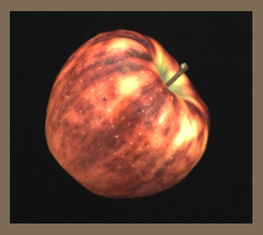
Antioxidants (phenolic acids and carotenoids) in selected apple varieties - harvested and stored



Zuzana Vondráková, Alena Trávníčková, Jiří Malbeck, Daniel Haisel and Milena Cvikrová

Institute of Experimental Botany of the Czech Academy of Sciences
Rozvojová 263, 165 02 Prague 6 - Lysolaje
Czech Republic

vondrakova@ueb.cas.cz

Introduction:

Apples are considered as one of the most important fruit crop with excellent health benefits and extensive area of cultivation. Majority of their benefits is associated with the content of antioxidants (including carotenoids and phenolic compounds).

Carotenoids are a major class of natural, coloured isoprenoid pigments that are synthesized by all photosynthetic organisms. In plants, they are essential for photosynthesis, photoprotection and the production of carotenoid-derived phytohormones. In mammalians, carotenoids represent a vital component of diets given both by their antioxidant activity and by providing precursors for vitamin A biosynthesis. Carotenoids together with other pigments give colours to fruit tissue which contributes to the sensory quality and enhance not only commercial value of fruits but also bring potential health benefits to consumers by reducing the risk of some diseases.

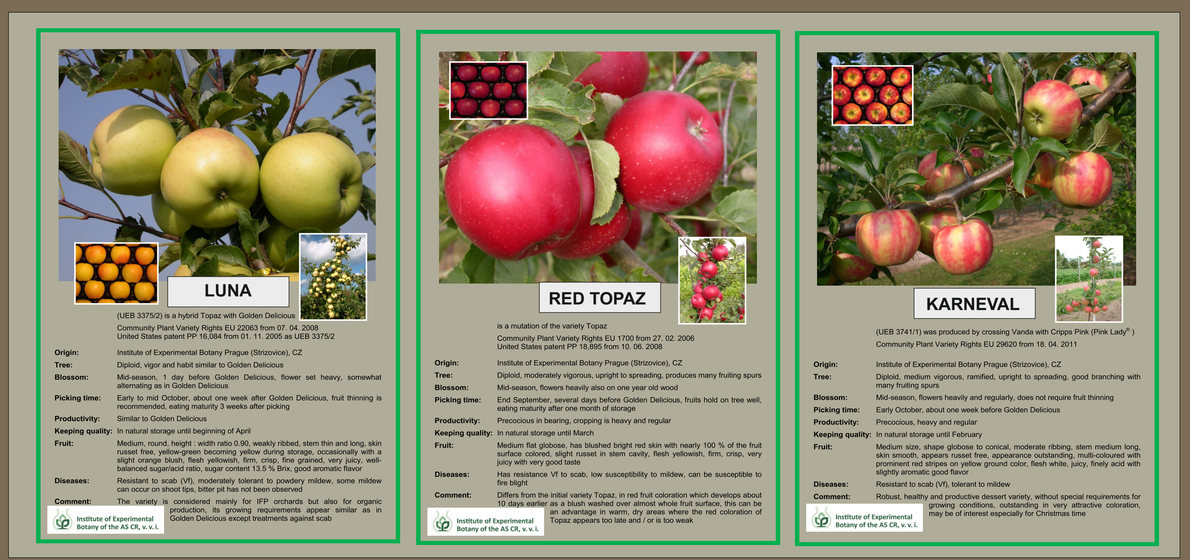
Phenolic compounds constitute a substantial and an important group of phenylpropanoids produced by plants as secondary metabolites. Phenolic functions in plants are as diverse as their structural variations and they play a crucial role in plant defence against both biotic and abiotic stresses. Recently, phenolic compounds have received considerable attention because of their antimicrobial and antioxidant properties, bioavailability and bioefficacy in humans.

The aim of this work was to determine the concentration of selected carotenoids (neoxanthin, violaxanthin, antheraxanthin, lutein, zeaxanthin and ß-carotene) and phenolic acids in selected scab resistant and powdery mildew tolerant apple varieties originating from the Station of apple breeding of the IEB. We investigated three different apple varieties - Luna (yellow), Red Topaz (red) and Karneval (streaked) for their antioxidant contents in peel and flesh immediately after the harvest and after 7 months of storage.

All concentrations of carotenoids and phenolic acids are stated in nmol/g DW.

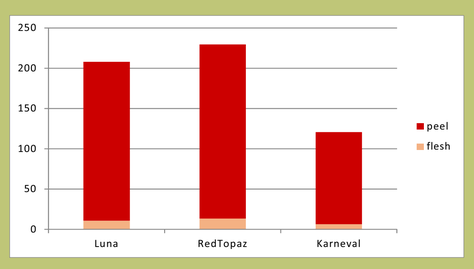
Material:

Carotenoid and phenolic acid contents were measured in three varieties of apples harvested in October 2016 - just after harvest and in June 2017 - after storage (darkness, 7 ± 2° C).
All material was obtained from the Station of apple breeding for disease resistence, IEB AS CR; ueb.strizovice@seznam.cz

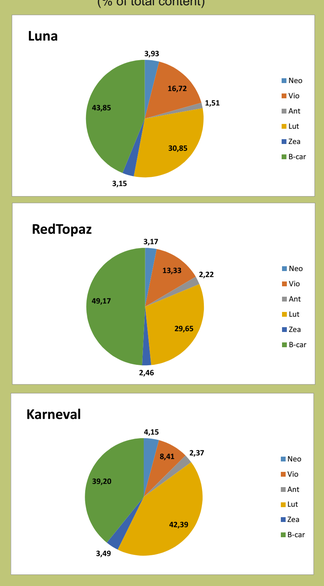


The content of selected carotenoids in apples just after harvest

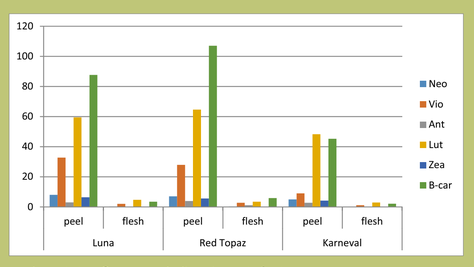
Total content of carotenoids in peel and flesh of three harvested apple varieties



Composition of carotenoids in whole harvested apples (% of total content)



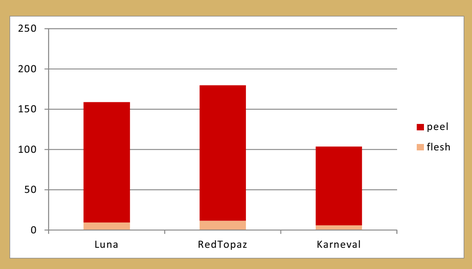
The comparison of selected carotenoid contents in three harvested apple varieties



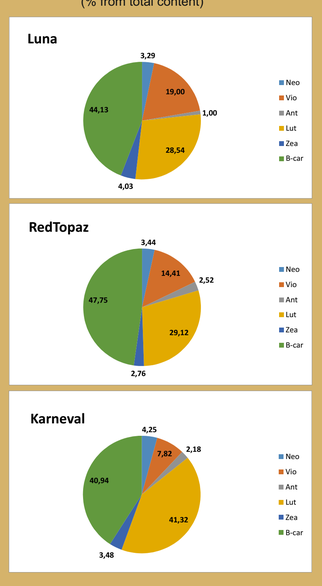
- extremely low Car concentrations in apple flesh
- ß-caroten (and lutein) represent the main components of total measured Car
- small difference between Car concentration in yellow (Luna) and red (Red Topaz) apples; lower concentration in streaked apples (Karneval)

The content of selected carotenoids in stored apples

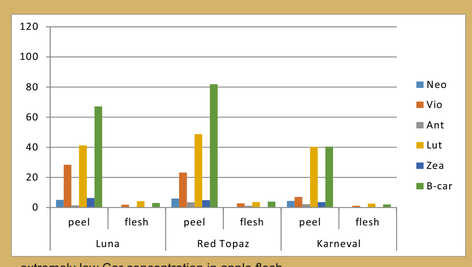
Total content of carotenoids in peel and flesh of three stored apple varieties



Composition of carotenoids in whole stored apples (% from total content)



The comparison of selected carotenoid contents in three stored apple varieties



- extremely low Car concentration in apple flesh
- ß-caroten (and lutein) represent the main components of total measured Car
- the same ratio of individual Car in their total content as in harvested apples
- small difference between Car concentration in yellow (Luna) and red (Red Topaz) apples; lower concentration in streaked apples (Karneval)

Chemical analysis:

Detection and quantification of carotenoids (ß-carotene, lutein, neoxanthin, violaxanthin, zeaxanthin and antheraxanthin) from acetone:ethylacetate (8:2) apple extracts were carried out using an HPLC (ECOM, Czech Republic). The analysis was performed using a reversed phase column (Watrex Nucleosil 120 5 C18, 5 µm particle size, 125×4 mm, ECOM, Czech Republic) with the solvent system acetonitrile:methanol:water (80:12:10 v:v:v) followed by methanol:ethylacetate (95:5 v:v). The total time of analysis was 25 min, the linear gradient run from 2 to 6 min (the flow rate 1 cm3min-1), the detection wavelength 445 nm). Data were captured and calculated by PC-software Clarity (DataApex, Czech Republic).

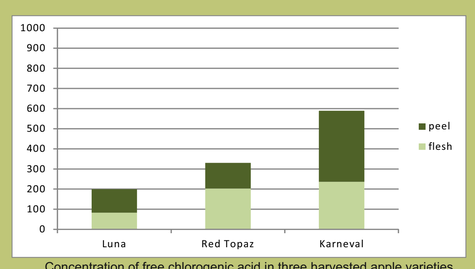
Two forms of phenolic acids (free and glycoside-bound) were analyzed. The samples were extracted with 80% methanol and the extract was subsequently evaporated to the aqueous phase. After acidification, free acids were extracted with diethyl ether and the aqueous residue was subjected to acid hydrolysis. The decomposed glycoside-bound phenolic acids were extracted with diethyl ether. All other extracts were evaporated in rotary vacuum concentrator. Evaporated samples were dissolved in 50% methanol and analyzed on LC-MS instrument. Chromatographic analyses were performed using 50x2.1 mm HPLC column Kinetex C18 with ternary gradient water/acetonitrile/1% acetic acid. The mass spectrometer was operated in the negative multiple SRM (single reaction monitoring) mode and the analytes were quantified by the calibration graph with deuterated compounds used as internal standards.

Conclusions

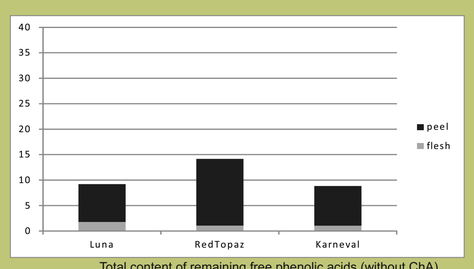
- **The concentration of carotenoids slightly decreased after the storage in all studied apple varieties.**
- **The qualitative composition of individual carotenoids and their percentage in the total content did not change after the storage.**
- **Conversely the increase in the content of phenolic acids was observed in all apples after the storage.**
- **Chlorogenic and protocatechuic acids represented most abundant free /resp. glycoside-bound phenolic acids both in harvested and stored apples.**
- **Both of these acids exhibit a strong scavenging activity and might contribute considerably to the total antioxidant potential of apples and increase their nutritional quality.**

The content of phenolic acids in apples just after harvest

The content of free phenolic acids in peel and flesh of three harvested apple varieties

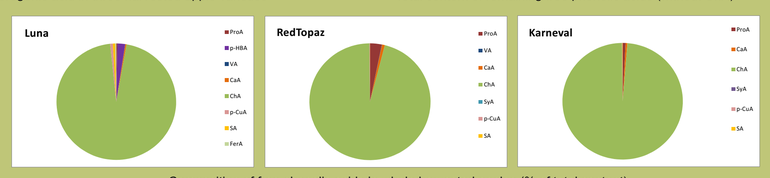


Concentration of free chlorogenic acid in three harvested apple varieties



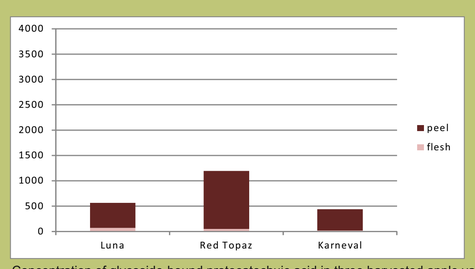
Total content of remaining free phenolic acids (without ChA)

- ChA represents the major part of free PhA (95 - 98%)
- the differences between the content of ChA in peel and flesh are not substantial
- the other PhA are present preferentially in apple peel

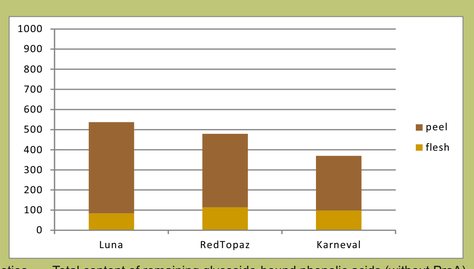


Composition of free phenolic acids in whole harvested apples (% of total content)

The content of glycoside-bound phenolic acids in peel and flesh of three harvested apple varieties

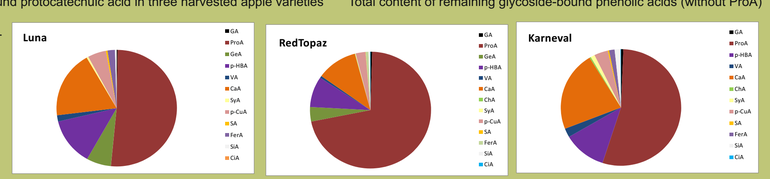


Concentration of glycoside-bound protocatechuic acid in three harvested apple varieties



Total content of remaining glycoside-bound phenolic acids (without ProA)

- higher concentration of glycoside-bound PhA (compared with free PhA) was detected in all apple varieties
- glycoside-bound PhA are prefentially present in apple peel
- ProA represents the most abundant glycoside-bound PhA (55-70%), followed by CaA (11 - 22%) and p-HBA (8 - 13%).



Composition of glycoside-bound phenolic acids in whole harvested apple (% of total content)

Abbreviations:

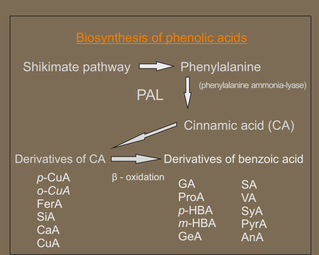
Car - carotenoids

Neo - neoxanthin
Vio - violaxanthin
Ant - antheraxanthin
Lut - lutein
Zea - zeaxanthin
B-car - ß-caroten

PhA - phenolic acids

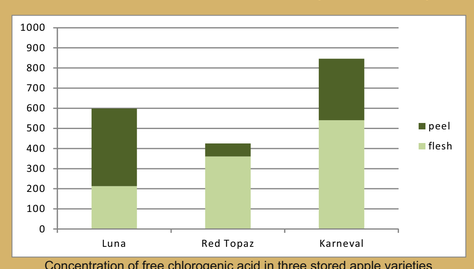
GA - gallic acid
ProA - protocatechuic acid
GeA - gentisic acid
p-HBA - *para*-hydroxy-benzoic acid
SA - salicylic acid
VA - vanillic acid
CaA - caffeic acid
ChA - chlorogenic acid
SyA - syringic acid
p-CuA - *para*-coumaric acid
FerA - ferulic acid
SiA - sinapic acid
CiA - cinnamic acid

Biosynthesis of phenolic acids

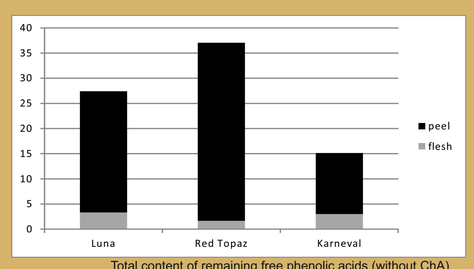


The content of phenolic acids in stored apples

The content of free phenolic acids in peel and flesh of three stored apple varieties

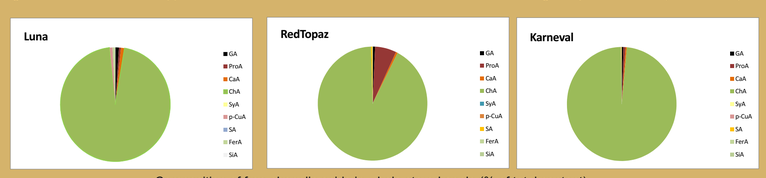


Concentration of free chlorogenic acid in three stored apple varieties



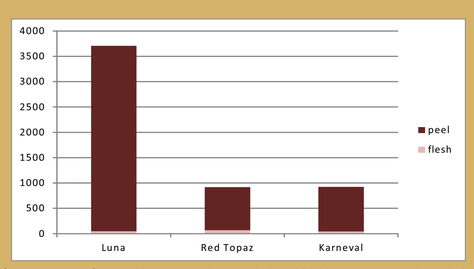
Total content of remaining free phenolic acids (without ChA)

- ChA represents the major part of free PhA (95 - 98%)
- the differences between the content of ChA in peel and flesh are variety-specific
- other PhA are present preferentially in apple peel
- the spectrum of individual free PhA detected in stored apples is higher compared with harvested ones

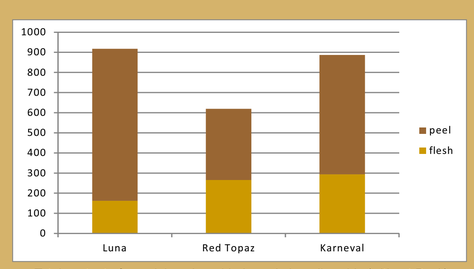


Composition of free phenolic acids in whole stored apple (% of total content)

The content of glycoside-bound phenolic acids in peel and flesh of three stored apple varieties

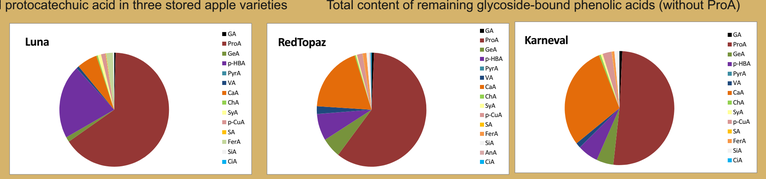


Concentration of glycoside-bound protocatechuic acid in three stored apple varieties



Total content of remaining glycoside-bound phenolic acids (without ProA)

- higher concentration of glycoside-bound PhA (compared with free PhA) was detected in all apple varieties
- glycoside-bound PhA are prefentially present in apple peel
- ProA represents the most abundant glycoside-bound PhA (51-65%), followed by CaA (6 - 19%) and p-HBA (6 - 21%)
- the number of other glycoside-bound PhA is higher than in harvested ones



Composition of glycoside-bound phenolic acids in whole stored apple (% of total content)

Acknowledgement: This work was supported by the Ministry of Education of the Czech Republic, project LTC 17034; within CA COST Action CA 15136 - Eurocaroten.