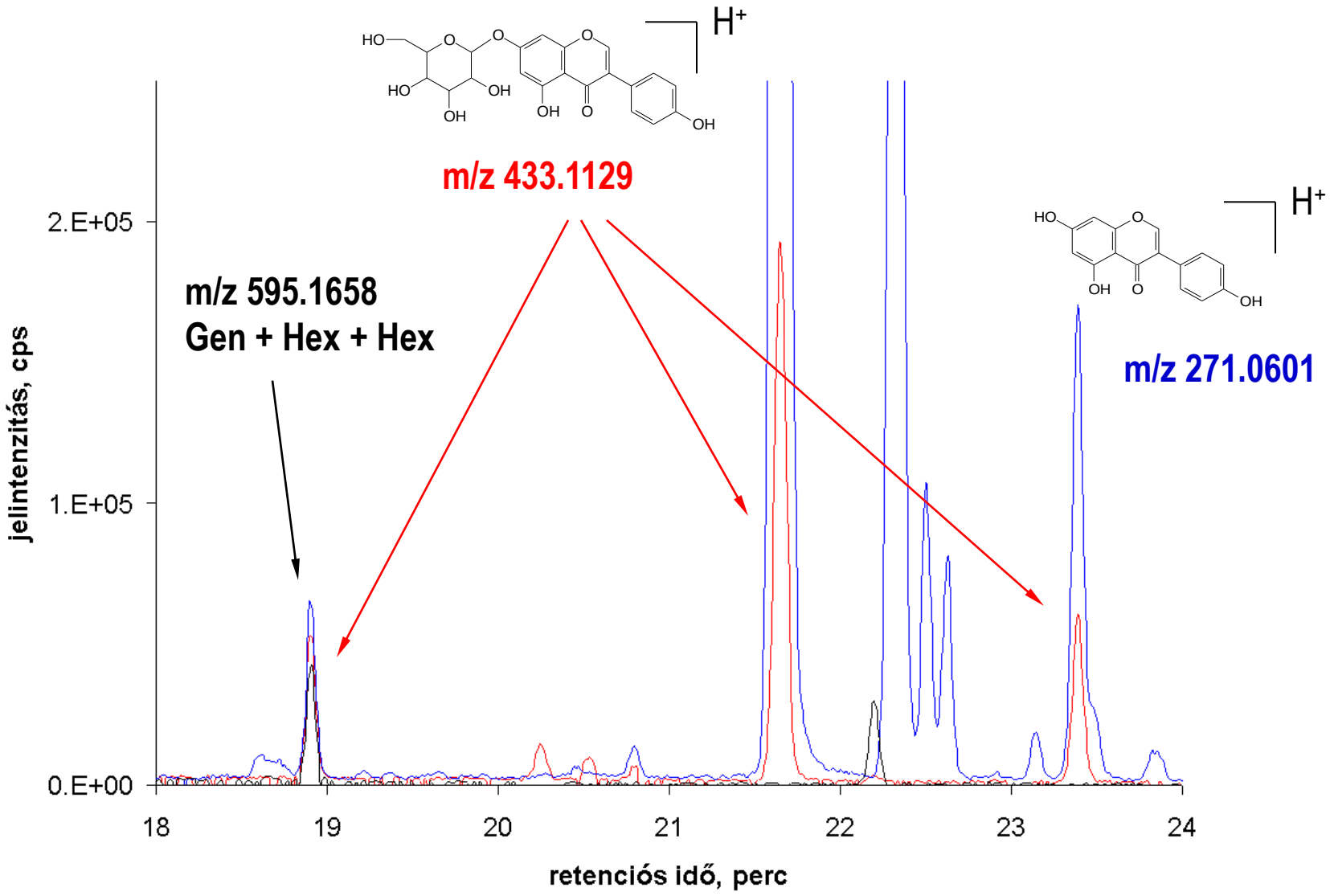
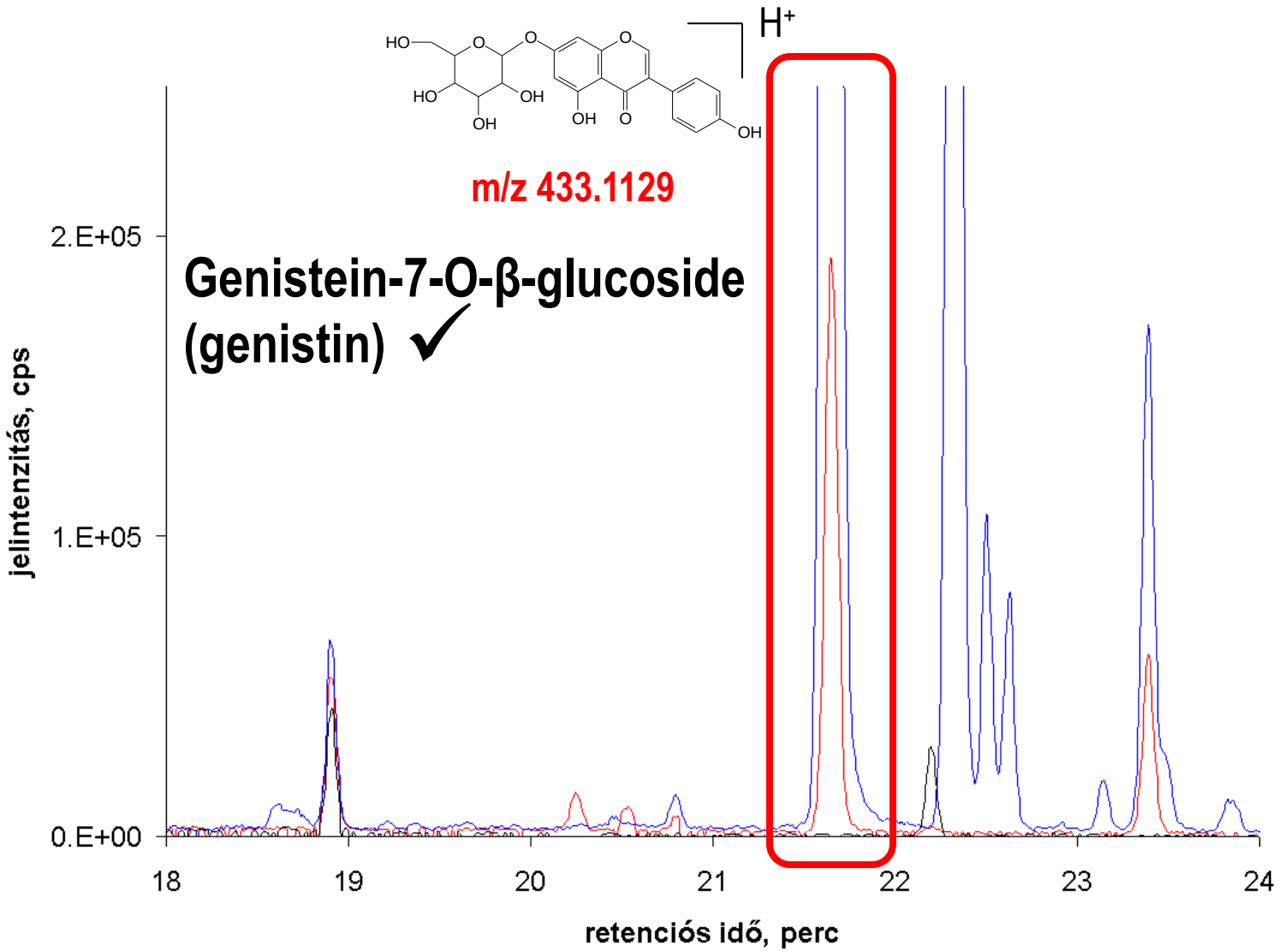


TOF chromatogram of genistein profiling (zoom)



TOF chromatogram of genistein profiling (zoom)



Concentrations of genistein compounds I.

(mg/100 g fresh weight)

Variety	Year	Mass spectrometry (MS/MS)		UV
		Genistin	Genistein	Gen-H (tR = 22.73)
Oblachiskha	2009	<0.02[a]	<0.02	n.d.
VN-7	2009	<0.02	<0.02	n.d.
Érdi bőtermő	2009	<0.02	<0.02	n.d.
Csengődi	2009	<0.02	<0.02	n.d.
Cigány404	2009	<0.02	<0.02	n.d.
Korai pipacs	2009	<0.02	<0.02	n.d.
VN-4	2009	<0.02	<0.02	n.d.
Sárdy SF	2010	<0.02	<0.02	n.d.

[a]limit of quantification, LOQ, n.d. = not detected

Concentrations of genistein compounds II.

(mg/100 g fresh weight)

Variety	Year	Mass spectrometry (MS/MS)		UV
		Genistin	Genistein	Gen-H (tR = 22.73)
Pipacs1	2009	4.49±0.62	1.39±0.05	0.39±0.12
Kántorjánosi	2009	0.54±0.01	0.11±0.01	0.08±0.001
Debreceni bőtermő	2009	0.52±0.01	0.06±0.001	0.03±0.01
Éva	2010	0.79±0.01	0.05±0.002	0.05±0.01
		1.94[a]	0.64[a]	
		43.0[b]	1.29[c]	
			2.30[d]	

[a] green soybean [Chan S.G., Murphy P.A., Ho S.C., Kreiger N., Darlington G., So E.K.F., Chong P.Y.Y. *J Agr Food Chem* **2009** 57:5386-90]

[b] [Wang H.J., Murphy P.A., *J Agr Food Chem* **1994** 42:1666-73]

[c] raw soybean sprout [Morandi S., D'Agostina A., Ferrario F., Arnoldi A., *Eur Food Res & Technol* **2005**, 221:84-91]

[d] [Chan S.G., Murphy P.A., Ho S.C., Kreiger N., Darlington G., So E.K.F., Chong P.Y.Y. *J Agr Food Chem* **2009** 57:5386-90]

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**.

Acknowledgment:

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Thank you for your attention!