

The effect of storage on the profile of phenolic compounds in selected apple varieties

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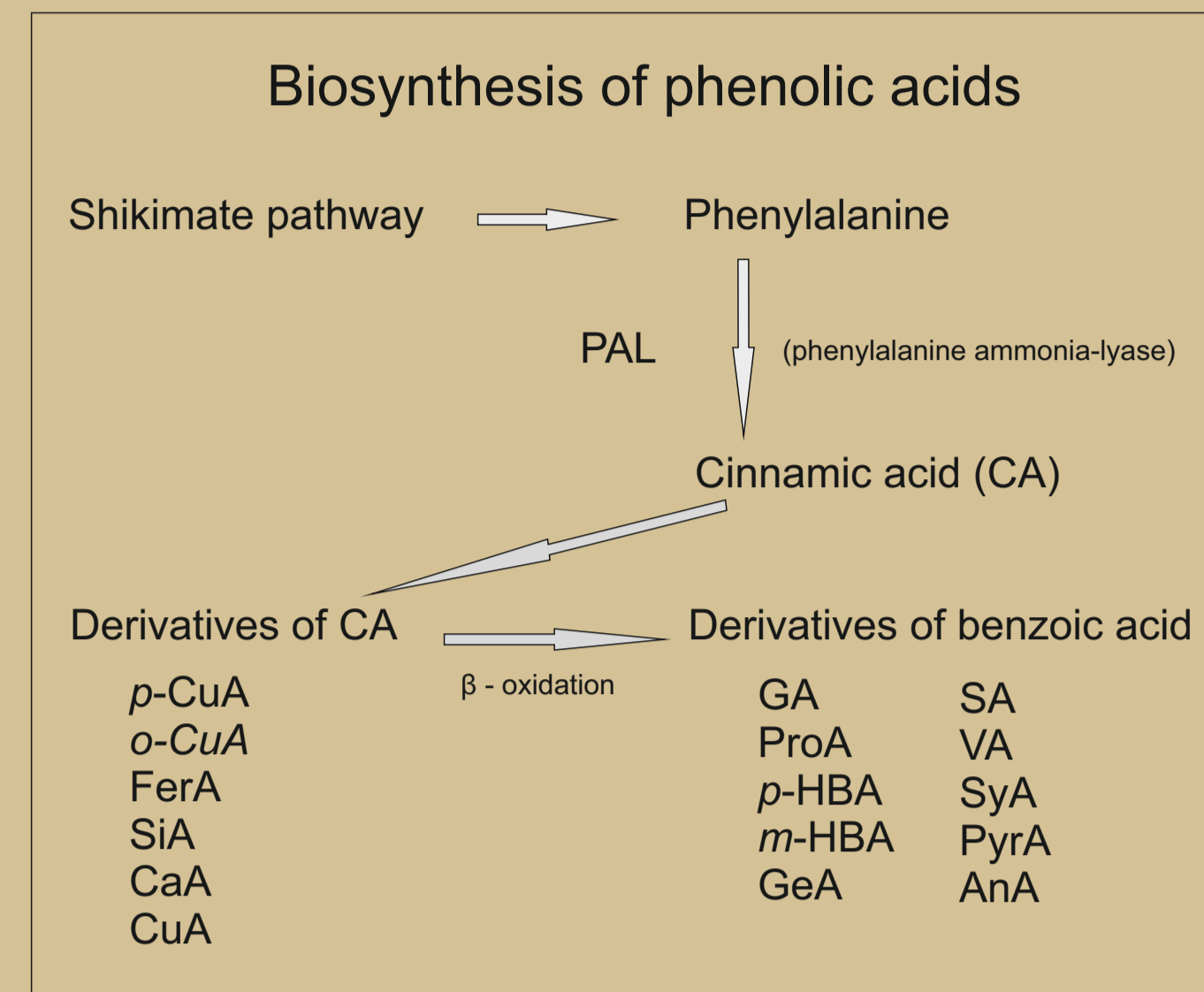


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Introduction:

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 a relatively high content of antioxidants including phenolic compounds that belong to the health-promoting phytochemicals. Phenolic compounds constitute a substantial and an important group of phenylpropanoids produced by plants as secondary metabolites. The health-promoting effects of phenolic compounds depend on their bioaccessibility from the food and their consequent bioavailability. However, many phenolics occur in nature as glycosylated derivatives. Thus in order to become bioactive in the human body, these bound forms must undergo transformations, which occur due to the action of digestive enzymes. The aim of this work was to determine the concentration of phenolic acids in selected apple varieties originating from the Station of apple breeding of the IEB. We investigated three different varieties of apples (red, yellow and streaked) for their phenolic acid contents in peel and flesh immediately after the harvest and after 7 months of storage. In the second part of investigation we detected the changes in phenolic acid contents in red, yellow and streaked apples during their maturation (from June to October, i.e. to harvest).



Abbreviations:

PhA - phenolic acids

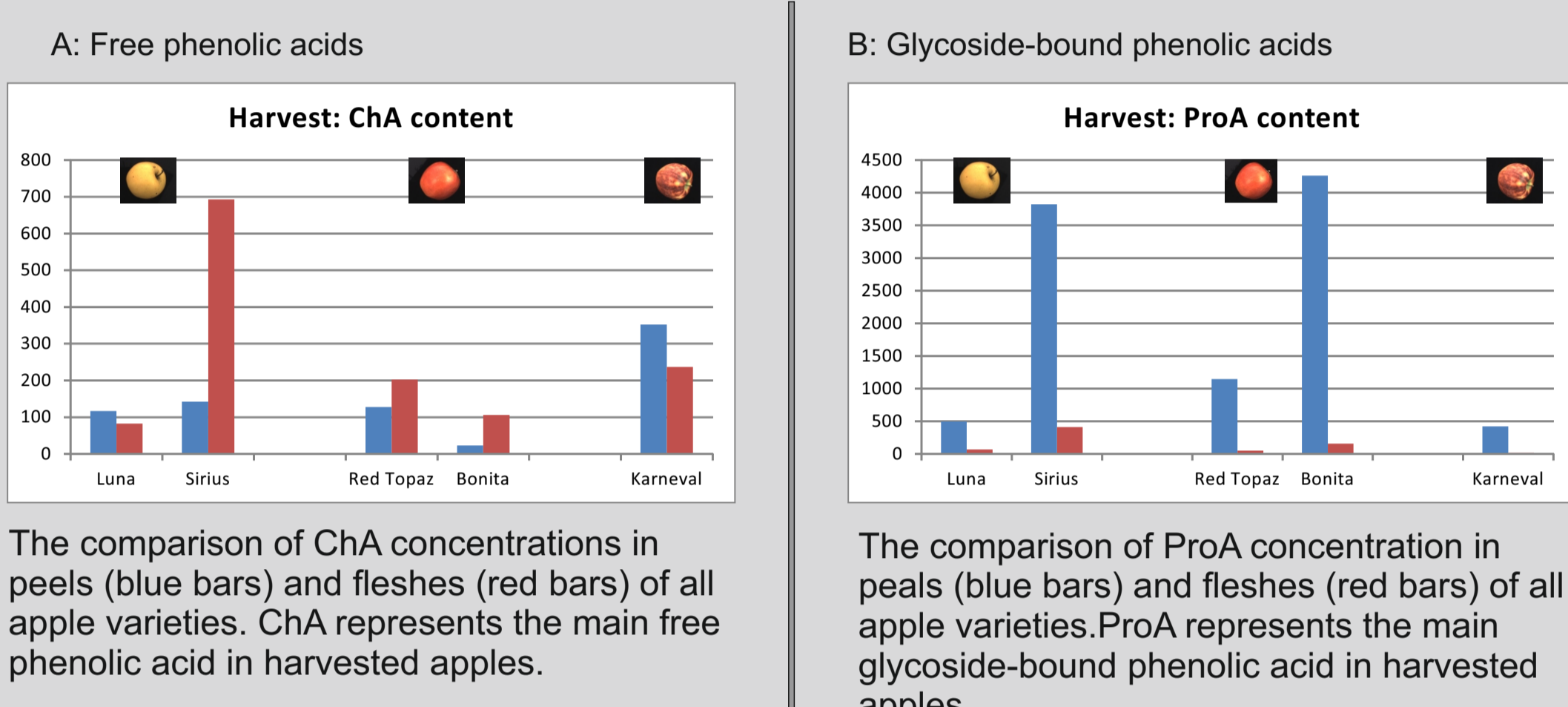
- GA - gallic acid
- ProA - protocatechuic acid
- GeA - gentisic acid
- PyrA - pyrocatechuic acid
- p-HBA - *para*-hydroxy-benzoic acid
- m-HBA - *meta*-hydroxy-benzoic acid
- SA - salicylic acid
- VA - vanillic acid
- CaA - caffeic acid
- ChA - chlorogenic acid
- SyA - syringic acid
- p-CuA - *para*-coumaric acid
- o-CuA - *ortho*-coumaric acid
- FerA - ferulic acid
- SiA - sinapic acid
- AnA - Anisic acid

Material:

Phenolic acid contents were measured in five varieties of apples harvested in years 2016 and 2017.

All concentrations of phenolic acids are in nmol/g DW. The content of phenolic acids was analysed in apple peels (blue bars) and fleshes (red bars).

The content of phenolic acids in all apple varieties just after harvest (in October)



The comparison of ChA concentrations in peels (blue bars) and fleshes (red bars) of all apple varieties. ChA represents the main free phenolic acid in harvested apples.

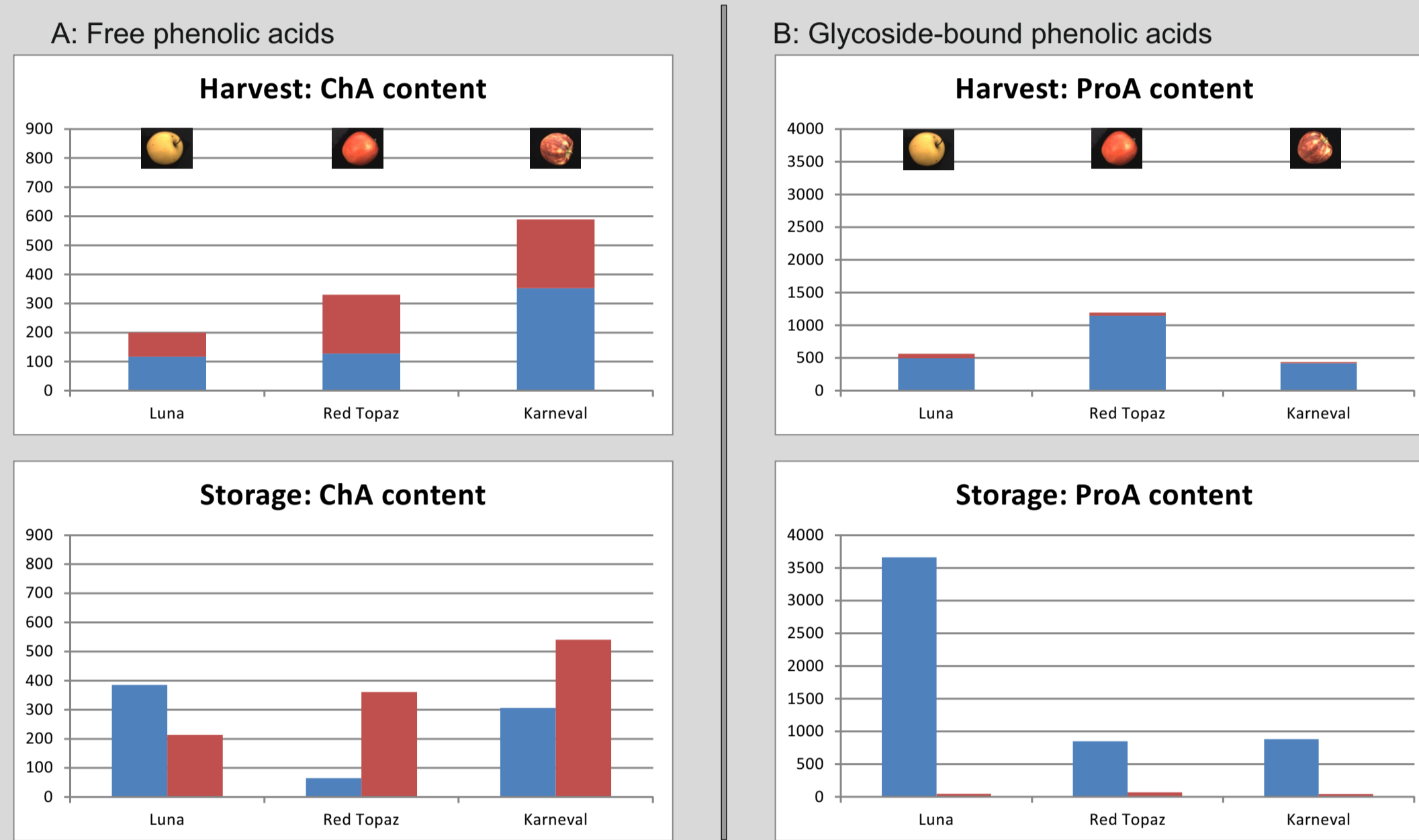
The comparison of ProA concentration in peels (blue bars) and fleshes (red bars) of all apple varieties. ProA represents the main glycoside-bound phenolic acid in harvested apples.

Further individual phenolic acids detected in harvested apples



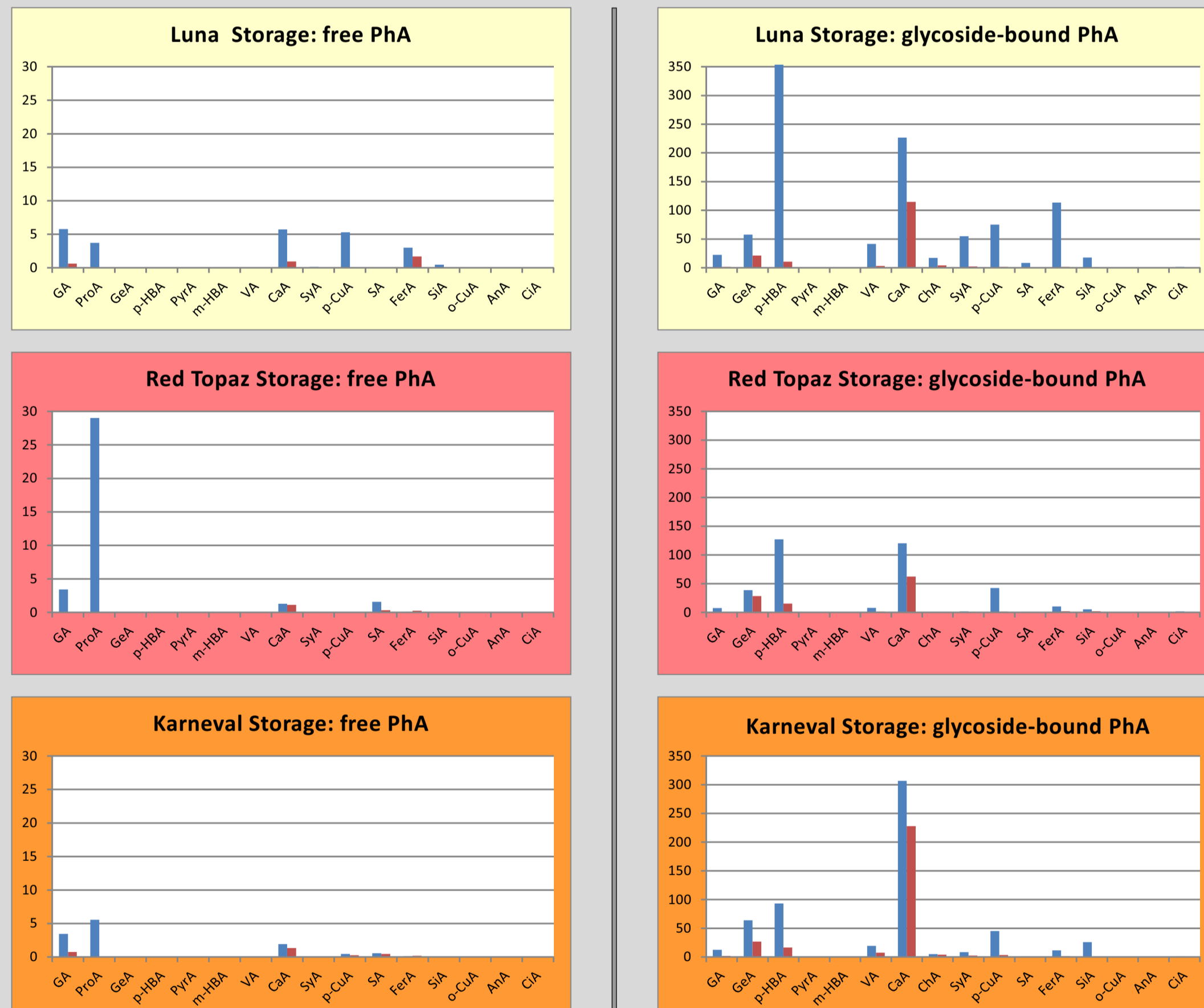
Free PhAs were present in low concentrations, relatively higher level of ProA was found in the red coloured varieties of apples. Glycoside-bound form PhA were detected in higher concentrations. The highest content of CaA, p-HBA, followed by lower contents of GeA, VA and p-CuA were found in all studied apple varieties.

The effect of storage on phenolic acid contents (7 month of storage under the darkness and temperature 5°C)



The content of free ChA and glycoside-bound ProA was higher in stored apples. The most substantial increase was observed in Luna cultivar - i.e. 3 and 7 multiple of the value determined in harvested apples, respectively.

Further individual phenolic acids detected in stored apples

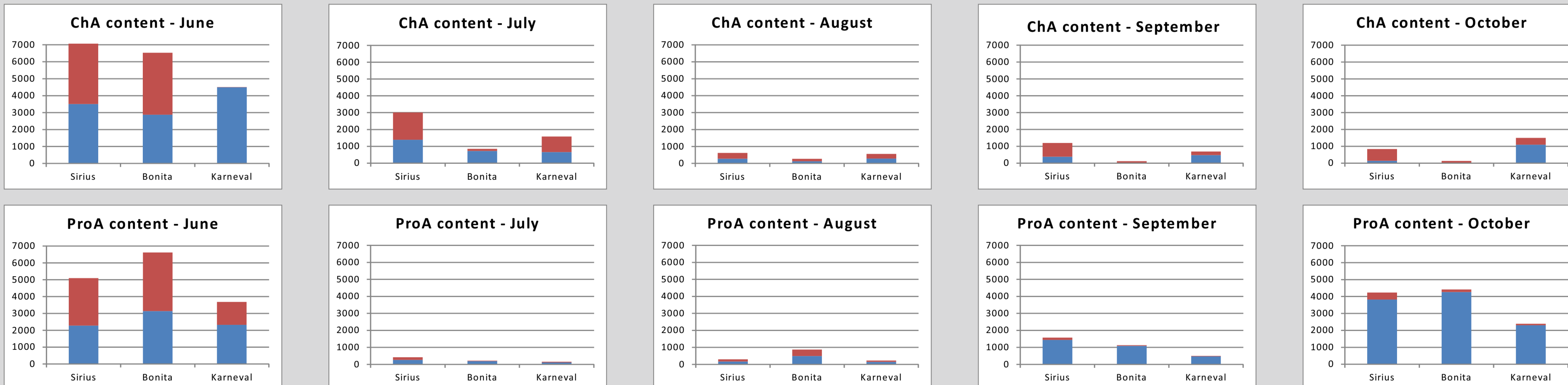


No marked differences in the contents of phenolic acids were observed in the course of storage.

Conclusion

The ChA was the major free PhA in both peels and fleshes of all the studied apple varieties. The most abundant glycoside-bound phenolic acid was protocatechuic acid. Both these acids exhibit a strong scavenging activity and thus may contribute considerably to the total antioxidant potential of apples.

Phenolic acid contents during maturation - from June till October



The content of the free ChA both in peels and fleshes of the studied varieties continuously decreased during the fruit maturation (from June to October). In a similar way a significant decrease of the glycoside-bound ProA was observed in July and August. However in the course of the continuing maturation over September and October the bound-ProA concentration increased in the peels of all the studied apple varieties.

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-quadrupole 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.

