Unveiling the molecular mechanisms behind non-browning phenotype in the apple cultivar 'Majda' (*Malus domestica* Borkh.) by a comprehensive investigation.

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Nicola Busatto[1], Anka Cebulj[2], Francesca Populin[1], Domenico Masuero[1], Urska Vrhovsek[1], Lucrezia Angeli[3], Ksenia Morozova[3], Matteo Scampicchio[3], Fabrizio Costa[4].

[1] Fondazione Edmund Mach, Research and Innovation Centre, Trento, Italy
[2] Agricultural Institute of Slovenia, Department of Fruit Growing, Viticulture and Oenology, Ljubljana, Slovenia
[3] Free University of Bolzano, Faculty of Science and Technology, Bolzano, Italy
[4] University of Trento, Center Agriculture Food Environment, Trento, Italy

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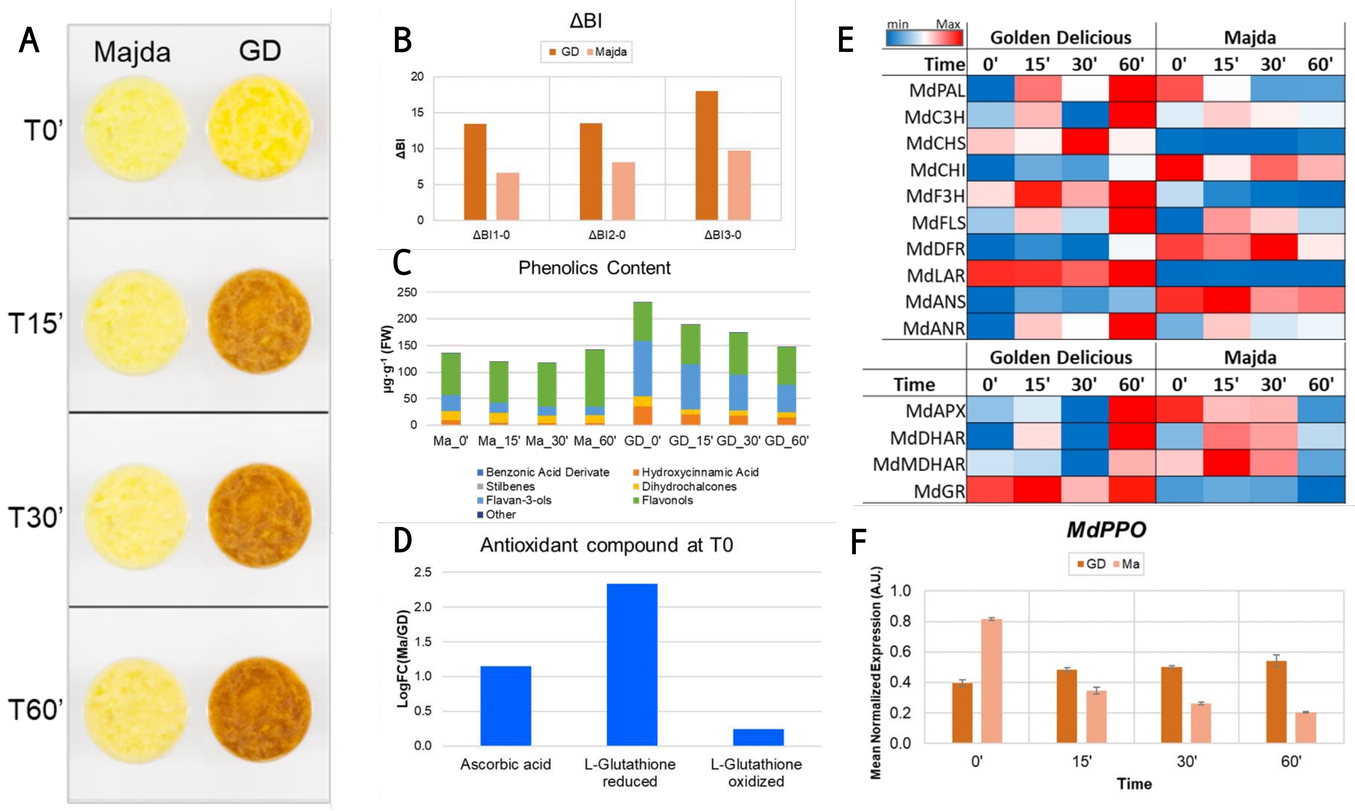
Background. Apple, a widely cultivated fruit globally, is not only enjoyed fresh but also processed into various products. Among these, the demand for fresh-cut options is rising due to its convenience for on-the-go consumption. However, enzymatic browning remains a significant hurdle in distributing fresh-processed apple products while preserving their quality attributes. Enzymatic flesh browning results from the interplay between polyphenol oxidase activity and polyphenol content. While multiple strategies exist to counteract browning, they can be costly (e.g., modified atmosphere packaging) or impact product quality and flavor (e.g., physical or chemical treatments). Therefore, a more optimal solution could lie in utilizing apple cultivars naturally resistant to enzymatic browning.

Materials and methods. For this purpose, we performed a metabolic and transcriptional study on 'Majda', a Slovenian apple cultivar known for its non-browning flesh even after extensive processing or prolonged exposure to air (Fig. 1 A, B). Our goal is to comprehend the molecular regulation (both metabolically and transcriptionally) behind Majda's non-browning trait, particularly in comparison to 'Golden Delicious,' during a browning progression after processing (Fig. 1 A).
We quantified prevalent phenolic compounds and organic acids using UHPLC-MSMS (Fig. 1 C, D). We also analyzed gene expression profiles related to polyphenol biosynthesis, ascorbic acid glutathione cycle, and polyphenol oxidase (*PPO*) in both apple varieties as browning progressed (Fig 1 E and F). Additionally, we evaluated the apple extract's antioxidant activity through a Kinetic-based DPPH assay (Fig 2 A)

Fig.1

A. Visual browning progression after pulp homogenization, after 0, 15, 30 and 60 min.
B. Evaluation of the Delta Browning Index (ΔBI), representing the evolution of brown coloration over the time course.
C. Content of each polyphenolic classes assessed for the two apple cultivars over the time course.
D. Antioxidant compounds quantification at T0 express as LogFC(Ma/ GD).
E. Heatmap showing the expression profile, of the most relevant genes involved in the polyphenol's biosynthesis (upper part) and in the ascorbic acid glutathione cycle (lower part).
F. Expression profile of the polyphenol oxidase Md*PPO* gene in the two cultivars during the browning progression.



Results and Conclusions. After processing, the Slovenian 'Majda' cultivar exhibited minimal browning, even after 60 minutes, as confirmed by ΔBI calculation (Fig 1 A, B). These cultivars displayed distinct polyphenol profiles. 'Golden Delicious' featured hydroxycinnamic acids, flavan-3-ols, and dihydrochalcones, while 'Majda' exhibited dihydrochalcones and flavonols. Additionally, 'Golden Delicious' had higher polyphenol content (Fig 1 C), whereas 'Majda' displayed elevated levels of antioxidants like ascorbic acid and glutathione (Fig 1 D).

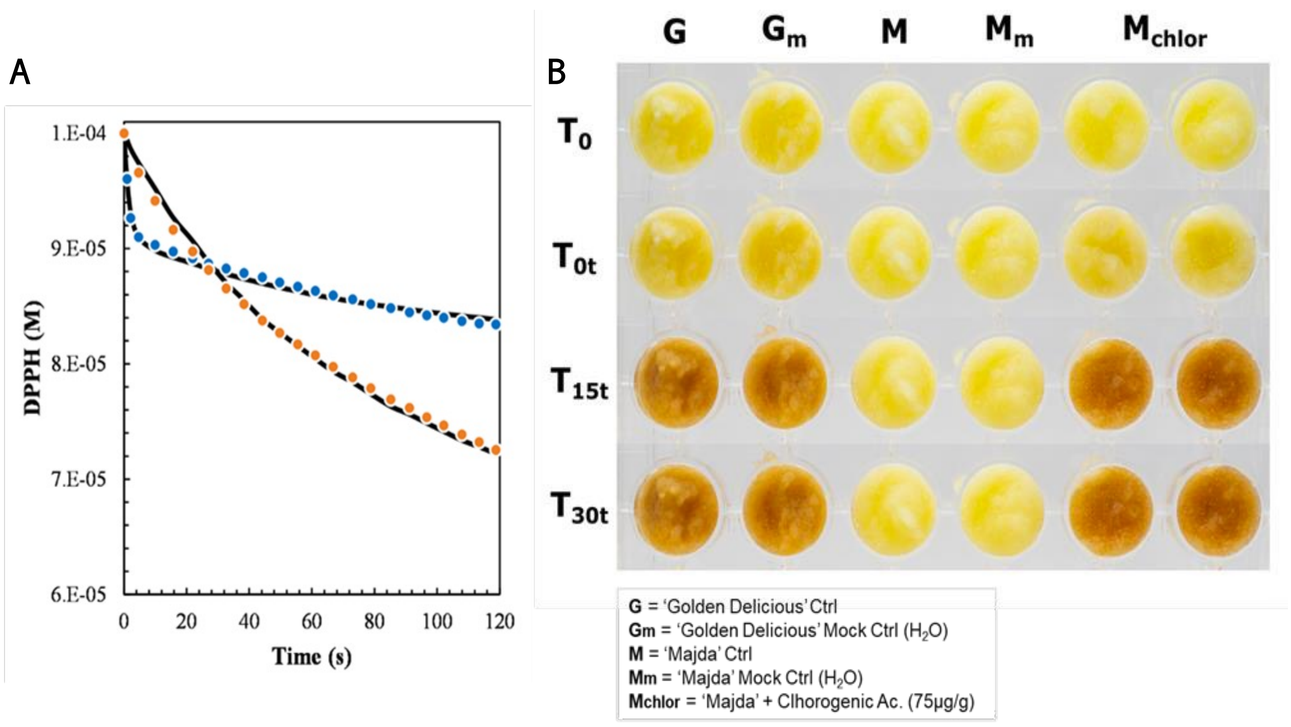


Fig. 2

A. Fitting curve of the reaction between DPPH• and 'Golden Delicious' (orange dots) and Majda ' (blue dots).
B. Effect of treatment with chlorogenic acid. T0: immediately after processing; $T0_t$: immediately after chlorogenic acid additions; $T15_t$ and $T30_t$ timepoints correspond to 15 and 30 min after chlorogenic acid addition.

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