Carotenoids and phenolic acids during ripening, harvest and storage in selected scab-resistent and mildew-tolerant apple cultivars

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Introduction

The aim of this study was to characterise the changes in concentration and composition of antioxidants during ripening, harvest and after 3 and 6 months of storage in three commercially successful scab-resistant and powdery mildew-tolerant apple cultivars selected in the Institute of Experimental Botany. The detailed description of free and glycosylated phenolic acid profiles and content of 6 selected carotenoids - neoxanthin, violaxanthin, antheraxanthin, lutein, zeaxanthin and ß-carotene will serve for outcomes of major characteristics of these apple cultivars. The information dealt with the concentration and composition of antioxidants in selected apple cultivars is aimed at consumers in view of health benefits of phenolic compounds and carotenoids.

Material

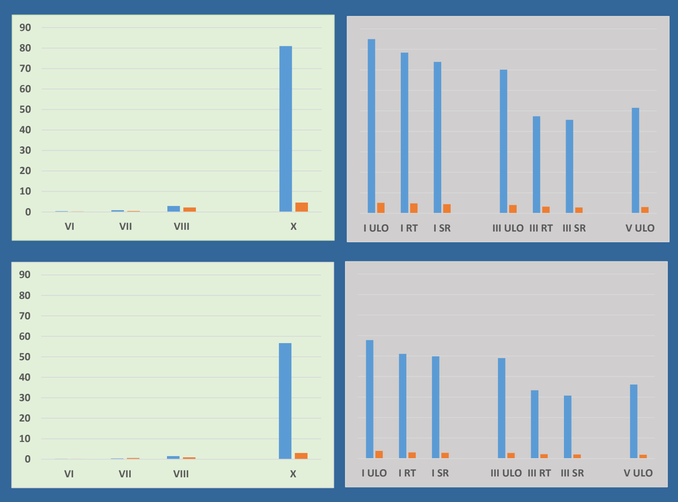
In our experiments we used three apple cultivars originating from the Institute of Experimental Botany, Station of apple breeding for disease resistence, Strizovice, CR: red Bonita, streaked Karneval and yellow Sirius.

Experimental design

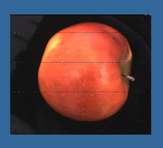
The samples of apple peels and fleshes were collected during ripening, , in June (VI), July (VII) and August (VIII) and during harvest in October (X). Apples were then stored under 3 different conditions: in ULO boxes under low oxygen (1,2%), CO2 (2,2%) and under the temperature 1° C; in boxes RT under the temperature 1° C; and in storerooms SR under the temperature fluctuated between 1° – 4° C. Samples of peels and fleshes from stored apples, , were collected in January (I), i.e. after 3 months of storage, in March (III), i.e. after 6 months of storage. Samples from apples stored in ULO were also used in May (V).

The content of carotenoids

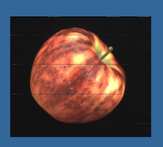
Total carotenoids during ripening and storage (µg /g DW)
Blue bars = peel; red bars = flesh

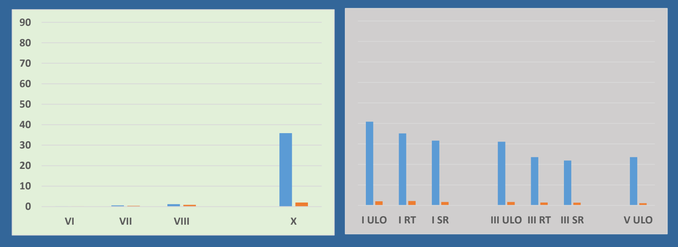


Bonita

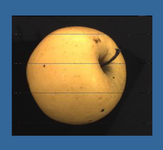


Karneval

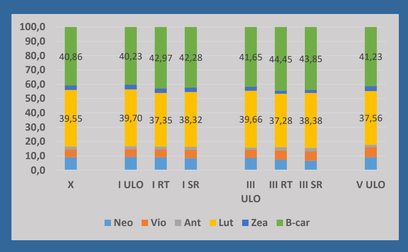


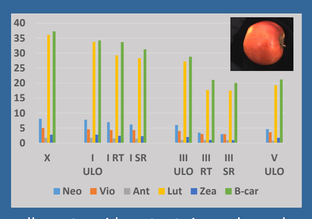


Sirius

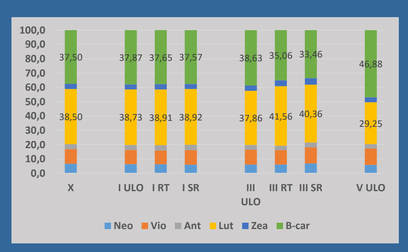


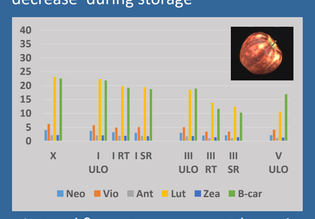
The content of neoxantin (Neo), violaxantin (Vio), antheraxantin (Ant), lutein (Lut), zeaxantin (Zea) and β-caroten (B-car) in peels of stored apples - in % from total carotenoids (left) and in µg /g DW (right))



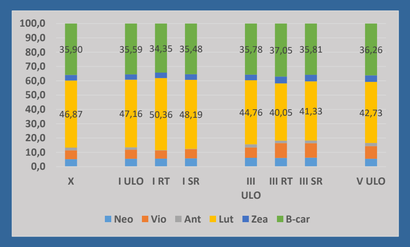


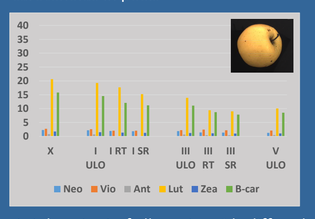
- all carotenoid contents in apple peels decrease during storage





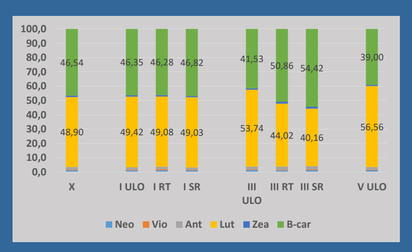
- Lut and ß-caroten represent the main carotenoids in peels

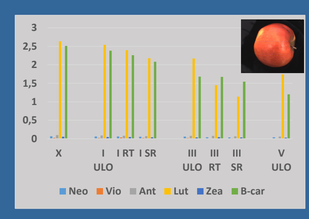




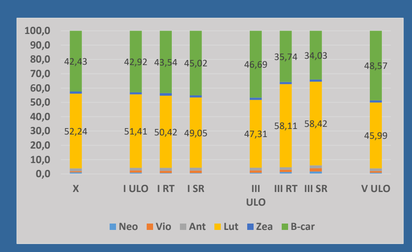
- total content of all carotenoids differs between cultivars but the changes during storage are similar

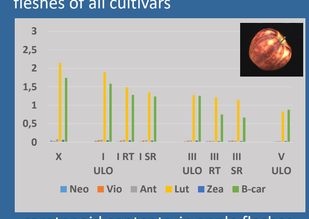
The content of neoxantin, violaxantin, antheraxantin, lutein , zeaxantin and β-caroten in fleshes of stored apples - in % from total carotenoids (left) and in µg /g DW (right))



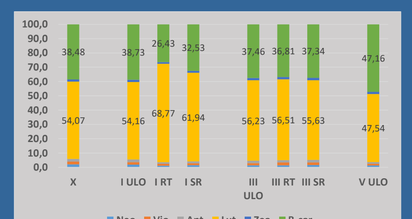


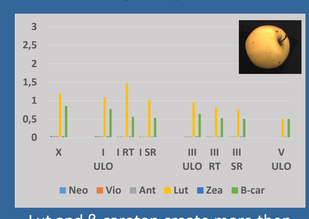
- carotenoid content is low in apple fleshes of all cultivars





- carotenoid contents in apple fleshes decrease during storage





- Lut and ß-caroten create more then 90% of carotenoids in apple fleshes

Conclusions:

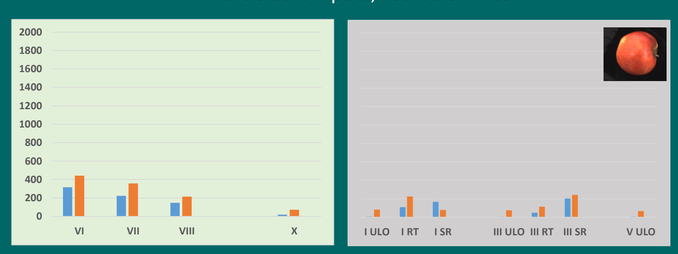
- carotenoids were mainly present in apple peels
- the highest content of carotenoids was found in harvested apples, it increased during ripening and then decreased during storage
- the ratio between individual carotenoids didn´t change during storage
- fully regulated storage conditions (in ULO boxes) were able to prolong the time of storage and moreover to reduce the lost of carotenoids in stored apples

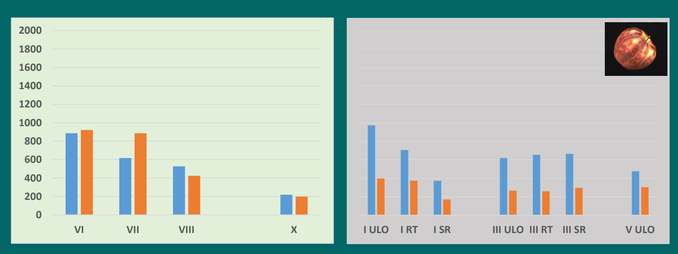
The content of phenolic acids

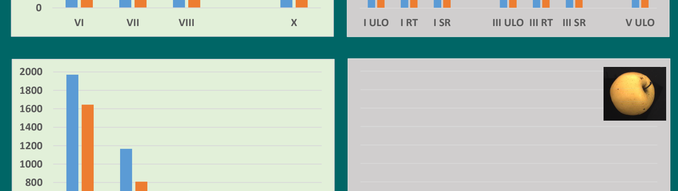
GA ProA GeA p-HBA VA CaA ChA SyA p-CuA FerA SiA CiA

spectrum of glycosylated phenolics acids

Total free phenolic acids during ripening and storage (µg /g DW)
Blue bars = peel; red bars = flesh



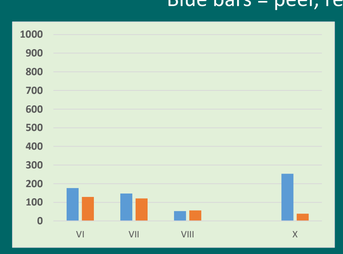


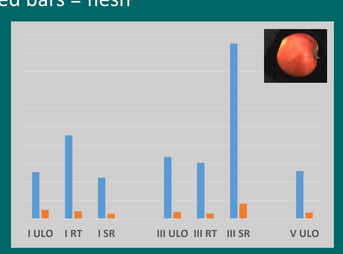


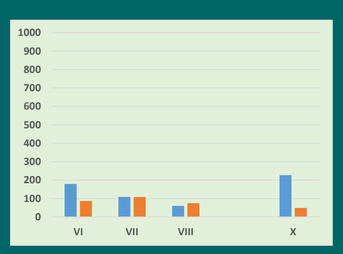
Abbreviations:

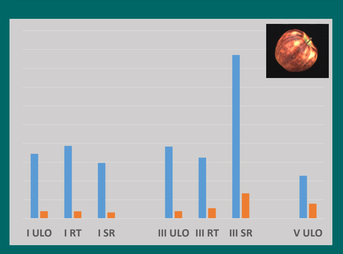
GA – gallic acid
ProA – protocatechuic acid
VA – vanillic acid
CaA – caffeic acid
ChA – chlorogenic acid
***p*-CouA** – *para*-coumaric acid
FerA – ferulic acid
SiA – sinapic acid
GeA – gentisic acid
***P*-HBA** – *para*-hydroxy-benzoic acid
SyA – syringic acid
CiA – cinnamic acid

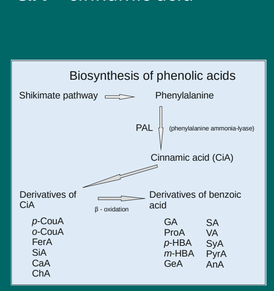
Total glycosylated phenolic acids during ripening and storage (µg /g DW)
Blue bars = peel; red bars = flesh

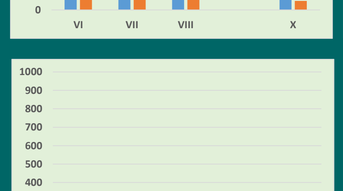




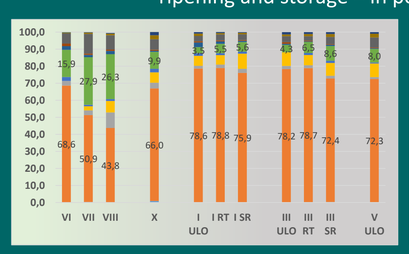


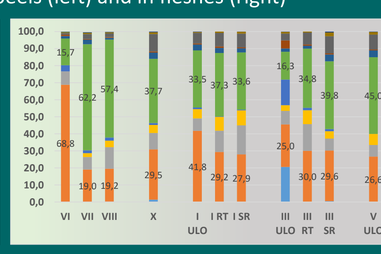


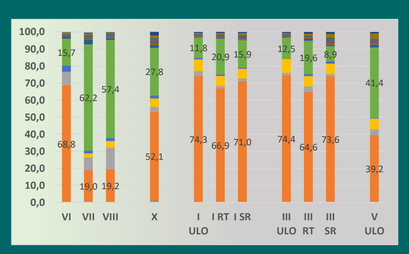


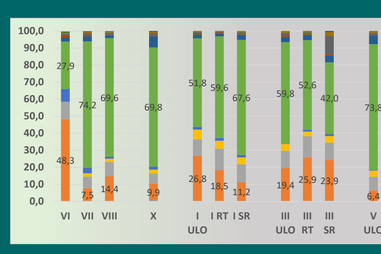


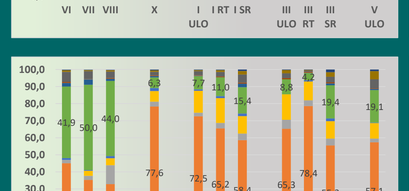
Individual glycosylated phenolic acids (% from total content of them) during ripening and storage – in peels (left) and in fleshes (right)

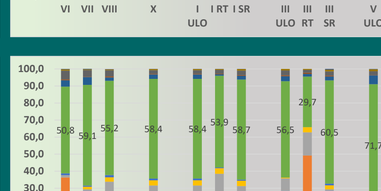












Conclusions:

- Chlorogenic acid represented 98 – 100% of free phenolic acids in peels and fleshes of apples during ripening, harvest and storage
- high level of free phenolic acids in non-mature apples declined during ripening
- the content of free phenolic acids increased again during storage
- the effect of different storage conditions was not found
- spectrum of glycosylated phenolic acids in peels and fleshes was broader then spectrum of free ones
- the changes in the content of glycosylated phenolic acids in apples during ripening were smaller then in free ones
- the concentration of glycosylated phenolic acids increased in harvest and during storage – especially in apple peels
- Protocatechuic acid was the main glycosylaed phenolic acid in peel; caffeic acid represented the main glycosylated phenolic acid in apple fleshes
- the effect of storage conditions was not obvious – the increase of glycosylated phenolic acid contents at the end of storage could be linked with the damage of fruits due to worse storage conditions

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/0.1% 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.

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