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

<u>Zuzana Vondráková</u> and Milena Cvikrová - Laboratory of Biologically Active Compounds Alena Trávníčková and Jiří Malbeck - Laboratory of Mass Spectrometry Radek Černý and Miloslav Juříček - Station of Apple Breeding for DiseaseResistance



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



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 Octoberi.e. to harvest).



Material:

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

Derivatives of CA		_ Derivatives of benzoic acid		VA - vanillic acid
<i>p</i> -CuA <i>o-CuA</i> FerA SiA CaA CuA	β - oxidation	GA ProA <i>p</i> -HBA <i>m</i> -HBA GeA	SA VA SyA PyrA AnA	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
				\square ANA - ANISIC ACIO

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 most substantial increase was observed in Luna cultivar - i.e. 3 and 7 multiple of the value

	(UEB 3375/2) is a hybrid Topaz with Golden Delicious			
	Community Plant Variety Rights EU 22063 from 07. 04. 2008 United States patent PP 16,084 from 01. 11. 2005 as UEB 3375/2			
rigin:	Institute of Experimental Botany Prague (Strizovice), CZ			
ree:	Diploid, vigor and habit similar to Golden Delicious			
lossom:	Mid-season, 1 day before Golden Delicious, flower set heavy, somewhat alternating as in Golden Delicious			
icking time:	Early to mid October, about one week after Golden Delicious, fruit thinning is recommended, eating maturity 3 weeks after picking			
roductivity:	Similar to Golden Delicious			
eeping quality:	In natural storage until beginning of April			
ruit:	Medium, round, height : width ratio 0.90, weakly ribbed, stem thin and long, skin russet free, yellow-green becoming yellow during storage, occasionally with a slight orange blush, flesh yellowish, firm, crisp, fine grained, very juicy, well-balanced sugar/acid ratio, sugar content 13.5 % Brix, good aromatic flavor			
iseases:	Resistant to scab (Vf), moderately tolerant to powdery mildew, some mildew can occur on shoot tips, bitter pit has not been observed			
omment: Institute of E Botany of th	The variety is considered mainly for IFP orchards but also for organic production, its growing requirements appear similar as in Golden Delicious except treatments against scab e AS CR, v. v. i.			



grained, very juicy, well balanced sugar (14,7 % Brix) and acid level, rich flavou Resistant to scab (Vf), tolerant to powdery mildew, absence of bitter pit he variety can be considered for organic production as well as for IF tems, growing requirements seem to be similar to Jonagold except treatments against scab, nice appearance, very interesting variety with many good qualities Botany of the AS CR, v. v. i. Institute of Experimental





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.

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.

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 concentation increased in the peels of all the studeid apple varieties.

	(UEB I-406/1) is a hybrid between Topaz and Cripps Pink Plant Variety Rights EU 45354, granted 06.02.2017 Applied for US Plant Patent, 24.03.2017					
1:	Institute of Experimental Botany Prague (Střížovice) CZ					
	Diploid, medium vigorous, ramified, spreading, good branching with many fruiting spurs					
om:	Mid-season, flowers heavily					
ng time:	About 1 week after Golden Delicious					
ctivity:	Precocious, high and mostly regular					
ng quality:	In cool storage about six months					
	Size medium, shape globose with broad eye basin, stem thin and long, ski smooth russet free, green yellow ground color is covered on 80 - 100 % with pink to brightly red overcolor, flesh firm, crisp, juicy with good, slightly soutaste					

smooth russet free, green yellow ground color is covered on 80 - 100 % with pink to brightly red overcolor, flesh firm, crisp, juicy with good, slightly sour taste				
Scab resistant based on Vf gen, low susceptibility to powdery mildew				
Late apple variety with very homogenous nicely red fruits outstanding an				
perimental AS CR, v. v. i.	thinning and with good keeping and eating qualities. Suitable for commercial apple growing.			
	smooth russ pink to brigh taste Scab resista Late apple perimental AS CR, v. v. i.			



- Origin: Institute of Experimental Botany Prague (Strizovice), CZ
- spreading, go many fruiting spurs
- Blossom: Mid-season, flowers heavily and regularly, does not require fruit thinning
- **Picking time:** Early October, about one week before Golden Delicious

Productivity: Precocious, heavy and regular Keeping quality: In natural storage until February

- Medium size, shape globose to conical, moderate ribbing, stem medium long, skin smooth, appears russet free, appearance outstanding, multi-coloured with prominent red stripes on yellow ground color, flesh white, juicy, finely acid with slightly aromatic good flavor **Diseases:** Resistant to scab (Vf), tolerant to mildew
- Institute of Experimental Botany of the AS CR, v. v. i.

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 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 HCI 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-bond 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.