

CHANGES DURING FRUIT DEVELOPMENT OF RED- AND WHITE-FLESHED APPLE CULTIVARS

Martina Kolarek^{1, 4*}, Michael Neumüller², Thomas Hoffmann¹, Johannes Hadersdorfer⁴, Heidi Halbwirth³, Wilfried Schwab¹

¹Biotechnology of Natural Products, Technical University of Munich, Freising, Germany ²Bavarian Centre of Pomology and Fruit Breeding, Hallbergmoos, Germany ³Institute of Chemical, Environmental and Bioscience Engineering, Technische Universität Wien, Vienna, Austria ⁴Associate Professorship of Fruit Science, Technical University of Munich, Freising, Germany

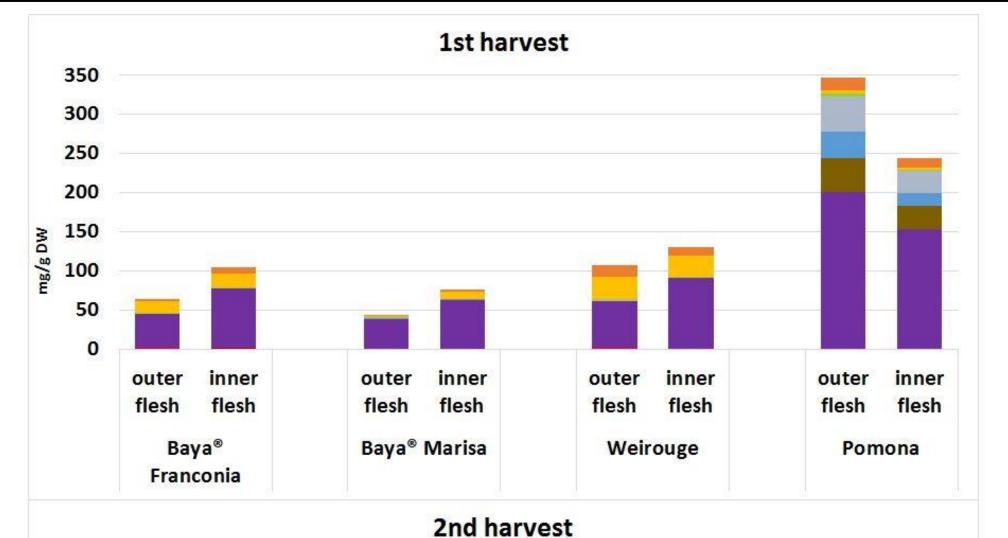
* kolarek@wzw.tum.de

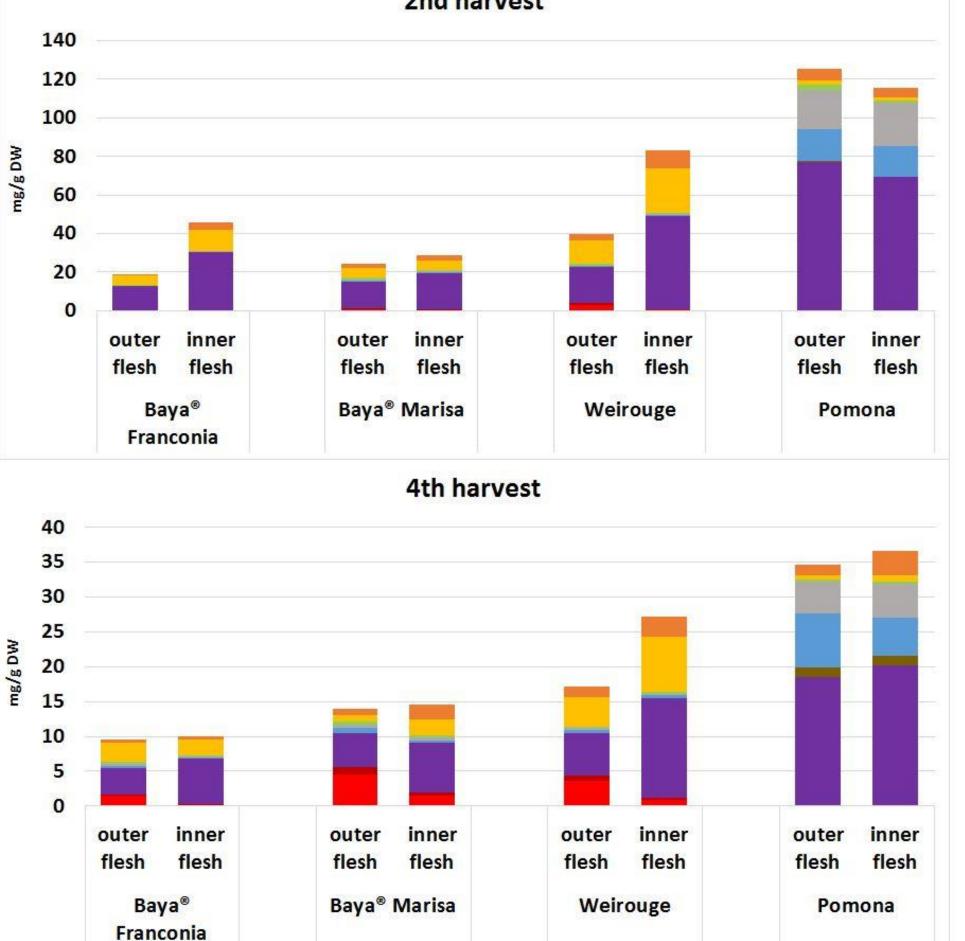
Red-fleshed apples are increasingly important for consumer preference and marketability, not only due to aesthetic reasons, but also due to the health benefits associated particularly with red pigments. Anthocyanins and other phenolic compounds are important components of red-flesh apples and are responsible for the colour and flavour of the fruit ^{1,2}. Due to their particular anthocyanin contents, red-fleshed apples have received increased awareness.

> 'Baya[®] Franconia' and 'Baya[®] Marisa' are modern and

The objective of this research

aromatic type-1 red-fleshed apple cultivars with an attractive bicolored fruit flesh. They were bred at the Bavarian Centre of Pomology and Fruit Breeding. The apple fruits of these cultivars develop a broad red colored stripe below the skin that turns into a marbled pattern towards the core of the fruit. These cultivars are progenies of a white flesh apple cultivar 'Pomona' and a type-1 red-fleshed apple cultivar 'Weirouge'. was to evaluate the polyphenolic profile between the cultivars and their parental lines during development by means of high performance liquid chromatography (HPLC). The acquired detailed information on phenolic pattern could sustain future breeding efforts towards additional improvement of apple fruit quality and could support the potential of red-fleshed apples as functional food.





Fruits were harvested at four different stages of fruit development (07.06, 27.6, 25.07 and 28.8.2018). The flesh was divided into outer (red) and inner (white) part. The separated sections were frozen in liquid nitrogen and stored at -20 °C. After Iyophilisation, extraction was done with 95% MeOH with 4-Methylumelliferyl-glucuronide (0,2 mg/ml) included as an internal standard.

To evaluate the phenolic profiles, reverse phase high performance liquid chromatography (RP-HPLC) analysis was performed with a Nucleosil C18 column followed by post derivatization with DMACA (p-Dimethylaminocinnamaldeyde) as described in ³ using the Agilent Infinity 1260 system. Chromatograms were evaluated with Agilent OpenLab 2.3 software.

5

Pomona
Weirouge
Baya® Marisa
Baya® Franconia

A)
Image: Comparison of the second second

Five phenolic subgroups were analysed in the outer and inner parts of the different apple genotypes during their development. Following metabolites were quantified within the subgroups: cyanidin-3-O-galactoside (anthocyanins); phloridzin and phloretin 2'-O-xylosy-glucoside (dihydrochalcones); chlorogenic acid (hydroxycinnamic acid); catechin, procyanidin B2 and epicatechin (flavan-3-ols) and flavonols as observed in previous studies ^{4,5}.

6

19

📕 ideain 📕 other anthocyanins 📕 chlorogenic acid 📕 catechin 📕 procyanidin B2 🔳 epicatechin 📕 total flavonols 📕 phloridzin 📕 phloretin 2-xylosyl-glucoside

Fig 2. RP-HPLC analysis of unripe and ripe apple fruits showing five phenolic subgroups in the outer and inner tissue from three red-flesh apple cultivars; 'Baya[®] Franconia', 'Baya[®] Marisa' and 'Weirouge' and a white flesh cultivar 'Pomona'.

Further steps include LC/MS, gene expression and transcriptomic analysis to uplift future breeding efforts towards further advancement of red-fleshed apple quality.



Fig 1. Fruit developmental stages of 'Pomona', 'Weirouge', 'Baya[®] Marisa' and 'Baya[®] Franconia'. (A) first harvest at 07.06; (B) second harvest at 27.06; (C) third harvest at 25.07 and (D) fourth harvest at 28.08.2018.

References

(1) Espley, R. V.; Hellens, R. P.; Putterill, J.; Stevenson, D. E.; Kutty-Amma, S.; Allan, A. C. Red colouration in apple fruit is due to the activity of the MYB transcription factor, MdMYB10, *The Plant journal : for cell and molecular biology.* 2007, 49, pp. 414–427.
(2) Malec, M.; Le Quéré, J.-M.; Sotin, H.; Kolodziejczyk, K.; Bauduin, R.; Guyot, S. Polyphenol profiling of a red-fleshed apple cultivar and evaluation of the color extractability and stability in the juice, Journal of agricultural and food chemistry. 2014, 62, pp. 6944–6954.
(3) Treutter, D.; Wang, D.; Farag, M. A.; Baires, G. D. A.; Rühmann, S.; Neumüller, M. Diversity of phenolic profiles in the fruit skin of Prunus domestica plums and related species, *Journal of agricultural and food chemistry*. 2012, 60, pp. 12011–12019.
(4) Bars-Cortina, D.; Macià, A.; Iglesias, I.; Garanto, X.; Badiella, L.; Motilva, M.-J. Seasonal Variability of the Phytochemical Composition of New Red-Fleshed Apple Varieties Compared with Traditional and New White-Fleshed Varieties, *Journal of agricultural and food chemistry*. 2018, 66, pp. 10011–10025.

(5) Bars-Cortina, D.; Macià, A.; Iglesias, I.; Romero, M. P.; Motilva, M. J. Phytochemical Profiles of New Red-Fleshed Apple Varieties Compared with Traditional and New White-Fleshed Varieties, *Journal of agricultural and food chemistry*. 2017, 65, pp. 1684–1696.
(6) Wang, X.; Li, C.; Liang, D.; Zou, Y.; Li, P.; Ma, F. Phenolic compounds and antioxidant activity in red-fleshed apples, *Journal of Functional Foods*. 2015, 18, pp. 1086–1094.
(7) Sadilova, E.; Stintzing, F. C.; Carle, R. Chemical quality parameters and anthocyanin pattern of red-fleshed Weirouge apples, *1.* 2006, *80*, pp. 82–87.

The main anthocyanin detected in all red-flesh genotypes (in both outer and inner sections of the fruit flesh) throughout the development was cyanidin-3-O-galactoside (ideain) which is the predominant anthocyanin found in most red-fleshed apple varieties ^{1,6,7}. Chlorogenic acid was the prevalent metabolite present during the development with the highest concentrations at the unripe stage and decreasing towards maturity.

ACKNOWLEDGMENT

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 675657.

Special thanks to our technician Anja Forstner for her expert technical assistance and support.