

Is phenolic acid content in apples influenced by environmental factors?

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Introduction:

Phenolic compounds (phenylpropanoids with an aromatic ring bearing one or more hydroxyl groups and various other substituents) are produced by plants as secondary metabolites, ranging from simple monomers to very large polymers. Due to their antioxidant activity they play crucial roles in plant defences against both biotic and abiotic stressors, moreover they are an important component of animal diet and they are highly beneficial for human health.

As fruits represent the key source of antioxidants the knowledge of health-promoting substance contents in fruits affects consumers' perceptions of fruits quality. Apples are a widely available fruit exhibiting significant concentrations of antioxidants, however the concentrations and composition of antioxidants vary during ripening and subsequent handling of harvested fruits.

Aims:

- to specify the effect of exogenous (environmental) factors on the content and composition of phenolic acids (PhAs) - free and glycoside-bound in three cultivars of apples during ripening, at the harvest and after 3 months of storage

- the comparison of results obtained in apples harvested in two years with marked differences in the weather conditions - in 2017 and 2018

Material:

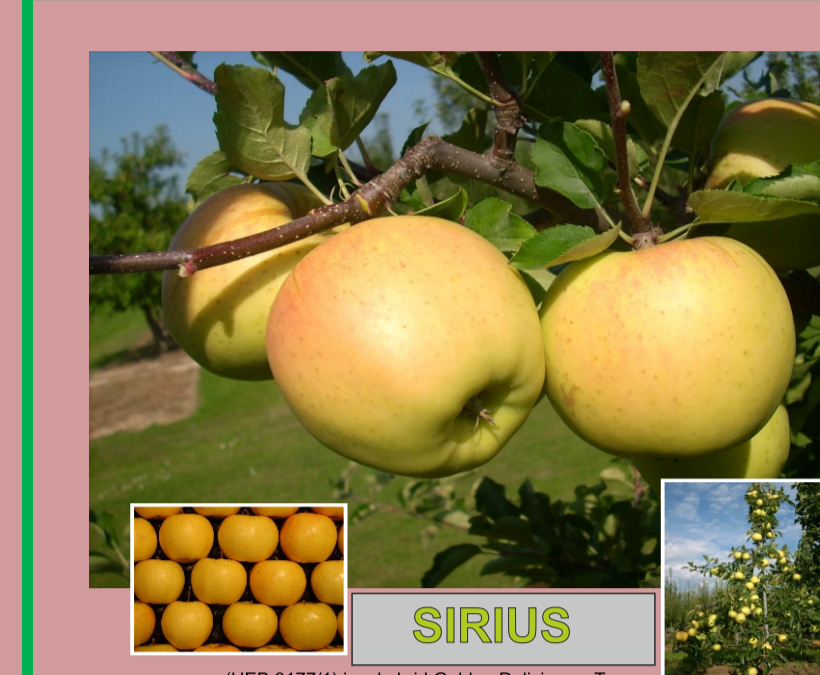
In our experiments we used three commercially successful scab-resistant and powdery mildew-tolerant apple cultivars originating from the Institute of Experimental Botany.



BONITA
(UEB 3147) is a hybrid between Topaz and Crisp Pink Plant Variety Rights, EU 42504, granted 06.02.2017. Applied for EU Plant Patent, 24.02.2017.
Origin: Institute of Experimental Botany Prague (Střelčice), CZ
Tree: Compact, medium vigorous, ramified, spreading, good branching with many fruiting spurs
Blossom: Mid-season, flowers heavily
Picking time: About 1 week after Golden Delicious
Productivity: Phytotoxic, high and mostly regular
Keeping quality: In cool storage about six months
Fruit: Size medium, shape globose with broad eye basin, stem thin and long, skin smooth, russet free, green yellow ground color is covered on 80 - 100 % with pink to brightly red overcoat, flesh firm, crisp, juicy with good, slightly sour taste
Diseases: Scab resistant based on V1 gene, low susceptibility to powdery mildew
Late ripening variety with very homogeneous russet red from overcoat, an appearance, high and regular yields, without need of fruit thinning and with good keeping and eating qualities. Suitable for commercial apple growing.

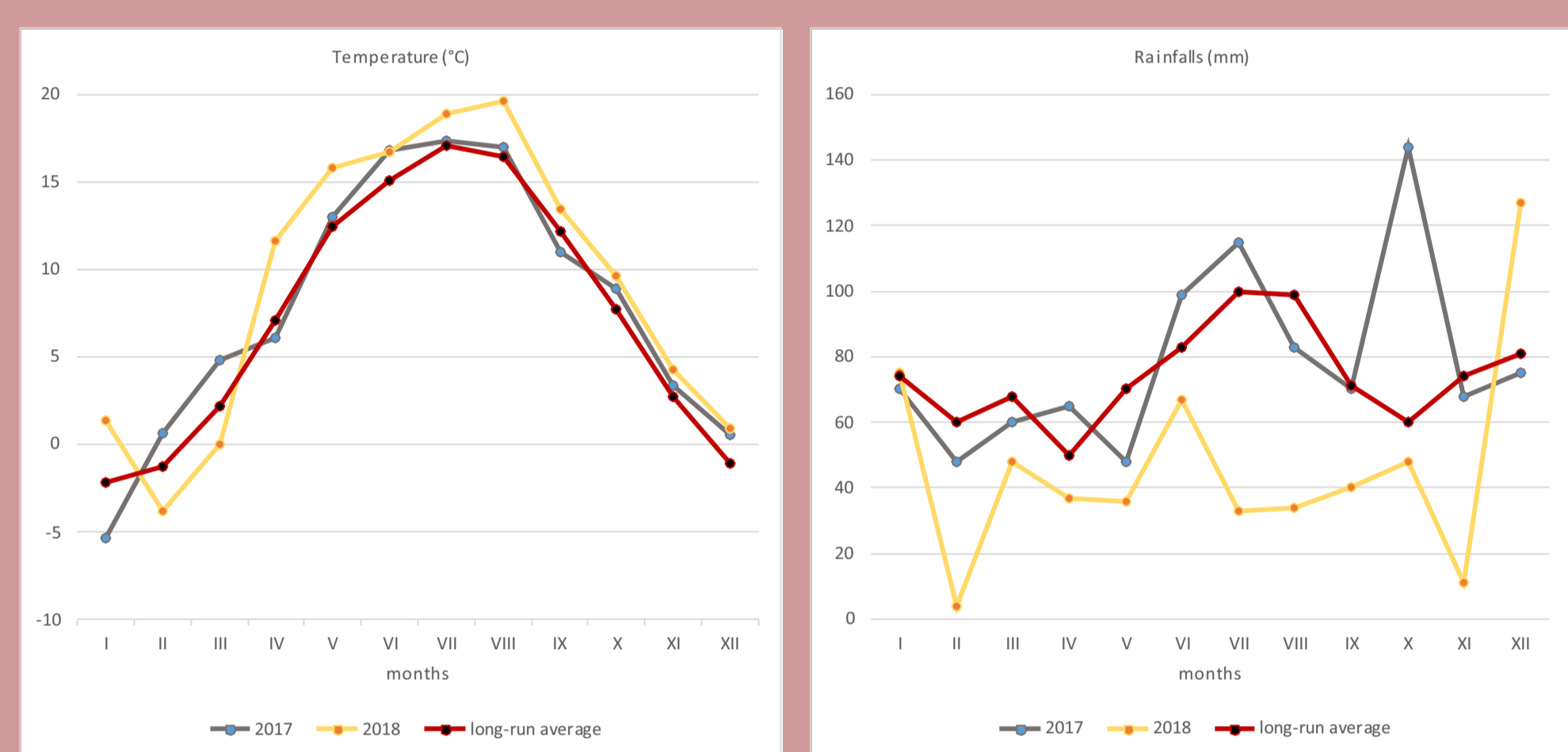


KARNEVAL
(UEB 3147) was produced by crossing Vanda with Crisp Pink (Pink Lady*) Community Plant Variety Rights EU 20005 from 24. 08. 2007
Origin: Institute of Experimental Botany Prague (Střelčice), CZ
Tree: Triplaxid, vigorous, spreading, branching medium, fruiting spurs medium to long
Blossom: Mid-season, flowers heavily and regularly, does not require fruit thinning
Picking time: Early October, about one week before Golden Delicious
Productivity: Phytotoxic, heavy and regular
Keeping quality: In natural storage until February
Fruit: Medium size, shape globose to conical, moderate ribbing, stem medium long, skin smooth, russet free, appearance outstanding, multicoloured with prominent red stripes on yellow ground color, flesh white, juicy, tender with slightly aromatic good taste
Diseases: Resistant to scab (V1), tolerant to mildew
Russet, healthy and productive dessert variety, without special requirements for growing conditions, outstanding in very attractive coloration, free of russet especially for Christmas time



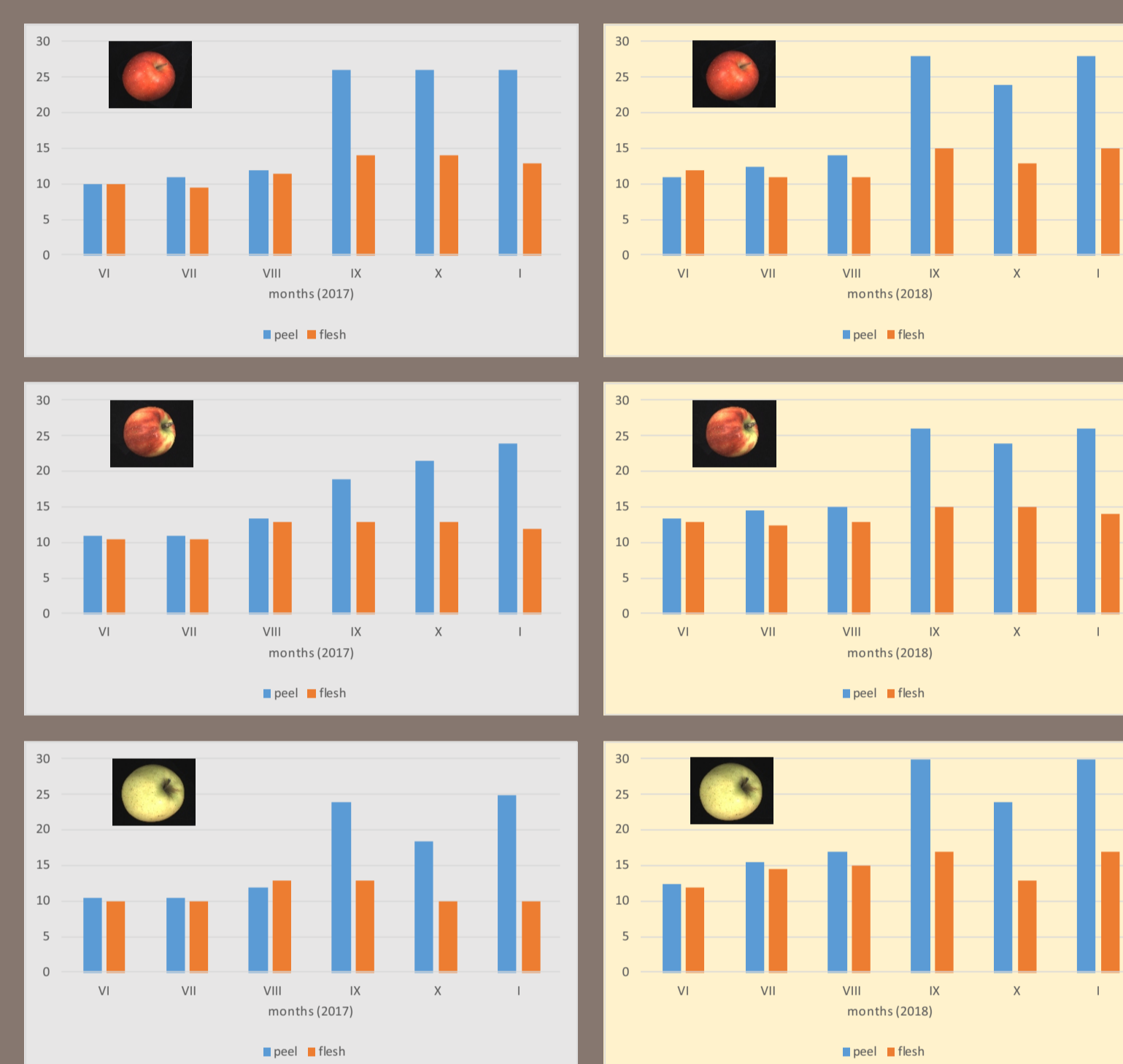
SIRIUS
(UEB 31771) is a hybrid Golden Delicious x Topaz
Community Plant Variety Rights EU 20005 from 24. 08. 2007
Origin: Institute of Experimental Botany Prague (Střelčice), CZ
Tree: Triplaxid, vigorous, spreading, branching medium, fruiting spurs medium to long
Blossom: Mid-season, slightly before Golden Delicious, flowers not medium, regular
Picking time: Towards mid-October, about 10 days after Golden Delicious, fruits hang mostly singly without thinning
Productivity: Phytotoxic, produces regular good crops
Keeping quality: In natural storage until April, eating maturity 4 weeks after picking
Fruit: Medium to large, round, height: width ratio 0.25, stem long and medium thick, some fine russet may be present in the stem cavity, ground color green yellow to yellow, occasionally with a slight red blush, flesh yellow, firm, crisp, fine grained, very juicy, well balanced sugar (14.7 % Brix) and acid level, rich flavour
Resistant to scab (V1), tolerant to powdery mildew, absence of bitter pit
Diseases: The variety can be considered for organic production as well as for IFF systems, growing requirements seem to be similar to Golden Delicious
Comment: Treatments against scab, rust appearance, very interesting variety with many good qualities

The values of temperature and rainfall in 2017 and 2018 years (according to Czech Hydrometeorological Institute http://portal.chmi.cz)
Long run average was estimated from the data obtained in 1981 - 2010 years.



The average month's temperature in 2017 was 7.9 and in 2018 - 9°C, i. e. 107% resp. 122% of long run temperature average.
The total water intake was 945 mm in 2017 in contrast to 559 mm in 2018, i. e., 106% resp. 63% of long run average.

The changes of peel and flesh dry weight (%) during ripening - from June(VI) to October (X) and after 3 months of storage (I)



Water content in peels and flesh of all cultivars was not affected by different weather conditions.

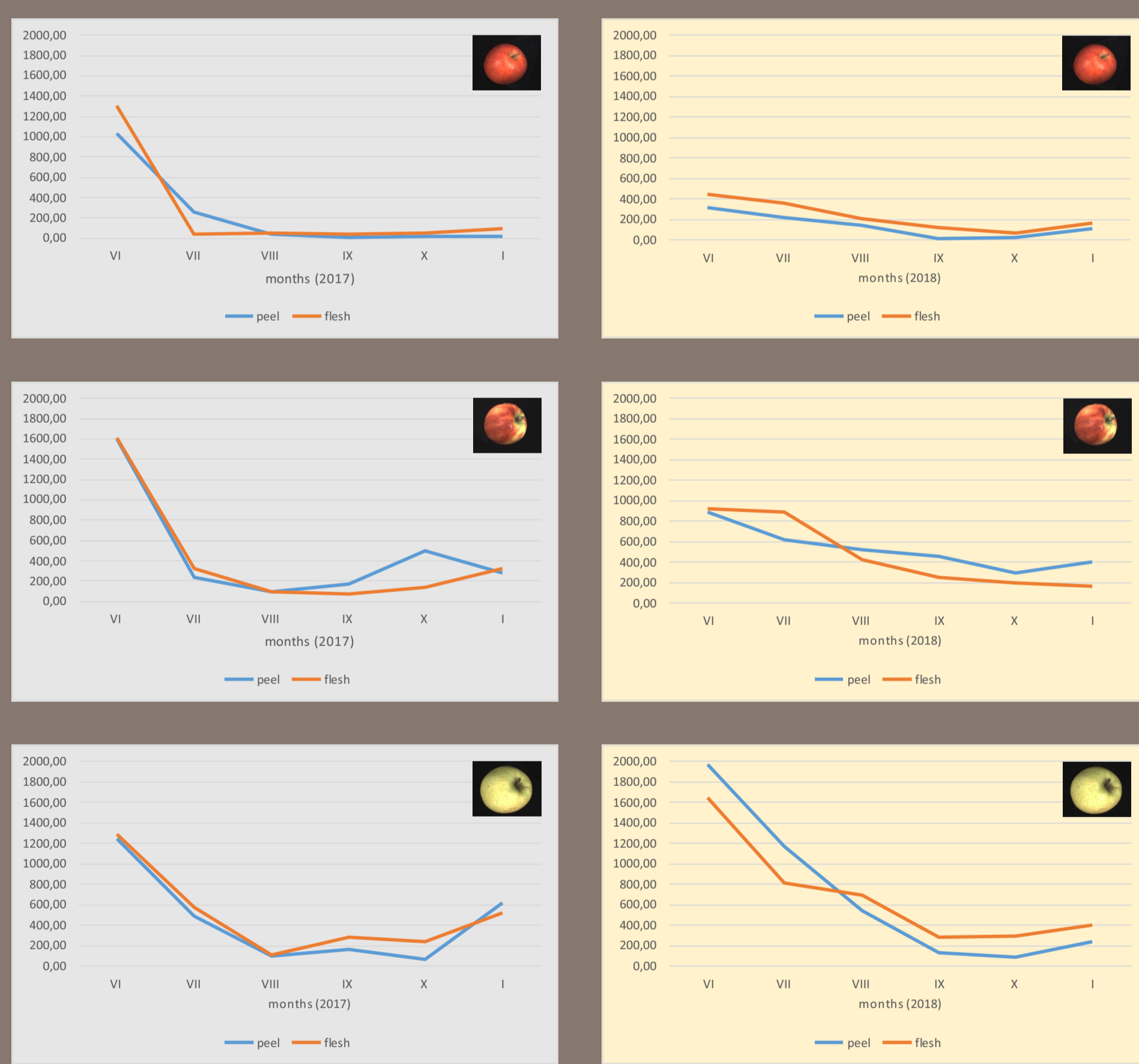
Summary:

- Free PhAs
 - the highest total content of free PhAs in non-matured apples (in June)
 - decrease in free PhAs content during ripening
 - ChA constitutes 98-99% of free PhAs in peels and flesh
 - similar content and spectrum of free PhAs in peels and flesh of harvested apples (in October) from both years
- Glycoside-bound PhAs
 - higher content of glycoside-bound PhAs in peels than in flesh
 - the highest concentration of glycoside-bound PhAs in peels of harvested and (stored) apples
 - lower total content of glycoside-bound PhAs in apples from 2018 than those from 2017
 - broader spectrum of glycoside-bound than free PhAs
 - CaA dominates in flesh of apples from 2018 (not from 2017)
 - difference in ratio between ProA and CaA in peels as well as in flesh of apples from 2017 and 2018
- The changes in the spectrum and content mainly of glycoside-bound PhAs might be induced by environmental factors.

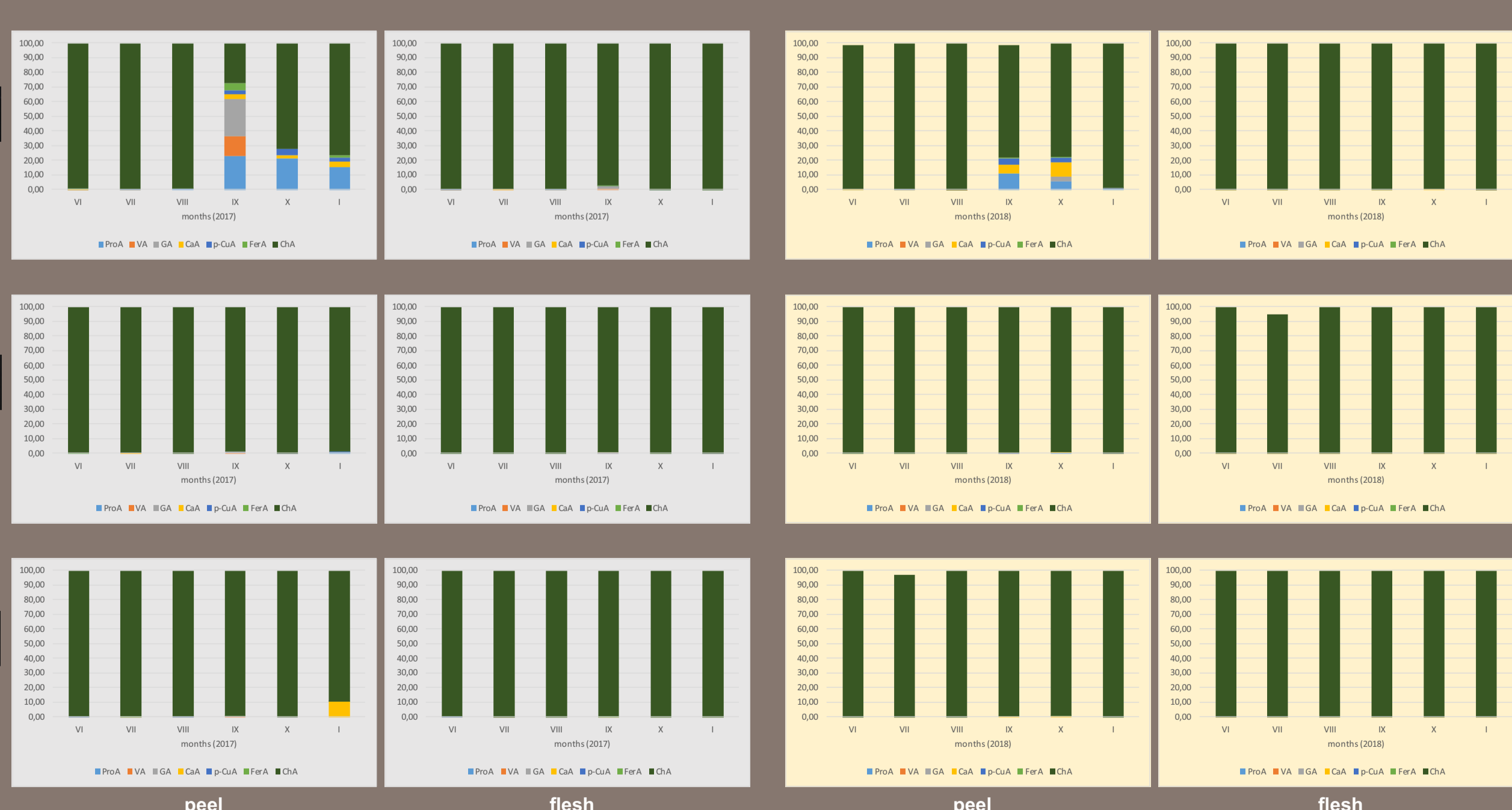
No differences in the course of total free PhAs content changes during ripening in apples from 2017 and 2018 were found. Total content in peels and flesh of harvested apples(X) of all three cultivars was similar in both years, though small differences in free PhA contents in non-matured apples(VI and VII) were obvious.

The total content of glycoside-bound PhAs was lower in peels of apples from 2018. No difference was found in flesh where the content was lower than in peels. The course of changes of glycoside-bound PhAs was similar in peels of apples from 2017 and 2018.

Total content of free phenolic acids in peels and flesh of apples (µg/g DW)



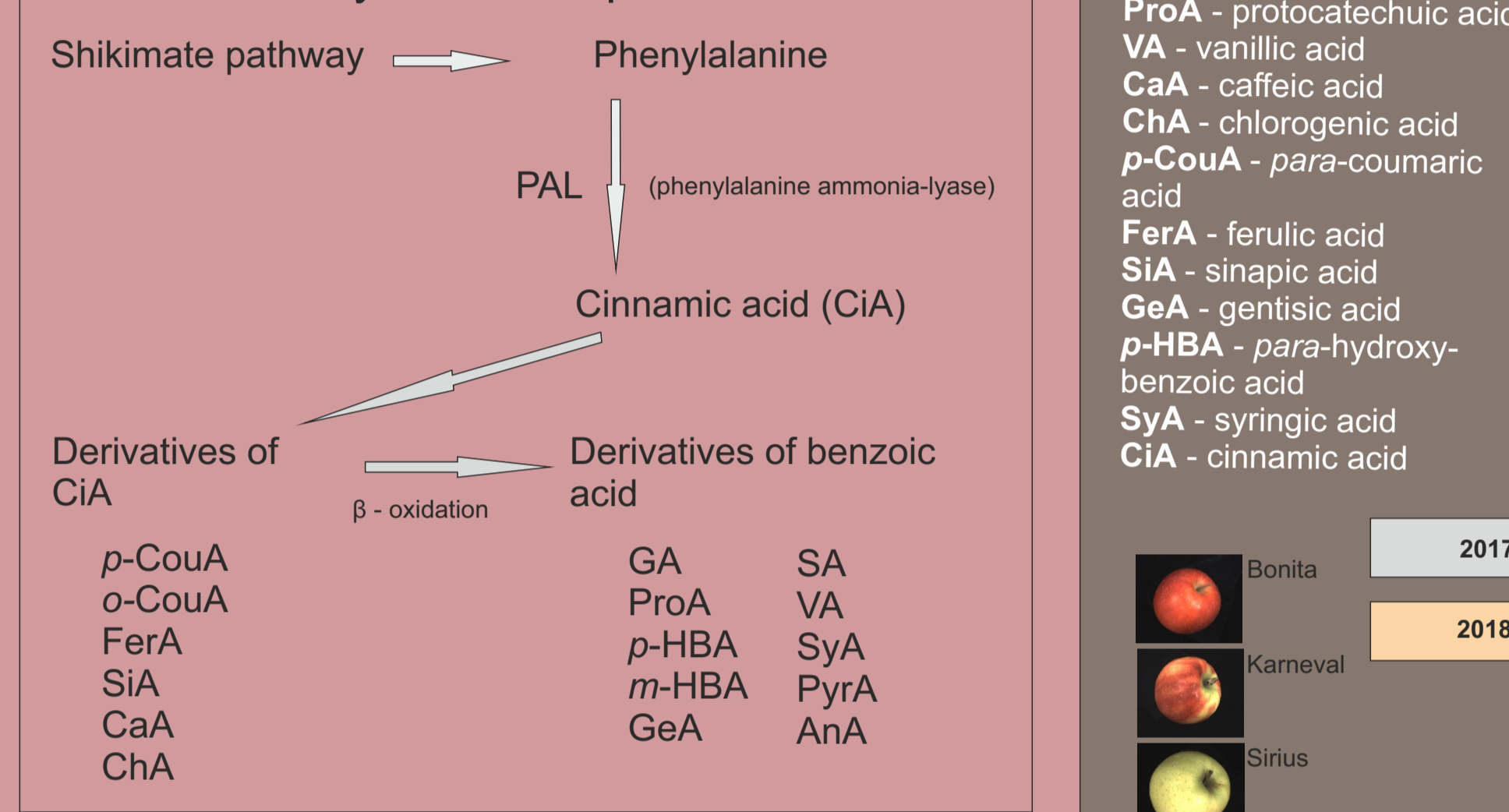
Spectrum of free phenolic acids presented in peels and flesh of apples (% of total content of free PhAs)



Chlorogenic acid represented the main free PhA in peels and flesh of all three apple cultivars gathered in 2017 and 2018. It constituted more than 98% whereas other PhAs less than 0.1% of total content of free PhAs (excepting cv. Bonita during harvest).

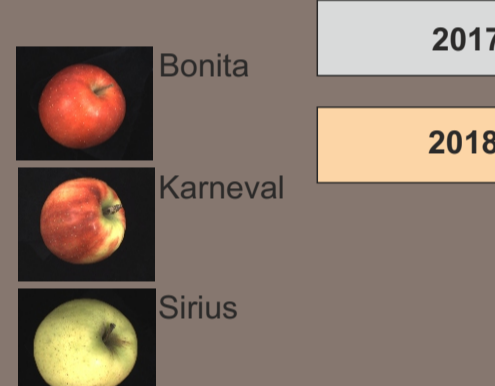
ProA and CaA were the main glycoside-bound PhAs in apple peels and flesh; the ratio between CaA and ProA was higher in apples from 2018. GeA and p-HBA constituted approx. 10-15% of total glycoside-bound PhAs, and the other individual PhAs represented only minor part of total content of glycoside-bound PhAs in all apples.

Biosynthesis of phenolic acids

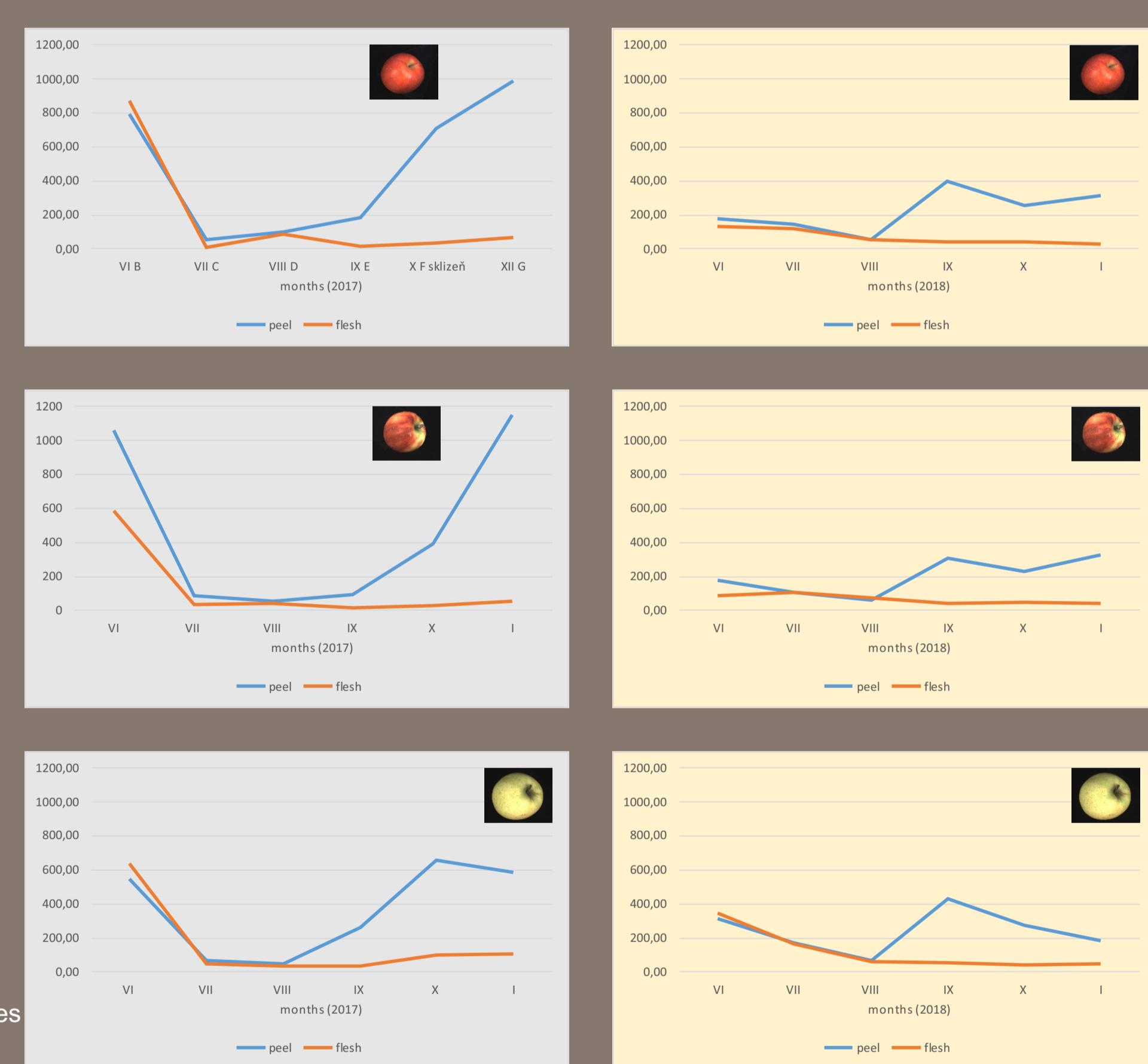


Abbreviations:

- 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-hydroxy-benzoic acid
- SyA - syringic acid
- CiA - cinnamic acid



Total content of glycoside-bound phenolic acids in peels and flesh of apples (µg/g DW)



Spectrum of glycoside-bound phenolic acids in peels and flesh of apples (% of total content of glycoside-bound PhAs)



Methods

Phenolic acid analysis
Sample preparation
Samples of approx. 50-200mg of fresh weight were homogenized in 80% (v/v) methanol in Eppendorf vial tubes using a mixer mill. After addition of isotopically labelled internal standards they were left in the fridge overnight. The mixture was then centrifuged and the solids were re-suspended in 80% methanol and extracted in ultrasonic bath. After centrifugation the combined supernatants were evaporated to water phase and acidified to pH 2. The acid solution was extracted three times by diethyl ether. This extract was prepared for free phenolic acid analysis.
The acidified water phase was left in fume for approx. 30 min to remove rest of diethylether and then transferred into crimp vial. Isotopically labelled internal standards and concentrated HCl were added, crimped and heated at 105°C for 1 hour. After cooling the reaction mixture was transferred into falcon tube; pH was adjusted to value 2 and the mixture was three times extracted by diethyl ether. This extract was prepared for glycoside-bound phenolic acids analysis.
The diethyl ether extracts were evaporated by the rotation vacuum concentrator (RVC) and stored in a freezer box to the final analysis.

LC-MS analysis
The evaporated samples were dissolved in 0.2 ml of 50% methanol, transferred into 0.5 ml polypropylene vials and placed into cooled stack of autosampler. The partition of 5 µl was injected on LC-MS system consisting of autosampler with cooling stack, quaternary HPLC pump and triple-quad mass spectrometer equipped with electrospray interface.
The chromatographic analysis was 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 with acquisition 3 to 8 transition for each compound. The most abundant ion was used for quantification, the others for identity confirmation. The analytes were quantified by the multilevel calibration graph with deuterated compounds used as internal standards.

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