

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



Z. Vondráková, A. Trávníčková, J. Malbeck, D. Haisel, R. Černý, M. Cvikrová

Institute of Experimental Botany of the Czech Academy of Sciences
Rozvojová 263, 16502 Prague 6, Lysolaje
Czech Republic

vondrakova@ueb.cas.cz

Introduction

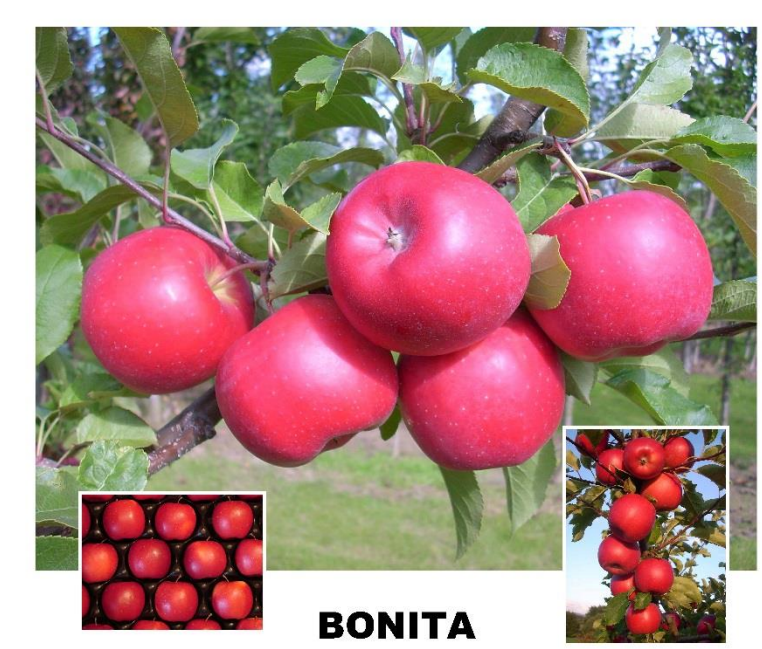
The aim of this study was to characterise the changes in concentration and composition of antioxidants during ripening, harvest and after 3 and 6 months of storage in three commercially successful scab-resistant and powdery mildew-tolerant apple cultivars selected in the Institute of Experimental Botany. The detailed description of **free and glycosylated phenolic acid profiles** and content of 6 selected carotenoids - **neoxanthin, violaxanthin, antheraxanthin, lutein, zeaxanthin and β -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 resistance, Strizovice, CR: red Bonita, streaked Karneval and yellow Sirius.

Experimental design

The samples of apple peels and fleshes were collected during ripening, **VI**, 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, **III**, 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).



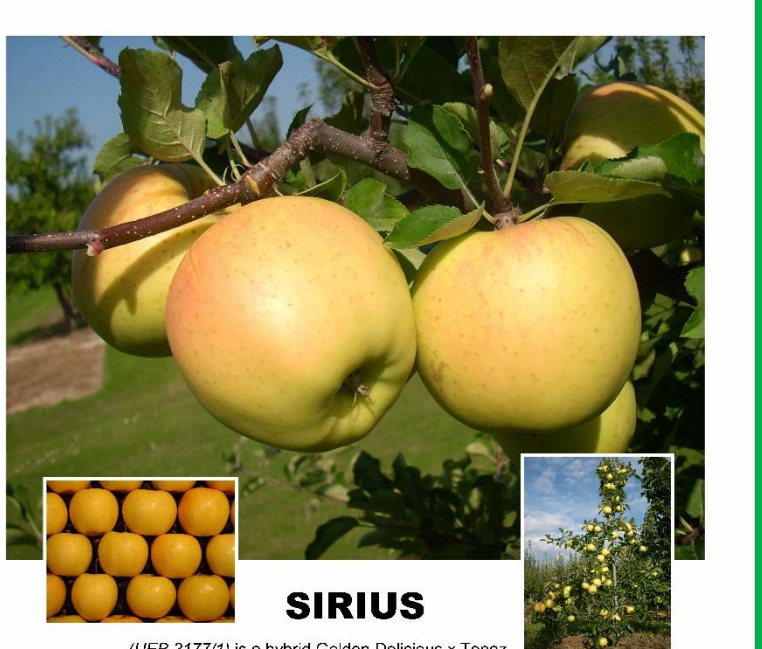
BONITA
(EU 27411) is a hybrid between Tonda and Golden Pippin
Plant Variety Rights EU 43354, granted 08.02.2017
Applied for EU Plant Patent, 04.03.2017

Origin: Institute of Experimental Botany Prague (Sbítovské) CZ
Tree: Diploid, medium vigorous, ramified, upright to spreading, good branching with many trifoliate leaves
Blossom: Mid-season, flowers heavily
Picking time: About 1 week after Golden Delicious
Productivity: Periodically high and mostly regular
Keeping quality: In cool storage about six months
Fruit: Size medium, single globose with small grey points, stem fat and long, skin smooth, russet free, often yellow ground color is covered on 85 - 100 % with deep red strongly variegated flesh firm, crisp, juicy with good, slightly sour taste
Diseases: Susceptible to scab (V), resistant to powdery mildew
Comment: Late apple variety with very homogeneous, mostly red fruits, outstanding appearance, high and regular yields, which make of this fruiting and with good keeping and eating qualities. Suitable for commercial apple growing.



KARNEVAL
(EU 27411) was produced by crossing Verde with Clappes Pink (Pink Lady)
Community Plant Variety Rights EU 26602 from 24. 08. 2007
Under-Scab resistant (V) 10.04. From 04. 03. 2008

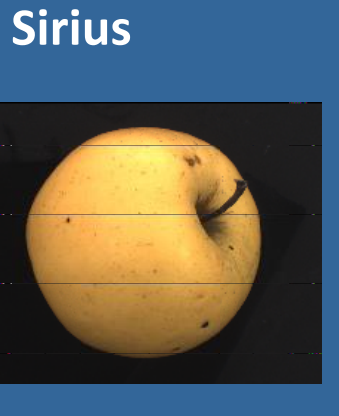
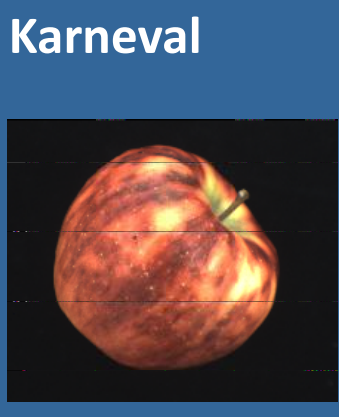
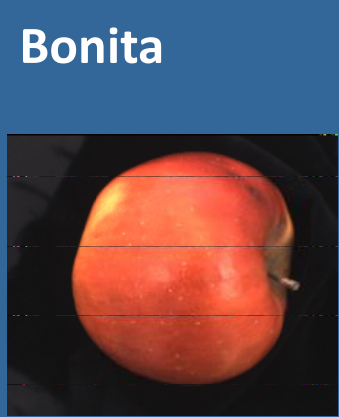
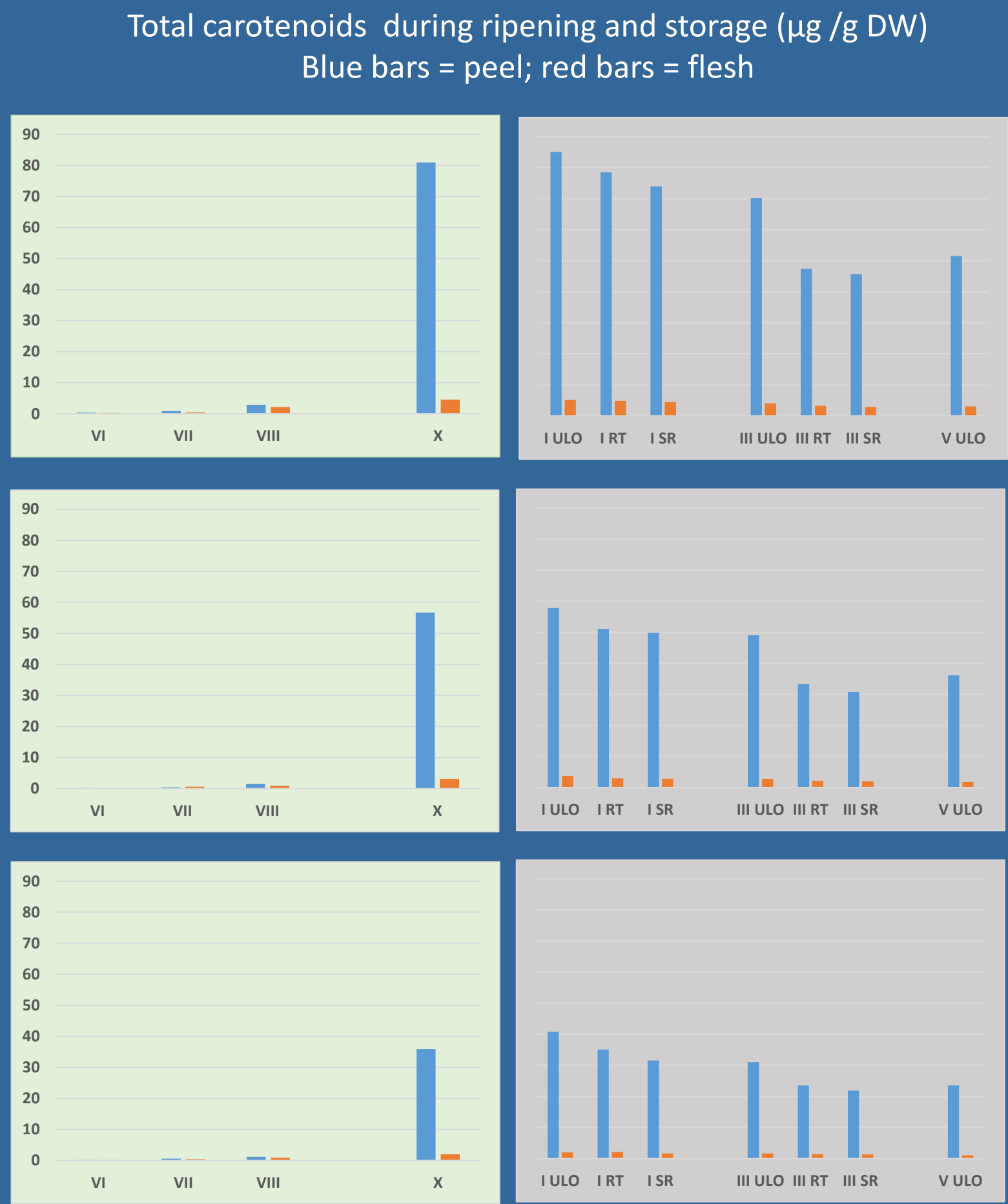
Origin: Institute of Experimental Botany Prague (Sbítovské) CZ
Tree: Diploid, medium vigorous, ramified, upright to spreading, good branching with many trifoliate leaves
Blossom: Mid-season, flowers heavily and regularly, does not require fruit thinning
Picking time: Early October, about one week before Golden Delicious
Productivity: Periodically heavy and regular
Keeping quality: In natural storage until February
Fruit: Medium size, single globose to ovoid, moderate ribbing, stem medium long, skin smooth, russet free, often yellow ground color is covered on 85 - 100 % with deep red strongly variegated flesh firm, crisp, juicy with good, slightly sour taste
Diseases: Susceptible to scab (V), resistant to powdery mildew
Comment: Medium, healthy and productive dessert variety, without special requirements for growing conditions, outstanding in very attractive coloration, may be of interest especially for Christmas time.



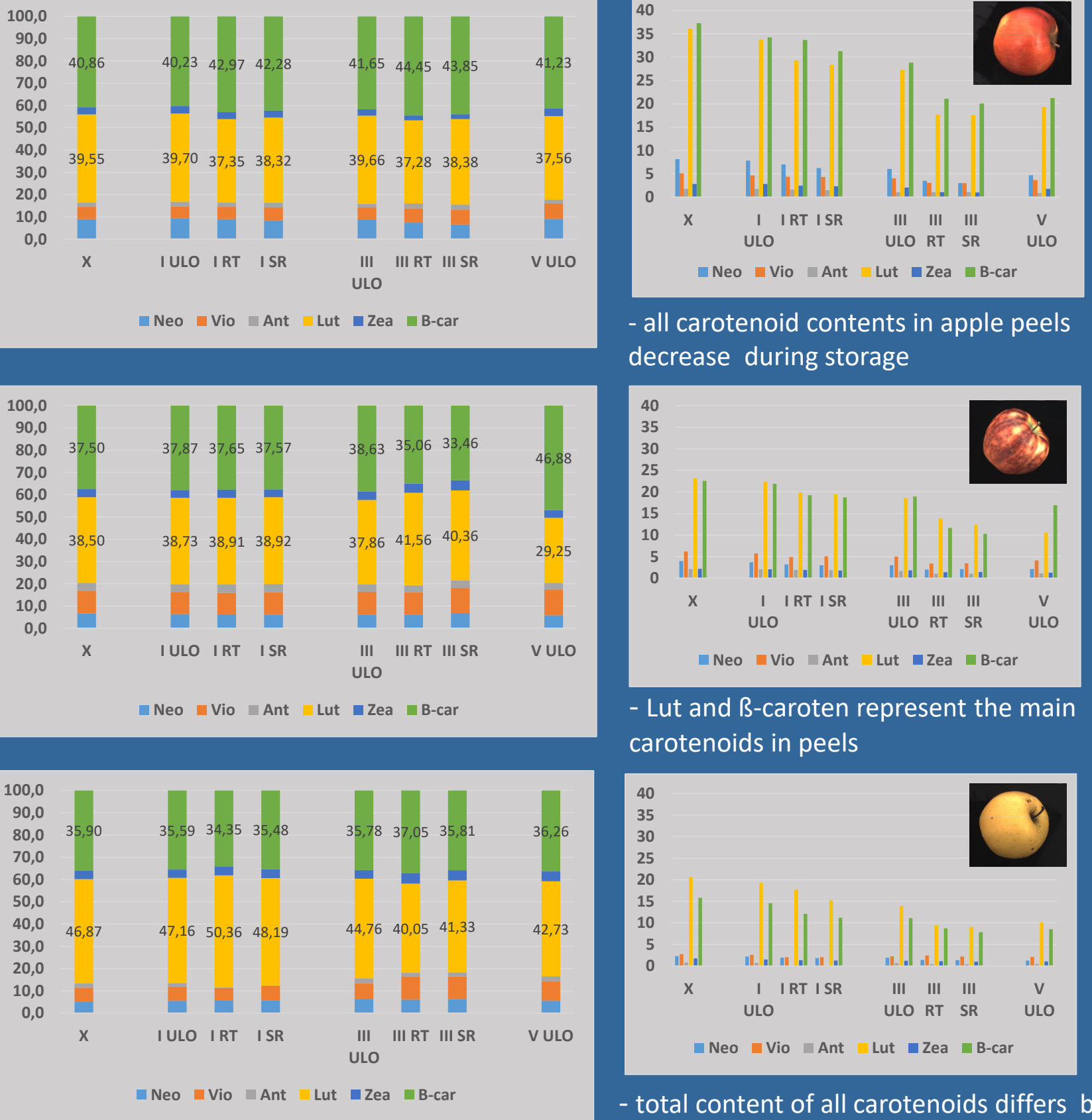
SIRIUS
(EU 31771) is a hybrid Golden Delicious x Tonda
Community Plant Variety Rights EU 26602 from 24. 08. 2007
Under-Scab resistant (V) 10.04. From 04. 03. 2008

Origin: Institute of Experimental Botany Prague (Sbítovské) CZ
Tree: Triplet, vigorous, spreading, branching medium, fruiting sparsely medium to long
Blossom: Mid-season, slightly before Golden Delicious, flower set medium, regular
Picking time: Towards mid-October, about 10 days after Golden Delicious, fruits hang mostly singly without thinning
Productivity: Periodically, produces regular good crops
Keeping quality: In natural storage until April, using moderately 1 week after picking
Fruit: Medium to large, round, height: width ratio 0.85, stem long and medium thick, skin has redness may be present in the stem, young ground color green yellow to yellow occasionally with a slight rosy tinge, light yellow, firm, crisp, the ground color very strong, with lustrous sugar (14.7 % Brix) and acid level, not Resistant
Diseases: Resistant to scab (V), tolerant to powdery mildew, absence of bitter pit
Comment: The variety can be considered for organic production as well as for IFF systems, growing requirements seems to be similar to Jonagold, except resistance against scab, low experience, very interesting variety with many good qualities.

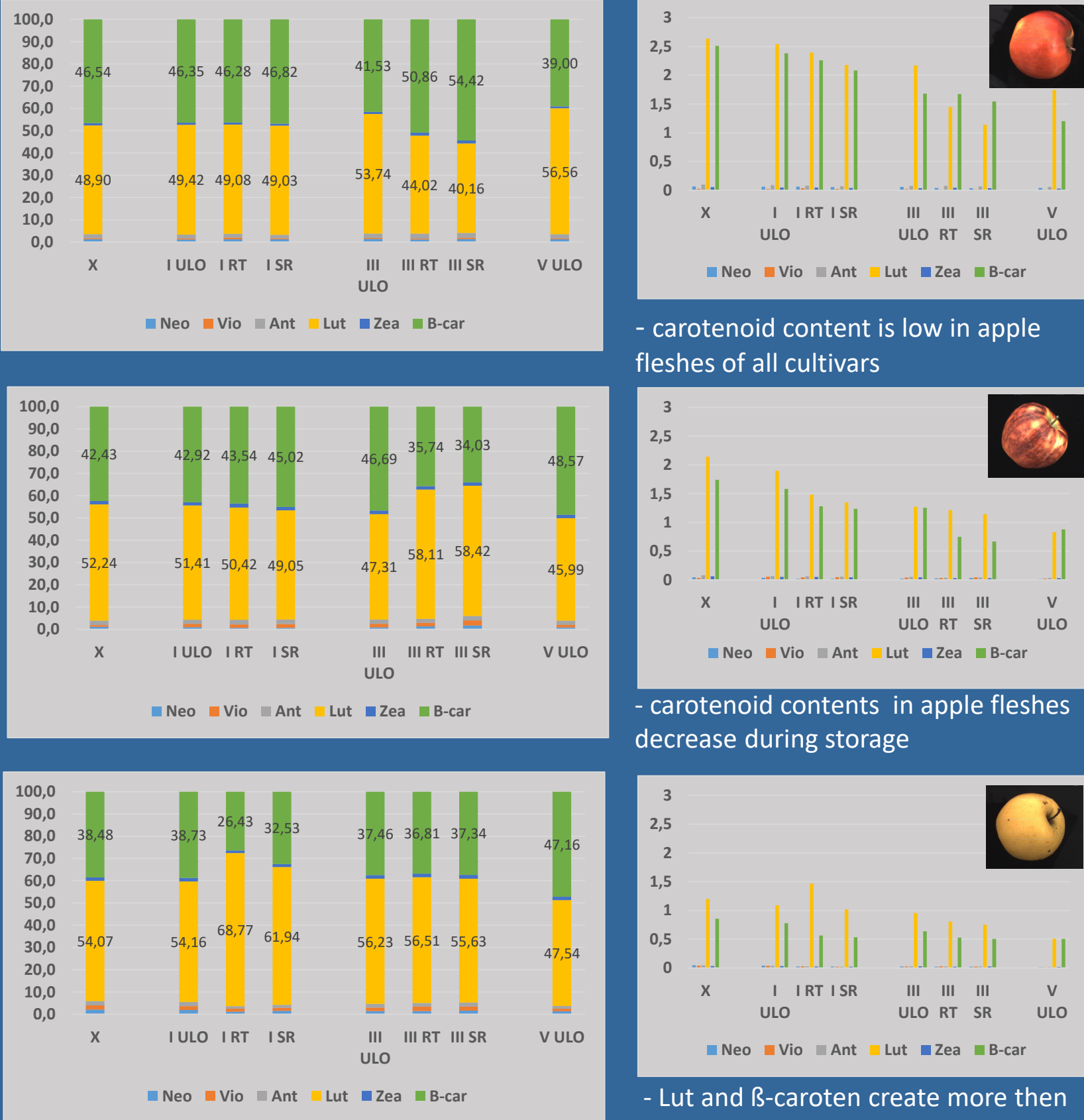
The content of carotenoids



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



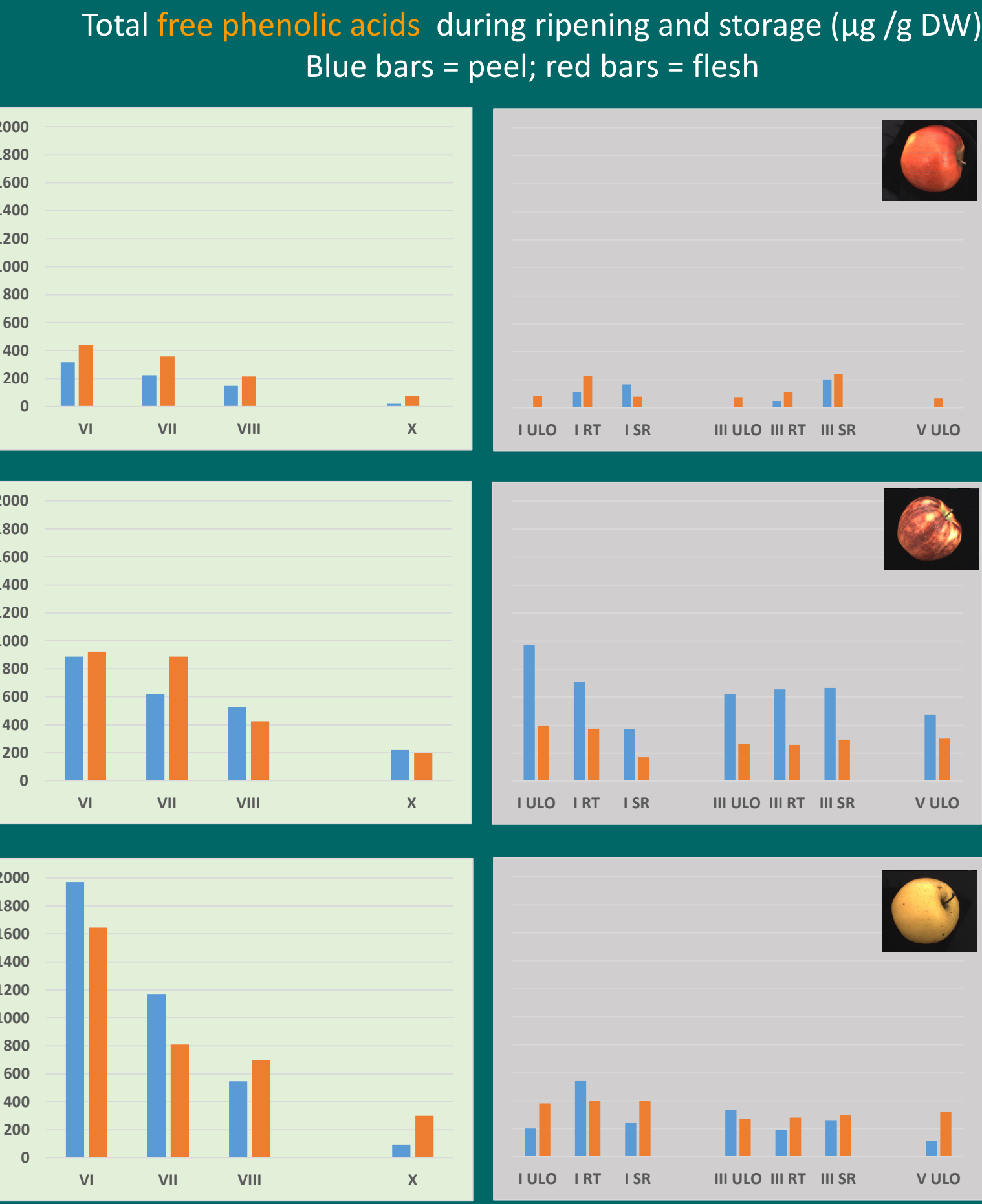
The content of neoxanthin, violaxanthin, antheraxanthin, lutein, zeaxanthin and β -caroten in fleshes of stored apples - in % from total carotenoids (left) and in µg / g DW (right)



Conclusions:

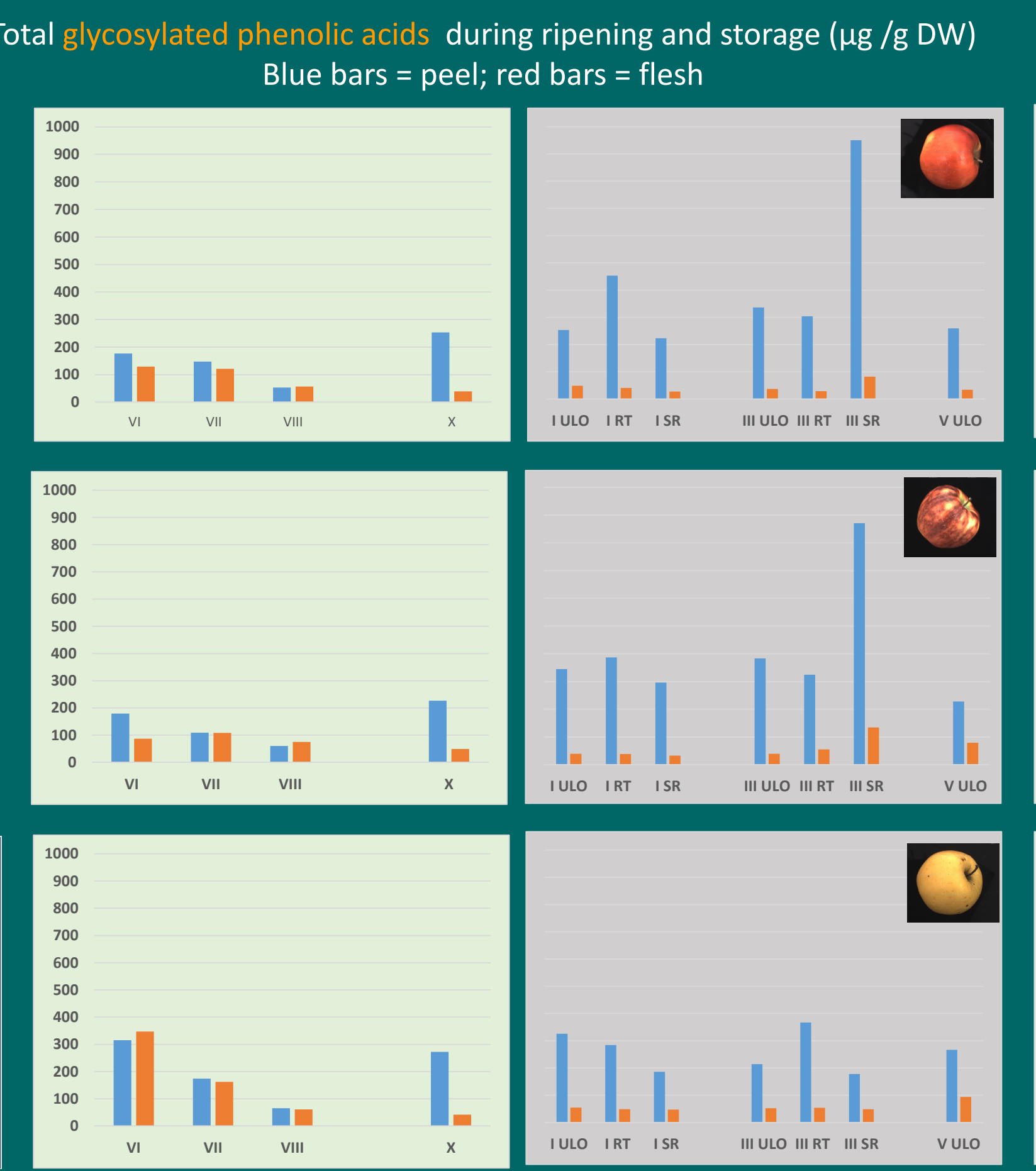
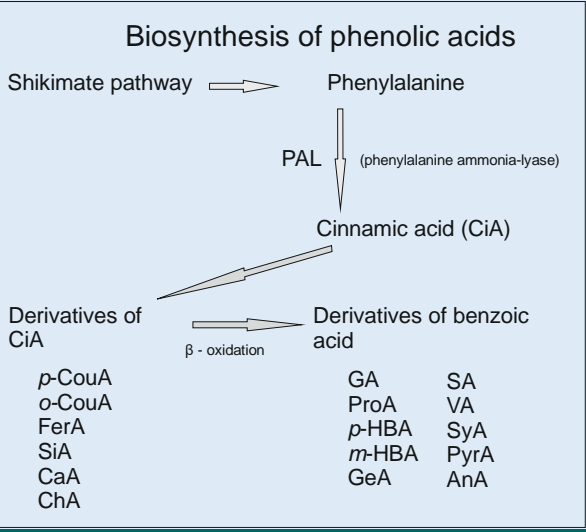
- carotenoids were mainly present in apple peels
- the highest content of carotenoids was found in harvested apples, it increased during ripening and then decreased during storage
- the ratio between individual carotenoids didn't change during storage
- fully regulated storage conditions (in ULO boxes) were able to prolong the time of storage and moreover to reduce the loss of carotenoids in stored apples

The content of phenolic acids



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-hydroxybenzoic acid
SyA – syringic acid
CiA – cinnamic acid



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 than spectrum of free ones
- the changes in the content of glycosylated phenolic acids in apples during ripening were smaller than in free ones
- the concentration of glycosylated phenolic acids increased in harvest and during storage – especially in apple peels
- Protocatechuic acid was the main glycosylated phenolic acid in peel; caffeic acid represented the main glycosylated phenolic acid in apple flesh
- 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 (β -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, 125x4 mm, ECOM, Czech Republic) with the solvent system acetonitrile:methanol:water (80:12:10 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.

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