



Comparative analysis of enzymatic activity, phenolic and ascorbic acid stability and browning dynamic in white and red-fleshed apple (*Malus domestica* Borkh.) cultivars post-air exposure

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ABSTRACT

This study investigates enzymatic browning in several white- and red-fleshed apple cultivars by analyzing polyphenol oxidase (PPO) and peroxidase (POX) activities, total phenolic content (TPC), anthocyanins, ascorbic acid, and color changes during air exposure. Six white-fleshed cultivars ('Topaz', 'Opal', 'Red Boskoop', 'Golden Delicious', 'Crown Prince Rudolph', 'Idared') and the red-fleshed cultivar 'Baya Marisa' were evaluated. Results revealed cultivar-specific enzymatic activity influencing browning susceptibility. 'Idared' exhibited the highest PPO activity and the strongest browning potential, while 'Baya Marisa' showed low PPO but unexpectedly high POX activity. All white-fleshed cultivars were prone to browning, as indicated by a significant increase in browning index (BI) from 0 to 480 min. In contrast, 'Baya Marisa' showed minimal color change and no significant BI variation, suggesting limited browning. However, due to high a^* values, BI may not be suitable for assessing browning in red-fleshed cultivars. 'Golden Delicious' had the highest initial ascorbic acid concentration and the slowest degradation rate. 'Baya Marisa' consistently exhibited the highest TPC, attributed to elevated anthocyanin levels. These findings emphasize the importance of cultivar selection in managing browning and highlight limitations of BI as a universal indicator, particularly for red-fleshed apples.

1. Introduction

The enzymatic browning process, commonly observed in fruits during the transition from ripening to over-ripening, poses a major challenge in preserving fruit quality (Murata et al., 1995). Apples are particularly susceptible to this phenomenon due to their high phenolic content (Ferreira-Holderbaum et al., 2010). Browning can also be induced by mechanical damage or wounds (Queiroz et al., 2008), while environmental conditions such as low temperature, reduced oxygen, and elevated CO₂ levels during storage may help mitigate browning reactions (Mellidou et al., 2014).

Phenolic compounds and related oxidative enzymes play a crucial role in fruit quality and postharvest behavior. Among these, polyphenol oxidase (PPO) and peroxidase (POX) are recognized as key contributors to browning, which can lead to undesirable changes in flavor, texture, nutritional value, and visual appearance. These effects are particularly problematic for the fresh-cut produce industry. Despite their negative impact on fruit quality, PPO also participates in physiological processes

such as defense responses to biotic and abiotic stress and photosynthesis (Walker and Ferrar, 1998).

PPO activity is a primary driver of enzymatic browning, with its effect dependent on both substrate availability and enzyme activity. PPO catalyzes the oxidation of phenolic compounds into o-quinones, which polymerize into brown pigments (Walker and Ferrar, 1998; Tang et al., 2020). PPO is a copper-containing oxidoreductase localized primarily in plastids, whereas its phenolic substrates are stored in the vacuole. Upon tissue disruption caused by cutting, peeling, or oxidative stress, the compartmentalized enzymes and substrates come into contact, initiating browning reactions (Singh et al., 2018; Sommano et al., 2020). In the presence of oxygen, PPO converts Monophenols to o-Diphenols and subsequently to o-quinones, which undergo non-enzymatic secondary reactions, including condensation and melanin formation (Singh et al., 2018).

POX also contributes to enzymatic browning, particularly in processed and stored fruits and vegetables. Although POX requires hydrogen peroxide (H₂O₂) to function, its role in apple browning has

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been documented (Jang and Moon, 2011). As a heat-stable oxidoreductase, POX can oxidize a wide range of substrates in the presence of H_2O_2 (Sikora et al., 2020). Notably, PPO-mediated reactions may generate H_2O_2 , potentially enhancing POX activity under certain conditions (Singh et al., 2018). However, POX typically has a less prominent role in apple browning compared to PPO, largely due to relatively low levels of H_2O_2 in apple tissues (Rodríguez-Arzuaga et al., 2019). Differences in the distribution of soluble versus bound enzyme forms may further explain cultivar-specific variation in enzyme activity (Singh et al., 2018).

Given the pivotal role of these oxidative enzymes, understanding their behavior across cultivars is essential. In this study, we aimed to evaluate and compare the activity levels of PPO and POX in six white-fleshed apple cultivars ('Topaz', 'Opal', 'Red Boskoop', 'Golden Delicious', 'Crown Prince Rudolph', and 'Idared') and one red-fleshed cultivar, 'Baya Marisa'. White-fleshed apples are typically susceptible to enzymatic browning, whereas red-fleshed apples like 'Baya Marisa' show a different response following exposure to air. In addition to enzyme activity, we investigated cultivar-dependent differences in total phenolic content (TPC), anthocyanin and ascorbic acid concentrations, and visible color changes over time. The results provide new insights into browning mechanisms and their biochemical drivers, with implications for postharvest quality management and fruit processing strategies.

2. Material and methods

2.1. Plant material

The high-density apple orchard of cultivars 'Baya Marisa', 'Red Boskoop', 'Opal', 'Idared', 'Crown Prince Rudolph', 'Golden Delicious' and 'Topaz' was cultivated in the southeastern region of Slovenia in Zdole (coordinates: 45°98'02" N; 15°52'07" E; elevation: 307 m above sea level). Cultivars were grafted onto M9 rootstocks with planting distance 3.2 m x 0.9 m, entering their seventh growing season. The orchard rows were aligned in a north-south orientation. Standard integrated pest management was applied to maintain tree health. Harvesting commenced with 'Golden Delicious' on 16th September 2023, followed by 'Crown Prince Rudolph' on 18th September 2023, 'Baya Marisa' on 21st September 2023, 'Opal' on 24th September 2023, 'Red Boskoop' on 1st October 2023, 'Idared' on 4th October 2023 and 'Topaz' on 28th October 2023. All the fruit were harvested at their technical maturity. They were then transported to the laboratory of the Department of Agronomy, Biotechnical Faculty, University of Ljubljana (Slovenia), where they were stored in controlled atmosphere (CA) cold storage at 1 °C, with 2 % O_2 , 2 % CO_2 , and 95 % humidity, which slowed the ripening and respiration of the fruit.

The analysis were conducted on 11th of November for all cultivars, at the laboratory of the Department of Agronomy, Biotechnical Faculty, University of Ljubljana (Slovenia). For the experiment, a total of 21 apples were chosen, washed and visually inspected to ensure they were free from mechanical damage, visible disease symptoms, and other defects that could influence the experimental results. Three apples of each cultivar were chosen as biological replicates, which were stored in cold storage after harvesting. The skin and core of apple fruits were removed, and the flesh grinded (3 apples per cultivar, Separately). We spread each grinded paste onto a separate standard kitchen desk (one apple per desk) in a 0.5-1.0 cm layer (one grinded apple represented a biological repetition). Each paste was divided in ten columns to correspond with the ten distinct time intervals at which measurements were taken. Enzyme activity, TPC, color, individual anthocyanins and ascorbic acid were measured at precise time intervals after air exposure (0 min, 5 min, 10 min, 15 min, 30 min, 60 min, 120 min, 240 min, 360 min and 480 min). The experiment was conducted in a climatized laboratory where the air temperature was maintained at 22 °C.

2.2. Chemicals

Milli-Q water purification system (Millipore, Bedford, MA, USA) was used for purification of bi-distilled water used for analysis and sample preparation. The chemicals used include sodium phosphate dibasic (Na_2HPO_4) (product code: 0876, reagent grade), pyrocatechol (product code: C9510, purity >99 %), methanol (purity >99,9 %), Ethylenediaminetetraacetic acid (EDTA, $C_{10}H_{12}N_2Na_4O_8$, $2H_2O$) (product code: ED4SS, purity ≥99 %, sodium), Tetraborate (Borax, $B_4Na_2O_7$), TRIS (Hydroxymethyl) tirsaminomethane ($H_2NC(CH_2OH)_3$) (product code: 1083820100 reagent grade), sand (SiO_2) (product code: 274739), McIlvaine buffer (product code: P4809), sephadex G-25 (product code: G25300) *o*-dianisidine ($C_{14}H_{16}N_2O_2$) (product code: D9143), Metaphosphoric acid (product code: 79613) and hydrogen peroxide 30 % (aqueous solution, H_2O_2) (product code: 1.07209.1000) all sourced from Sigma-Aldrich (Steinheim, Germany). Additionally, potassium Dihydrogen phosphate (KPi, KH_2PO_4) is obtained from Fluka Chemie GmbH (Buchs, Switzerland; product code: 60230), Formic acid was HPLC-MS grade (Fluka Chemie GmbH; product code: F0507, purity ≥95 %) and polyclar AT (product code: 33162) from SERVA Electrophoresis GmbH, Heidelberg, Germany).

Standards used for analysis were cyanidin-3-*O*-galactoside (Fluka Chemie GmbH, Buchs, Switzerland; product code: PHL89463 purity ≥95 %) and cyanidin-3-*O*-arabinoside (Extrasynthese, Genay, France; product code: 27214-72-8, purity ≥95 %).

2.3. Enzyme activity

The extraction was made as previously described in literature (Simkova et al., 2023).

The grinded samples were placed directly into a mortar filled with liquid nitrogen, after processing. Furthermore, to prevent the oxidation, grinded samples were frozen and stored at -80 °C. Each extraction comprised a mixture of 1.0 g of sample, 0.25 g of Polyclar combined with 0.25 g of sand, and 3 mL of extraction buffer consisting of 0.01 M TRIS, 0.007 M EDTA, and 0.01 M Borax. This mixture was vortexed for 30 s and then centrifuged for 10 min at 10,000 g at 4 °C using an Eppendorf Centrifuge 5810 R (Germany). Following centrifugation, the resulting supernatant (400 µl) underwent purification via a Sephadex G-25 gel column to eliminate low molecular weight compounds before subsequent analysis.

The assays were measured on a Synergy HTX multi-mode reader (Agilent Technologies, Santa Clara, California, USA), and data were collected using Gen5 software (version 3.12.08). The POX assay consisted of 180 µL H_2O_2 -KPi buffer, 20 µL sample extract and 20 µL 0.004 M *o*-dianisidine solution in methanol and was measured for 20 min at 460 nm. The PPO assay consisted of 100 µL McIlvaine buffer (pH 6.5), 75 µL sample extract and 70 µL 0.2 M pyrocatechol solution and was measured for 15 min at 410 nm. The maximum absorbance change per minute was calculated as an average from three maximum values.

2.4. Extraction and analytical methods used for determination of ascorbic acid and phenolic compounds

The extraction process followed previous protocols (Medic et al., 2021). The extract for phenolic compounds was prepared from 1 g of sample extracted with 5 mL of 80 % Methanol, 1 % formic acid and 19 % of bi-distilled water. Tubes were then placed onto ultrasonic bath for 60 min, followed by centrifugation at 8000 g for 8 min. The supernatant was then filtered by 0.2 µm polyamide filters (Chromafil AO -20/25; Macherey-Nagel, Düren, Germany) into vials and stored at -20 °C for further analysis.

Ascorbic acid was extracted following a procedure similar to previously described methods (Bizjak et al., 2013). In summary, 1.0 g of the sample was extracted with 5 mL of 3 % metaphosphoric acid solution

prepared in bi-distilled water, maintaining an extraction ratio of 1:5 (w/v). Samples were then put in centrifuge (Eppendorf Centrifuge 5810 R) at 4 °C and 5000 g, and filtered through 0.2 µm polyamide filters (Chromafil XTRA MV -20/25; Macherey-Nagel, Düren, Germany) into vials and transferred to freezer (-20 °C) until start of further analysis.

The total phenolic content (TPC) was determined using a UV/VIS spectrometer (Lambda Bio 20, Perkin Elmer, Waltham, USA). The assessment was performed following the Folin–Ciocalteu phenol reagent method as described by Singleton et al. (1999). For the analysis, a 10 mL test tube was filled with 7.9 mL of bi-distilled water. Then, 100 µL of extract (diluted 1:2 v/v with methanol) and 500 µL of Folin–Ciocalteu reagent was added. After allowing the mixture to stand at room temperature for 4 min, 1.5 mL of sodium carbonate (20 % w/v) and the remaining volume of water were added to bring the total volume to exactly 10 mL. The tube was sealed, shaken, and put to oven at 40 °C for 30 min. A control sample was also prepared, replacing the extract with 100 µL of methanol. Absorbance was measured at 765 nm. The TPC of apple fruits was expressed as gallic acid equivalents (GAE) mg GA/kg FW (fresh weight) of fruit.

2.5. Analytical analysis of anthocyanins and ascorbic acid

The anthocyanins were quantified using a UHPLC system (Thermo Scientific, San Jose, CA, USA). The diode array detector was set to 530 nm. The analysis followed established protocols (Medic et al., 2022), with an injection volume of 20 µL. Spectral data were recorded within the range of 200–600 nm. A C18 column (Gemini; 150 × 4.60 mm, 3 µm; Phenomenex, Torrance, CA, USA) was used for compound separation at a constant temperature of 25 °C. Tandem mass spectrometry (MS/MS; LCQ DECA XP MAX; Thermo Scientific, Waltham, MA, USA) was used for compound identification, utilizing heated electrospray ionization in positive ion mode for anthocyanins. MS scans were acquired over a range of 50–2000 *m/z*. Data acquisition was carried out using Xcalibur 2.2 software (Thermo Fisher Scientific, Waltham, MA, USA). External standards were used for compound quantification and identification.

Ascorbic acid analysis was performed at 20 °C using a UV detector set at 210 nm on the same UHPLC system, following the method described by Bizjak et al. (2013).

2.6. Fruit color and browning index

The color parameters were measured using the CR-300 Chroma colorimeter (Minolta Co., Osaka, Japan). The parameters included L* (lightness), ranging from 0 (black) to 100 (white); a* and b*, where positive a* values indicate red and negative values indicate green, while positive b* values represent yellow and negative values represent blue. Attribute of visual perception (hue angle: h°) was also measured. The C* value, corresponding to color intensity, reflects greater colorfulness with higher values, as outlined by Medic et al. (2021).

Browning index (BI) was calculated with the help of the following equation (Subhashree et al., 2017):

$$BI = \frac{x - 0.31}{0.172} \times 100$$

$$x = \frac{a^* + 1.75 L^*}{5.645 L^* + a^* - 0.3012 b^*}$$

2.7. Statistical analysis

Data collection was performed utilizing Microsoft Excel 2016, while statistical analysis was done with R commander (x64 4.1.2, R Foundation for Statistical Computing, Vienna, Austria). Significant variations within the dataset were assessed via one-way analysis of variance (ANOVA) with Tukey tests. The establishment of significance ($p < 0.05$)

was based on statistical mean calculations with a confidence level of 95 %. The presentation of all data includes means and standard errors (SE).

3. Results and discussion

3.1. Enzyme activity

Peroxidase (POX) activity varied significantly among the seven apple cultivars studied, with no consistent pattern over time (Table 1). This variability aligns with findings by Serra et al. (2021). At time zero, ‘Baya Marisa’ showed the highest POX activity (39.53 U/(min·kg FW)), peaking at 5 min (43.40 U/(min·kg FW)) before declining by nearly 50 % at 480 min (19.39 U/(min·kg FW)). Except at 360 and 480 min, ‘Baya Marisa’ consistently exhibited significantly higher POX activity than other cultivars, closely matching ‘Topaz’ at later time points.

Conversely, ‘Crown Prince Rudolph’ displayed the lowest initial POX activity (8.11 U/(min·kg FW)) and minimal temporal variation, remaining low throughout (6.29 U/(min·kg FW) at 480 min). ‘Idared’ showed the highest activity at 0 min but dropped sharply at 10 min (6.44 U/(min·kg FW)) before a gradual rise to 15.97 U/(min·kg FW) by 480 min. POX activity in all cultivars was notably variable over time. ‘Golden Delicious’ peaked early (0 min: 18.53; 5 min: 17.32 U/(min·kg FW)), declined until 120 min, then rose slightly to 12.70 U/(min·kg FW) at 480 min. ‘Opal’ peaked at 30 min (34.28 U/(min·kg FW)), decreasing until 120 min and stabilizing thereafter. ‘Red Boskoop’ exhibited an early peak (0 min: 24.50; 5 min: 23.81 U/(min·kg FW)), decreased until 60 min, then rose slightly at 240 min (21.89 U/(min·kg FW)) before plateauing. ‘Topaz’ showed an initial decline (32.08–22.42 U/(min·kg FW) at 5 min), a rebound at 120 min (27.07 U/(min·kg FW)), followed by a gradual decrease to 21.45 U/(min·kg FW) at 480 min.

These temporal differences in POX activity likely reflect cultivar-specific enzymatic potentials tied to phenolic compound polymerization (Serra et al., 2021). The elevated POX activity in ‘Baya Marisa’ may indicate higher antioxidant capacity or an inherent stress response, consistent with its richer phenolic content (Espley et al., 2019). This corroborates Espley et al. (2019), where red-fleshed apples exhibited higher POX activity than white-fleshed cultivars. Serra et al. (2021) also reported cultivar-dependent browning variability linked to phenolic composition and polymerization, highlighting oxidative browning’s complex biochemical basis.

Polyphenol oxidase (PPO) activity patterns differed markedly (Table 2). ‘Idared’ exhibited the highest PPO activity initially, peaking at 0 min (162.94 U/(min·kg FW)) and 5 min (132.02 U/(min·kg FW)), before sharply declining by 30 min. After this, ‘Crown Prince Rudolph’ surpassed other cultivars in PPO activity, peaking at 60 min (69.15 U/(min·kg FW)) and remaining relatively stable thereafter. ‘Red Boskoop’ had the second highest initial PPO activity (97.84 U/(min·kg FW) at 0 min), decreasing significantly by 15 min (46.88 U/(min·kg FW)). ‘Topaz’ showed moderate initial activity (60.30 U/(min·kg FW)) with a rapid decline by 15 min (22.36 U/(min·kg FW)), a transient increase at 30 min, and then a steady decrease to 11.24 U/(min·kg FW) by 480 min.

‘Golden Delicious’ had relatively low PPO activity at 0 min (30.26 U/(min·kg FW)), which fluctuated slightly before stabilizing near 10 U/(min·kg FW) after 240 min. ‘Baya Marisa’ and ‘Opal’ consistently exhibited the lowest PPO activity, peaking modestly at 15 min (13.97 U/(min·kg FW)) and 0 min (17.95 U/(min·kg FW)), respectively. ‘Opal’ showed a secondary peak at 120 min (16.86 U/(min·kg FW)). ‘Crown Prince Rudolph’ presented an initial low PPO activity (26.99 U/(min·kg FW)) with a peak at 60 min and a gradual decline afterward.

Overall, ‘Baya Marisa’ maintained the lowest PPO activity, followed by ‘Opal’, suggesting reduced browning potential. Hutabarat and Halbwirth (2019) similarly reported lower PPO activity in red apple cultivars, attributing this to substrate availability. Conversely, ‘Idared’, ‘Red Boskoop’, ‘Topaz’, and ‘Crown Prince Rudolph’ frequently exhibited moderate to high PPO activity, underscoring the importance

Table 1
Activity of POX (U/(min kg) FW) for 7 different apple cultivars, measured at different time intervals, ranging from 0 to 480 min.

Enzyme activity of POX (U/(min kg) FW)							
Time (min)	'Baya Marisa'	'Idared'	'Opal'	'Red Boskoop'	'Topaz'	'Golden Delicious'	'Crown Prince Rudolph'
0	39.53 ± 0.64 aA	18.33 ± 0.93 aD	27.04 ± 0.91 bC	24.50 ± 0.47 aC	32.08 ± 0.64 aB	18.53 ± 1.26 aD	8.11 ± 0.68 aE
5	43.40 ± 0.28 aA	15.17 ± 0.25 bcC	22.80 ± 0.07 cB	23.81 ± 0.19 aB	22.42 ± 1.41 cB	17.32 ± 1.53 aC	6.49 ± 0.38 bD
10	36.03 ± 0.51 bcA	6.44 ± 0.19 eD	19.95 ± 0.41 cB	18.27 ± 0.60 cB	13.96 ± 0.46 eC	14.21 ± 1.59 bC	6.35 ± 0.41 bD
15	37.31 ± 1.34 bcA	14.38 ± 0.80 cC	20.66 ± 1.35 cB	16.06 ± 1.20 dC	20.60 ± 0.36 cB	9.83 ± 1.50 cD	7.83 ± 0.32 abD
30	33.68 ± 0.52 cA	16.71 ± 0.13 bB	34.28 ± 0.54 aA	17.09 ± 0.54 dB	17.71 ± 0.16 dB	8.81 ± 1.36 cC	6.74 ± 0.32 bC
60	28.23 ± 0.85 dA	11.51 ± 0.40 dD	17.43 ± 1.55 dB	15.94 ± 0.20 dC	17.61 ± 0.04 dB	9.17 ± 0.27 cE	6.56 ± 0.81 bF
120	35.19 ± 0.79 cA	10.95 ± 0.71 dD	10.75 ± 0.44 eD	19.57 ± 0.21 cC	27.07 ± 0.35 bB	10.00 ± 0.79 cD	8.58 ± 0.84 aD
240	36.16 ± 0.32 bcA	11.84 ± 0.73 dD	11.86 ± 0.31 eD	21.89 ± 0.70 bB	16.32 ± 0.36 dC	14.42 ± 1.03 bC	4.35 ± 0.44 cE
360	22.70 ± 0.11 eA	12.47 ± 0.49 dC	11.82 ± 0.33 eC	22.11 ± 0.11 bA	19.55 ± 1.17 cdA	14.59 ± 1.06 bB	4.91 ± 0.62 cD
480	19.39 ± 1.07 fA	15.97 ± 0.56 bB	11.51 ± 0.60 eC	22.10 ± 0.33 bA	21.45 ± 0.36 cA	12.70 ± 1.17 bcC	6.29 ± 0.87 bD

Presented data are means ± standard error. Different lowercase letters (within column) indicate statistically significant differences ($p < 0.05$) over time for the same cultivar. Different uppercase letters (within a row) indicate significant differences ($p < 0.05$) between cultivars at the same time point.

Table 2
Activity of PPO (U/(min kg) FW) for seven different apple cultivars, measured at different time intervals, ranging from 0 to 480 min.

Enzyme activity of PPO (U/(min kg) FW)							
Time (min)	'Baya Marisa'	'Idared'	'Opal'	'Red Boskoop'	'Topaz'	'Golden Delicious'	'Crown Prince Rudolph'
0	12.41 ± 0.02 bF	162.94 ± 1.17 aA	17.95 ± 1.23 aE	97.84 ± 1.07 aB	60.30 ± 1.44 aC	30.26 ± 1.52 aD	26.99 ± 1.88 eD
5	11.25 ± 0.32 bD	132.02 ± 0.31 bA	12.94 ± 1.03 cD	53.95 ± 0.44 bB	54.64 ± 1.21 bB	25.34 ± 1.43 bC	28.39 ± 1.37 eC
10	6.98 ± 0.15 cE	95.67 ± 0.54 cA	3.21 ± 0.13 fF	48.96 ± 1.54 cB	23.93 ± 0.13 dD	30.22 ± 1.78 aC	26.97 ± 1.45 eD
15	13.97 ± 0.50 abE	98.49 ± 1.08 cA	14.85 ± 0.45 bE	46.88 ± 0.10 cC	22.36 ± 1.55 dD	20.39 ± 1.51 cD	53.26 ± 1.69 bB
30	10.85 ± 1.48 bcF	53.65 ± 0.58 dB	15.24 ± 0.38 bE	31.06 ± 0.29 dD	39.14 ± 1.03 cC	12.39 ± 1.20 deE	65.64 ± 1.08 aA
60	5.58 ± 0.23 dE	24.39 ± 0.71 fC	9.61 ± 0.36 dD	32.71 ± 0.28 dB	24.75 ± 0.95 dC	12.59 ± 1.51 deD	69.15 ± 1.40 aA
120	15.71 ± 0.97 aE	26.80 ± 0.35 fC	16.86 ± 0.47 abD	28.18 ± 0.04 eC	39.90 ± 0.37 cB	15.50 ± 1.41 dD	57.48 ± 1.36 abA
240	7.79 ± 0.11 cE	25.87 ± 0.08 fB	10.98 ± 0.09 cdD	28.93 ± 0.42 eB	14.52 ± 0.71 efC	10.89 ± 0.78 eD	39.24 ± 1.23 dA
360	5.53 ± 0.27 dF	31.10 ± 0.82 efB	6.14 ± 0.06 eF	28.46 ± 0.08 eC	17.29 ± 0.37 eD	9.94 ± 0.89 eE	43.47 ± 1.012 cA
480	4.66 ± 0.12 dF	27.99 ± 0.61 efB	7.45 ± 0.17 eE	20.61 ± 0.49 fC	11.24 ± 0.09 fD	11.69 ± 1.01 eD	44.68 ± 1.18 cA

Presented data are means ± standard error. Different lowercase letters within column indicate statistically significant differences ($p < 0.05$) over time for the same cultivar. Different uppercase letters within a row indicate significant differences ($p < 0.05$) between cultivars at the same time point.

of cultivar selection in minimizing enzymatic browning during post-harvest handling (Tang et al., 2020).

The relatively low PPO activity in 'Baya Marisa', 'Opal', and 'Golden Delicious' correlates with their reduced browning susceptibility, potentially extending shelf life and decreasing reliance on anti-browning treatments. Notably, 'Opal's' slow browning is consistent with its low PPO activity (Wang et al., 2023). These findings suggest factors beyond enzyme activity influence browning, as reported by Joshi et al. (2007) and Persic et al. (2017). For example, cultivars 'Majda' and 'Golden Delicious' share similar PPO and POX activities, yet 'Majda' resists browning due to low chlorogenic acid content (Cebulj et al., 2021, 2023).

'Baya Marisa', a red-fleshed cultivar rich in anthocyanins (Juhart et al., 2022), retains color after processing and prolonged storage (Juhart et al., 2023). Since anthocyanins are susceptible to oxidative degradation, browning mechanisms likely involve both enzymatic activity and genetic factors (Espley et al., 2019). Indeed, apples with higher anthocyanin content exhibit enhanced antioxidant capacity and stress tolerance, as seen in 'Royal Gala' which shows minimal browning (Espley et al., 2019).

High initial PPO activity in 'Idared' may result from its elevated chlorogenic acid content—constituting 77 % of total polyphenols (Can et al., 2014)—a principal substrate in enzymatic browning (Tang et al., 2020). 'Topaz', despite moderate PPO and POX activity, is reported to have high polyphenol content, implying alternative biochemical pathways modulate its browning (Serra et al., 2021). 'Red Boskoop' demonstrated moderate to high PPO and POX activities, particularly with extended air exposure, consistent with reports of accelerated browning in this cultivar (Luo and Barbosa-Cánovas, 1997).

Distinct PPO and POX activity profiles among cultivars indicate varied physiological and metabolic responses. Generally, PPO activity exceeded POX during the first 240 min in most cultivars, except 'Baya

Marisa' and 'Opal', where POX activity predominated. While both enzymes often fluctuated synchronously, 'Red Boskoop', 'Idared', and 'Crown Prince Rudolph' showed decoupled activities, a phenomenon previously observed (Hutabarat and Halbwirth, 2019).

3.2. Total phenolic content

At time 0 (Table 3), substantial differences in total phenolic content (TPC) were observed among the cultivars. 'Baya Marisa' exhibited the highest TPC at all measured time points, peaking at 5 and 10 min with approximately 629.52 mg GAE/kg FW. The TPC significantly decreased at later time points, dropping to 229.22 mg GAE/kg FW at 360 min and remaining stable thereafter. This suggests that 'Baya Marisa' inherently possesses higher phenolic content, potentially due to its genetic makeup and pigmentation, as red-fleshed apples are often richer in phenolics owing to the presence of anthocyanins in the flesh (Wang et al., 2015).

'Golden Delicious' had the second-highest initial TPC (496.76 ± 15.07 mg GAE/kg FW) and retained relatively high levels throughout the study, decreasing to 194.37 mg GAE/kg FW after 480 min. Compared to other cultivars, 'Golden Delicious' showed statistically significant differences and generally outperformed all but 'Baya Marisa' at each time point. Its slower rate of decline suggests better phenolic stability.

The TPC of 'Idared' was moderate at the start (436.81 mg GAE/kg FW) but declined significantly over time, reaching 180.29 mg GAE/kg FW at 480 min. The sharpest decline occurred within the first 30 min. Compared to other cultivars, 'Idared' showed consistently lower TPC values than 'Baya Marisa' and 'Golden Delicious' at all time points but higher values than 'Topaz', 'Opal', 'Crown Prince Rudolph', and 'Red Boskoop'.

'Crown Prince Rudolph' had a moderate TPC of 396.03 mg GAE/kg FW but experienced the sharpest decline among all cultivars, reaching

Table 3

Total phenolic content (mg GAE/kg FW) in seven different apple cultivars, measured at different time intervals after grinding, ranging from 0 to 480 min.

TPC (mg GAE/kg FW)							
Time (min)	'Baya Marisa'	'Idared'	'Opal'	'Red Boskoop'	'Topaz'	'Golden Delicious'	'Crown Prince Rudolph'
0	533.61 ± 16.47 bA	436.81 ± 12.59 aC	295.02 ± 6.45 aE	297.08 ± 9.80 aE	269.69 ± 16.12 aF	496.76 ± 15.07 aB	396.03 ± 18.70 aD
5	629.52 ± 25.61 aA	332.17 ± 11.75 bD	261.28 ± 8.37 bE	318.93 ± 7.40 aD	250.87 ± 16.04 aE	435.09 ± 8.13 bB	389.81 ± 18.91 aC
10	617.66 ± 17.10 aA	309.12 ± 9.62 bD	308.35 ± 12.65 aD	311.04 ± 10.47 aD	212.02 ± 12.80 bE	411.81 ± 10.89 bB	369.56 ± 14.47 abC
15	549.30 ± 8.23 bA	276.80 ± 1.67 cC	338.88 ± 9.78 aB	268.90 ± 5.32 bC	192.36 ± 9.36 bD	356.56 ± 10.68 cB	267.50 ± 9.00 cC
30	457.43 ± 14.05 cA	233.05 ± 5.18 dE	318.81 ± 8.38 aC	273.53 ± 6.58 bD	173.74 ± 9.19 cF	351.47 ± 10.49 cB	161.19 ± 14.31 dF
60	404.88 ± 16.25 dA	215.08 ± 1.90 dC	200.51 ± 8.39 cC	167.22 ± 8.19 cD	154.95 ± 8.14 dD	281.81 ± 10.19 dB	122.62 ± 10.94 eE
120	355.49 ± 9.68 eA	186.98 ± 1.75 eC	179.42 ± 7.10 dC	126.98 ± 6.89 dE	150.40 ± 7.98 dD	298.86 ± 9.84 dB	101.67 ± 8.93 fF
240	298.18 ± 5.74 fA	186.33 ± 8.71 eC	180.07 ± 8.41 dC	60.21 ± 1.58 eE	80.80 ± 7.31 eD	210.76 ± 8.30 eB	84.11 ± 5.37 gD
360	229.22 ± 7.67 gA	172.44 ± 5.50 eC	113.23 ± 10.16 eD	58.44 ± 1.69 eE	76.56 ± 5.76 eF	209.65 ± 8.14 eB	84.24 ± 5.52 gE
480	231.86 ± 16.96 gA	180.29 ± 8.92 eB	128.15 ± 6.49 eC	57.82 ± 2.53 eE	60.16 ± 5.09 eE	194.37 ± 10.92 eB	73.78 ± 6.52 hD

Presented data are means ± standard error. Different lowercase letters within column indicate statistically significant differences ($p < 0.05$) over time for the same cultivar. Different uppercase letters within a row indicate significant differences ($p < 0.05$) between cultivars at the same time point.

73.78 mg GAE/kg FW by 480 min. The rapid degradation, particularly after the first 30 min, suggests limited phenolic stability. Interestingly, TPC and polyphenol oxidase (PPO) activity displayed opposite trends, which may explain the observed TPC loss over time, suggesting phenolic oxidation driven by PPO activity. However, [Persic et al. \(2017\)](#) reported that oxidation of apple pulp depends more on TPC than on PPO and peroxidase (POX) activity.

'Red Boskoop' demonstrated a steep decline in TPC, starting at 297.08 mg GAE/kg FW and dropping significantly to 126.98 mg GAE/kg FW by 120 min. After 240 min, the TPC stabilized at very low levels (60.21 mg GAE/kg FW at 240 min and 57.82 mg GAE/kg FW at 480 min). Compared to other cultivars, 'Red Boskoop' consistently exhibited significantly lower TPC than 'Baya Marisa', 'Golden Delicious', 'Idared', and 'Opal'.

With an initial TPC of 295.02 mg GAE/kg FW, 'Opal' exhibited moderate phenolic stability. While TPC decreased significantly over time, it remained stable between 0 and 30 min (peaking at approximately 338.88 mg GAE/kg FW), then steadily declined to 128.15 ± 6.49 mg GAE/kg FW at 480 min. Compared to other cultivars, 'Opal' had significantly lower values than 'Baya Marisa', 'Golden Delicious', and 'Idared' at most time points but generally higher values than 'Red Boskoop' and 'Topaz'.

With the lowest initial TPC (269.69 mg GAE/kg FW), 'Topaz' showed a consistent and significant reduction over time. By 480 min, 'Topaz' and 'Red Boskoop' exhibited the lowest final TPC among all cultivars (60.16 mg GAE/kg FW), indicating high susceptibility to phenolic degradation.

3.3. Anthocyanin identification and content

Anthocyanin content was measured only for the red-fleshed cultivar 'Baya Marisa'. Two anthocyanins were identified in the flesh: cyanidin-3-O-galactoside and cyanidin-3-O-arabinoside. Cyanidin-3-O-galactoside exhibited a retention time (Rt) of 8.9 min, with a protonated molecular ion $[M + H]^+$ at m/z 449 and a major fragment ion at m/z 287. Cyanidin-3-O-arabinoside eluted earlier, with an Rt of 1.9 min, a protonated molecular ion at m/z 419, and the same MS^2 fragment ion at m/z 287.

Cyanidin-3-O-galactoside was the most abundant anthocyanin at all measured time points (Table 4), consistent with previous studies on red-fleshed apples ([Bars-Cortina et al., 2017](#)).

Both anthocyanins followed a similar trend as the TPC of 'Baya Marisa', peaking at 5 min (250.90 mg/kg FW and 31.32 mg/kg FW, respectively), followed by a steady decline. At 480 min, cyanidin-3-O-galactoside and cyanidin-3-O-arabinoside reached their lowest levels (20.76 mg/kg FW and 2.75 mg/kg FW, respectively). This alignment suggests that the early increase in TPC is likely driven by the high levels of anthocyanins, which are key phenolic compounds contributing to the total phenolic content of red-fleshed apples ([Bars-Cortina et al., 2017](#);

Table 4

Individual anthocyanin content (mg/kg FW) in flesh of cultivar 'Baya Marisa'.

Time (min)	Anthocyanins	
	Cyanidin-3-O-galactoside	Cyanidin-3-O-arabinoside
0	172.57 ± 2.22 cA	29.83 ± 1.72 aB
5	250.39 ± 2.14 aA	31.32 ± 0.61 aB
10	184.22 ± 2.73 bA	25.14 ± 0.74 bB
15	185.41 ± 2.89 bA	24.60 ± 0.18 bB
30	177.16 ± 2.98 cA	22.90 ± 0.59 bB
60	153.14 ± 2.46 dA	17.51 ± 0.59 cB
120	101.15 ± 2.99 eA	13.97 ± 0.61 dB
240	67.58 ± 2.49 fA	8.82 ± 0.94 eB
360	46.49 ± 2.09 gA	4.86 ± 0.41 fB
480	20.76 ± 1.18 hA	2.75 ± 0.59 gB

Presented data are means ± standard error. Different lowercase letters within column indicate statistically significant differences ($p < 0.05$) over time for the same anthocyanin. Different uppercase letters within a row indicate significant differences ($p < 0.05$) between anthocyanins at the same time point.

[Juhart et al., 2023](#)).

The TPC decreased from 629.52 mg GAE/kg FW at 5 min to 231.86 mg GAE/kg FW at 480 min, indicating that anthocyanin degradation significantly contributes to the reduction in TPC over time, as anthocyanins are important phenolic constituents in red-fleshed apples ([Juhart et al., 2023](#)).

Significant anthocyanin degradation at 480 min indicates that the remaining phenolic content (including non-anthocyanin phenolics) becomes a more significant contributor to TPC at later time points.

The paradox of Baya Marisa exhibiting high POX activity yet low browning can be mechanistically explained by its distinct phenolic substrate composition. The cultivar's flesh is rich in anthocyanins particularly cyanidin-3-O-galactoside and cyanidin-3-O-arabinoside, which are potent antioxidants and may act as competitive substrates or inhibitors for POX and PPO enzymes ([Espley et al., 2019](#)). Unlike chlorogenic acid, a major substrate in browning reactions for cultivars like 'Idared' ([Can et al., 2014](#)), anthocyanins and other flavonoids can scavenge reactive oxygen species generated during enzymatic oxidation, thereby mitigating pigment polymerization responsible for visible browning. Moreover, POX in Baya Marisa might preferentially catalyze phenolic cross-linking reactions that stabilize tissue structure without forming brown pigments ([Hutabarat and Halbwirth, 2019](#)). Genetic factors influencing phenolic biosynthesis pathways and enzyme expression profiles likely contribute further to this cultivar-specific response ([Espley et al., 2019](#)). Consequently, the high antioxidant capacity conferred by the phenolic substrate composition in Baya Marisa counteracts the browning effect despite elevated POX activity.

3.4. Ascorbic acid

At the start of the experiment, substantial variation in ascorbic acid levels was observed among the cultivars. 'Golden Delicious' exhibited the highest initial concentration (0.38 mg/kg FW), significantly greater than all other cultivars. Conversely, 'Opal' and 'Crown Prince Rudolph' consistently displayed the lowest ascorbic acid levels (Table 5).

'Baya Marisa' displayed slight fluctuations over time. A notable increase was observed at 15 min (0.23 mg/g FW), however ascorbic acid levels declined steadily after 15 min, reaching the lowest ascorbic acid content at 60 min (0.16 mg/g FW). Ascorbic acid levels in 'Idared' were stable throughout the experiment, with only minor declines observed at later time points. Cultivar 'Opal' exhibited minimal fluctuations over time. Ascorbic acid content in 'Red Boskoop' was stable during the first 15 min and showed a slight decline over time, with contents ranging from 0.14 mg/g FW at 15 min to 0.11 mg/g FW at 480 min. The ascorbic acid content in 'Topaz' remained relatively stable over time, showing minimal fluctuations. Its content ranged around 0.14–0.15 mg/g FW across most time points, with a slight decline to 0.12 mg/g FW at 480 min. Although ascorbic acid content declined over time, 'Golden Delicious' consistently maintained the highest content of ascorbic acid at all time points. Cultivar 'Crown Prince Rudolph' showed stable ascorbic acid content over time, with minimal changes.

Each cultivar showed a different vitamin C decay over time, its content decreasing up to 50 % in 'Golden Delicious' during the exposure time, despite maintaining the highest ascorbic acid content throughout the study. Similar decreases were observed in 'Idared', 'Baya Marisa' and 'Topaz'. 'Opal', 'Crown Prince Rudolph' and 'Red Boskoop' were cultivars with overall the lowest content of ascorbic acid and the least change at all measured time intervals.

3.5. Color and browning index

Apple color represents a significant role in determining the quality of fresh-cut apples, making it an important area of study. Research has shown that the darkening of apples occurs due to an enzymatic reaction driven by polyphenol oxidase (PPO) and peroxidase (POX), which oxidizes polyphenols into quinones, leading to browning (Tang et al., 2020). We measured the color parameters of all cultivars in the study and calculated the browning index (Table 6).

Lightness (L^*) significantly and steadily decreased in all cultivars throughout the study (0–480 min). 'Golden Delicious' had the highest initial L^* (66.25), while 'Baya Marisa' had the lowest (26.35), consistent with its red flesh. Red color (a^*) significantly increased in all cultivars, peaking at varying times depending on the cultivar: 60 min for 'Opal' (17.60), 'Crown Prince Rudolph' (19.30), and 'Golden Delicious' (15.25); 120 min for 'Idared' (17.05); and 240 min for 'Topaz' (15.35) and 'Red Boskoop' (17.65). The red-fleshed 'Baya Marisa' had the highest redness (a^*) at time 0 (27.50) immediately after grinding and air

exposure, with only minor fluctuations thereafter.

'Idared' was the only cultivar where red color did not decline after peaking at 120 min, whereas all other cultivars showed a decrease in redness following their peak, reaching the lowest values at 480 min.

Anthocyanins in 'Baya Marisa' flesh are the primary cause of its red color (Allan et al., 2008). The observed decrease in a^* values and anthocyanin content over time suggests a correlation between red color intensity and anthocyanin levels (Bouillon et al., 2024).

Yellow color (b^*) in 'Baya Marisa' did not significantly change throughout the study. 'Idared' and 'Red Boskoop' also showed stable b^* values, except for a transient peak at 240 min. Minor fluctuations were observed in 'Crown Prince Rudolph', 'Opal', 'Topaz', and 'Golden Delicious', with each cultivar peaking at different times.

Color intensity (C^*) decreased in 'Baya Marisa', 'Crown Prince Rudolph', 'Topaz', 'Golden Delicious', 'Opal', and 'Red Boskoop', but increased in 'Idared' from 0 to 480 min, with minor fluctuations in between.

The browning index (BI), which reflects the purity of brown color, increased significantly in all white-fleshed cultivars over time, with 'Crown Prince Rudolph' exhibiting the highest BI, indicating the most intense browning. In contrast, the red-fleshed 'Baya Marisa' showed no significant change in BI values, indicating no browning after air exposure.

However, 'Baya Marisa' had the highest BI values throughout the experiment (ranging from 68.48 at 0 min to 61.35 at 480 min). Evaluating BI for red-fleshed cultivars using the same formula as for white-fleshed apples may not be accurate due to their lower L^* and b^* values and higher a^* values (Sadilova et al., 2006). Our results align with previous studies (Shimizu et al., 2021), which found red-fleshed apples exhibit significantly higher BI values than white-fleshed ones. Moreover, while BI increased significantly in white-fleshed cultivars, it remained stable in red-fleshed cultivars.

Overall, 'Baya Marisa' showed no visible color change even after 480 min of air exposure, unlike the other cultivars. According to Abedi et al. (2019), high anthocyanin content in red-fleshed apples like 'Bakran' may inhibit enzymatic browning due to anthocyanins' strong antioxidant properties.

The low PPO activity in 'Baya Marisa' may explain the absence of enzymatic browning after air exposure. High POX activity was not accompanied by high PPO activity, consistent with findings by Hutarbarat and Halbwirth (2019). The TPC decreased in all cultivars during the study; in 'Baya Marisa,' this loss was mainly due to rapid anthocyanin degradation. However, the red flesh color persisted without browning, as reported by Wagner et al. (2022).

This suggests factors beyond anthocyanins, POX, and PPO contribute to color stability in red-fleshed apples. Some red-fleshed cultivars (Espley et al., 2019) are prone to flesh browning, indicating a complex balance between enzymatic browning, anthocyanin degradation, and pigment stabilization mechanisms. Anthocyanin degradation may be

Table 5

Ascorbic acid content (mg/g FW) for all seven cultivars studied in different time points (0–480 min).

Ascorbic acid content (mg/kg FW)							
Time (min)	'Baya Marisa'	'Idared'	'Opal'	'Red Boskoop'	'Topaz'	'Golden Delicious'	'Crown Prince Rudolph'
0	0.20 ± 0.00 abB	0.17 ± 0.00 aC	0.11 ± 0.00 cE	0.12 ± 0.00 bE	0.15 ± 0.00 aC	0.38 ± 0.03 aA	0.12 ± 0.00 bcE
5	0.20 ± 0.00 abB	0.17 ± 0.00 aC	0.13 ± 0.00 aD	0.11 ± 0.00 bE	0.14 ± 0.00 aD	0.33 ± 0.01 bA	0.14 ± 0.01 bD
10	0.21 ± 0.01 abB	0.17 ± 0.00 aC	0.12 ± 0.00 abD	0.12 ± 0.00 bD	0.15 ± 0.00 aC	0.30 ± 0.00 bA	0.13 ± 0.00 bD
15	0.23 ± 0.00 aA	0.16 ± 0.00 aB	0.12 ± 0.00 abC	0.14 ± 0.00 aB	0.14 ± 0.00 aB	0.23 ± 0.01 cA	0.13 ± 0.00 bC
30	0.20 ± 0.00 abA	0.15 ± 0.01 abB	0.13 ± 0.00 aC	0.11 ± 0.00 bD	0.14 ± 0.00 aC	0.20 ± 0.00 cA	0.16 ± 0.00 aB
60	0.18 ± 0.01 cAB	0.15 ± 0.00 abB	0.14 ± 0.00 aBC	0.12 ± 0.00 bC	0.13 ± 0.00 abBC	0.21 ± 0.01 cA	0.12 ± 0.00 bcC
120	0.17 ± 0.01 cB	0.15 ± 0.01 abC	0.11 ± 0.00 cE	0.13 ± 0.00 aD	0.13 ± 0.00 abD	0.22 ± 0.01 cA	0.13 ± 0.00 bD
240	0.18 ± 0.01 cB	0.13 ± 0.01 cC	0.14 ± 0.00 aC	0.13 ± 0.00 aC	0.14 ± 0.00 aC	0.25 ± 0.00 cA	0.14 ± 0.00 bC
360	0.16 ± 0.01 cAB	0.15 ± 0.00 abB	0.11 ± 0.00 cCD	0.12 ± 0.00 bC	0.13 ± 0.00 abC	0.18 ± 0.01 cA	0.13 ± 0.00 bC
480	0.16 ± 0.00 cB	0.14 ± 0.00 cC	0.11 ± 0.00 cD	0.11 ± 0.00 bD	0.12 ± 0.00 bcCD	0.20 ± 0.01 cA	0.13 ± 0.00 bC

Presented data are means ± standard error. Different lowercase letters within column indicate statistically significant differences ($p < 0.05$) over time for the same cultivar. Different uppercase letters within a row indicate significant differences ($p < 0.05$) between cultivars at the same time point.

Table 6

The color parameters (represented as mean $\pm$ standard error) and browning index (BI) of seven studied apple cultivars were assessed for all time intervals after air exposure (0–480 min).

Cultivar	Time (min)	Color parameters					BI
		L*	a*	b*	C*	h°	
'Idared'	0	59.55 $\pm$ 0.03 a	5.25 $\pm$ 0.09 d	25.95 $\pm$ 0.20 ab	26.45 $\pm$ 0.20 c	78.60 $\pm$ 0.29 a	9.38 $\pm$ 0.06 e
	5	45.95 $\pm$ 0.84 b	12.30 $\pm$ 0.17 c	27.20 $\pm$ 0.17 ab	29.85 $\pm$ 0.09 ab	65.60 $\pm$ 0.40 b	23.19 $\pm$ 0.04 d
	10	36.55 $\pm$ 1.59 c	12.80 $\pm$ 0.29 c	24.05 $\pm$ 0.89 b	27.25 $\pm$ 0.89 bc	61.90 $\pm$ 0.35 c	29.28 $\pm$ 0.51 c
	15	37.15 $\pm$ 0.84 c	14.30 $\pm$ 0.75 b	25.00 $\pm$ 1.33 ab	28.75 $\pm$ 1.53 bc	60.30 $\pm$ 0.01 c	31.57 $\pm$ 0.96 bc
	30	37.55 $\pm$ 1.63 c	15.25 $\pm$ 0.60 b	26.60 $\pm$ 1.50 ab	31.15 $\pm$ 1.59 ab	58.50 $\pm$ 0.46 d	35.14 $\pm$ 1.00 bc
	60	36.40 $\pm$ 0.81 c	14.70 $\pm$ 0.35 b	25.30 $\pm$ 0.58 ab	29.30 $\pm$ 0.69 bc	59.85 $\pm$ 0.03 cd	33.04 $\pm$ 0.04 bc
	120	35.40 $\pm$ 0.12 c	17.05 $\pm$ 0.78 a	26.70 $\pm$ 0.46 ab	31.70 $\pm$ 0.81 ab	57.50 $\pm$ 0.75 d	38.53 $\pm$ 1.62 a
	240	38.85 $\pm$ 0.66 c	17.75 $\pm$ 0.20 a	30.15 $\pm$ 0.09 a	35.50 $\pm$ 0.17 a	58.15 $\pm$ 0.20 d	38.87 $\pm$ 0.10 a
	360	33.70 $\pm$ 1.62 c	17.00 $\pm$ 1.56 a	26.55 $\pm$ 1.05 ab	31.55 $\pm$ 1.69 ab	57.55 $\pm$ 0.38 d	40.14 $\pm$ 1.62 a
'Baya Marisa'	480	35.50 $\pm$ 0.75 c	17.10 $\pm$ 0.58 a	28.15 $\pm$ 0.84 ab	32.90 $\pm$ 1.04 ab	58.75 $\pm$ 0.14 d	38.95 $\pm$ 0.45 a
	0	26.35 $\pm$ 0.89 a	27.50 $\pm$ 1.62 a	14.10 $\pm$ 0.92 ab	30.90 $\pm$ 1.85 a	27.10 $\pm$ 0.17c	68.48 $\pm$ 1.52 a
	5	22.75 $\pm$ 0.14 b	23.00 $\pm$ 0.12 b	12.15 $\pm$ 0.20 b	26.00 $\pm$ 0.02 b	27.90 $\pm$ 0.52 c	66.91 $\pm$ 0.53 a
	10	21.45 $\pm$ 0.95 bc	21.45 $\pm$ 1.13 c	11.50 $\pm$ 0.40 b	24.35 $\pm$ 1.18 c	28.25 $\pm$ 0.43 c	66.33 $\pm$ 0.36 a
	15	21.10 $\pm$ 0.06 bc	22.50 $\pm$ 0.23 bc	11.85 $\pm$ 0.20 b	25.45 $\pm$ 0.32 bc	27.80 $\pm$ 0.17 c	64.75 $\pm$ 0.48 a
	30	21.90 $\pm$ 1.15 bc	20.15 $\pm$ 0.55 c	10.90 $\pm$ 0.06 b	22.95 $\pm$ 0.43 cd	28.55 $\pm$ 0.72 c	61.91 $\pm$ 1.58 ab
	60	22.40 $\pm$ 0.69 bc	18.90 $\pm$ 0.17 cd	11.85 $\pm$ 0.20 b	22.30 $\pm$ 0.06 cd	32.00 $\pm$ 0.69 b	58.09 $\pm$ 1.29 ab
	120	19.95 $\pm$ 0.60 bc	17.50 $\pm$ 1.62 cd	10.70 $\pm$ 0.87 b	20.55 $\pm$ 1.82 c	31.55 $\pm$ 0.32 b	59.66 $\pm$ 3.24 ab
	240	19.60 $\pm$ 0.23 bc	17.65 $\pm$ 0.78 cd	10.75 $\pm$ 0.66 b	18.90 $\pm$ 0.06 d	31.60 $\pm$ 0.64 b	61.23 $\pm$ 1.87 ab
'Crown Prince Rudolph'	360	20.05 $\pm$ 0.03 bc	16.00 $\pm$ 0.06 d	10.45 $\pm$ 0.66 b	19.15 $\pm$ 0.32 d	33.10 $\pm$ 1.79 a	55.43 $\pm$ 3.14 ab
	480	20.55 $\pm$ 0.14 bc	20.95 $\pm$ 0.32 c	11.25 $\pm$ 0.03 b	19.30 $\pm$ 0.29 d	34.25 $\pm$ 0.38 a	61.35 $\pm$ 0.40 ab
	0	56.85 $\pm$ 2.40 a	5.25 $\pm$ 0.32 f	19.00 $\pm$ 0.35 e	19.75 $\pm$ 0.43 g	74.55 $\pm$ 0.61 a	9.79 $\pm$ 0.04
	5	58.25 $\pm$ 0.26 a	9.60 $\pm$ 0.12 e	24.60 $\pm$ 0.12 d	26.40 $\pm$ 0.06 f	68.70 $\pm$ 0.35 b	15.67 $\pm$ 0.04
	10	55.25 $\pm$ 0.66 a	13.55 $\pm$ 0.95 d	30.70 $\pm$ 1.44 b	33.50 $\pm$ 1.73 ce	66.35 $\pm$ 0.49 c	22.44 $\pm$ 1.15
	15	45.80 $\pm$ 0.23 b	14.00 $\pm$ 0.52 d	29.70 $\pm$ 0.81 b	32.85 $\pm$ 0.95 e	64.75 $\pm$ 0.26 d	27.44 $\pm$ 1.06
	30	40.90 $\pm$ 0.23 c	18.25 $\pm$ 0.20 ab	32.85 $\pm$ 0.14 a	37.60 $\pm$ 0.23 b	61.00 $\pm$ 0.17 e	38.06 $\pm$ 0.14
	60	40.45 $\pm$ 0.43 c	19.30 $\pm$ 0.35 a	33.00 $\pm$ 0.23 a	33.25 $\pm$ 1.51 ce	59.70 $\pm$ 0.29 ef	40.13 $\pm$ 1.00
	120	30.45 $\pm$ 0.49 e	15.60 $\pm$ 0.40c	26.00 $\pm$ 0.69 cd	30.35 $\pm$ 0.78 e	59.10 $\pm$ 0.01 f	42.68 $\pm$ 0.41
'Red Boskoop'	240	35.25 $\pm$ 0.72 d	18.55 $\pm$ 0.38 ab	30.20 $\pm$ 0.98 b	35.50 $\pm$ 1.04 c	58.35 $\pm$ 0.32 f	43.57 $\pm$ 0.11
	360	30.55 $\pm$ 0.95 e	16.95 $\pm$ 0.43c	27.85 $\pm$ 0.78 c	32.60 $\pm$ 0.87 c	58.60 $\pm$ 0.06 f	40.13 $\pm$ 1.00
	480	41.75 $\pm$ 0.38 c	19.95 $\pm$ 0.26 a	33.90 $\pm$ 0.52 a	39.35 $\pm$ 0.61 a	59.55 $\pm$ 0.03 f	45.93 $\pm$ 0.22
	0	59.35 $\pm$ 0.61 b	5.75 $\pm$ 0.49 g	30.10 $\pm$ 0.25 bc	37.30 $\pm$ 0.29 a	75.35 $\pm$ 0.09 b	11.90 $\pm$ 0.61 g
	5	62.30 $\pm$ 0.01 a	9.45 $\pm$ 0.03 e	36.13 $\pm$ 0.27 a	30.65 $\pm$ 0.43 b	79.15 $\pm$ 1.07 a	16.43 $\pm$ 0.08 f
	10	49.40 $\pm$ 0.46 c	7.80 $\pm$ 0.11 f	28.90 $\pm$ 0.52 bc	30.00 $\pm$ 0.52 b	74.85 $\pm$ 0.03 b	16.91 $\pm$ 0.43 f
	15	47.65 $\pm$ 0.26 cd	10.35 $\pm$ 0.09 e	28.70 $\pm$ 0.11 bc	30.20 $\pm$ 0.12 b	70.45 $\pm$ 0.20 c	21.10 $\pm$ 0.26 e
	30	45.05 $\pm$ 0.03 cd	12.60 $\pm$ 0.17 d	28.75 $\pm$ 0.26 bc	31.40 $\pm$ 0.29 b	66.30 $\pm$ 0.06 d	25.62 $\pm$ 0.33 d
	60	37.85 $\pm$ 1.53 f	14.10 $\pm$ 0.23 c	27.70 $\pm$ 1.21 bc	31.05 $\pm$ 1.18 b	65.95 $\pm$ 0.66 d	32.68 $\pm$ 0.55 bc
'Opal'	120	39.80 $\pm$ 0.29 e	14.90 $\pm$ 0.64 b	27.65 $\pm$ 1.30 bc	31.40 $\pm$ 1.50 b	61.65 $\pm$ 0.09 e	32.33 $\pm$ 1.14 bc
	240	41.30 $\pm$ 0.06 e	17.65 $\pm$ 0.09 a	33.05 $\pm$ 0.32 b	37.50 $\pm$ 0.35 a	61.90 $\pm$ 0.12 e	36.85 $\pm$ 0.17 a
	360	36.65 $\pm$ 1.93 fg	15.40 $\pm$ 0.98 b	30.00 $\pm$ 1.48 bc	33.75 $\pm$ 1.77 ab	62.75 $\pm$ 0.38 e	36.30 $\pm$ 0.60 a
	480	35.60 $\pm$ 0.06 g	14.95 $\pm$ 0.26 b	28.20 $\pm$ 0.29 bc	31.90 $\pm$ 0.40 b	62.10 $\pm$ 0.12 e	36.56 $\pm$ 0.53 a
	0	59.25 $\pm$ 1.41 a	9.75 $\pm$ 0.26 de	35.90 $\pm$ 0.40 b	37.70 $\pm$ 0.75 b	74.85 $\pm$ 0.20 a	17.58 $\pm$ 0.05 e
	5	55.90 $\pm$ 1.27 b	9.35 $\pm$ 0.20 de	33.80 $\pm$ 0.89 b	35.05 $\pm$ 0.89 bc	74.50 $\pm$ 0.06 a	17.75 $\pm$ 0.01 e
	10	54.50 $\pm$ 0.46 b	9.10 $\pm$ 0.01 de	32.80 $\pm$ 0.17 bc	34.05 $\pm$ 0.14 c	74.50 $\pm$ 0.06 a	17.71 $\pm$ 0.12 e
	15	49.00 $\pm$ 0.17 c	8.70 $\pm$ 0.17 e	30.20 $\pm$ 0.12 c	31.40 $\pm$ 0.06 d	73.90 $\pm$ 0.40 a	18.58 $\pm$ 0.15 d
	30	47.65 $\pm$ 0.32 c	10.15 $\pm$ 0.03 d	30.40 $\pm$ 0.64 c	32.10 $\pm$ 0.64 cd	71.50 $\pm$ 0.35 b	21.20 $\pm$ 0.04 c
'Topaz'	60	51.65 $\pm$ 1.01 bc	17.60 $\pm$ 0.52 a	39.90 $\pm$ 1.10 a	43.60 $\pm$ 1.21 a	66.15 $\pm$ 0.03 c	31.04 $\pm$ 0.29 b
	120	38.70 $\pm$ 0.17 de	13.00 $\pm$ 0.29 c	29.25 $\pm$ 0.61 c	32.00 $\pm$ 0.69 cd	66.10 $\pm$ 0.06 c	30.56 $\pm$ 0.52 b
	240	41.25 $\pm$ 0.14 d	14.85 $\pm$ 0.20 b	30.45 $\pm$ 0.20 c	33.50 $\pm$ 0.29 c	64.30 $\pm$ 0.23 d	31.91 $\pm$ 0.37 b
	360	36.85 $\pm$ 1.36 e	14.85 $\pm$ 0.32 b	30.25 $\pm$ 1.13 c	33.70 $\pm$ 1.15 c	63.80 $\pm$ 0.35 d	35.58 $\pm$ 0.39 a
	480	34.90 $\pm$ 0.29 e	14.70 $\pm$ 0.17 b	29.55 $\pm$ 0.14 cd	33.05 $\pm$ 0.20 c	63.55 $\pm$ 0.14 d	36.99 $\pm$ 0.06 a
	0	49.95 $\pm$ 2.97 a	7.00 $\pm$ 0.52 e	25.10 $\pm$ 1.09 e	30.45 $\pm$ 1.30 bc	75.65 $\pm$ 0.03 a	14.79 $\pm$ 0.27 g
	5	50.25 $\pm$ 2.51 a	7.50 $\pm$ 0.35 e	29.50 $\pm$ 1.27 c	26.10 $\pm$ 1.14 d	74.50 $\pm$ 0.06 b	16.34 $\pm$ 0.08 f
	10	47.65 $\pm$ 0.95 ab	8.20 $\pm$ 0.23 de	27.40 $\pm$ 0.64 de	28.55 $\pm$ 0.66 c	73.30 $\pm$ 0.06c	17.76 $\pm$ 0.12 f
	15	49.90 $\pm$ 0.87 a	10.00 $\pm$ 0.17 d	30.90 $\pm$ 0.29 ab	32.50 $\pm$ 0.35 b	72.10 $\pm$ 0.17c	20.15 $\pm$ 0.05 e
'Golden Delicious'	30	47.60 $\pm$ 0.92 ab	12.50 $\pm$ 0.35 c	32.75 $\pm$ 0.60 a	35.00 $\pm$ 0.69 a	69.10 $\pm$ 0.23 d	25.03 $\pm$ 0.14 d
	60	42.25 $\pm$ 1.01 b	12.45 $\pm$ 0.49 c	29.55 $\pm$ 1.13 c	32.05 $\pm$ 1.24 b	67.15 $\pm$ 0.03 e	27.25 $\pm$ 0.41c
	120	39.55 $\pm$ 0.78 c	13.80 $\pm$ 0.29 b	30.30 $\pm$ 0.58 abc	33.30 $\pm$ 0.64 b	65.50 $\pm$ 0.01 f	31.51 $\pm$ 0.02 b
	240	39.60 $\pm$ 1.13 c	15.35 $\pm$ 0.89 a	31.55 $\pm$ 1.70 ab	35.10 $\pm$ 1.96 a	64.10 $\pm$ 0.12 g	34.30 $\pm$ 0.11 a
	360	33.70 $\pm$ 0.23 d	12.50 $\pm$ 0.06 c	26.25 $\pm$ 0.26 e	29.10 $\pm$ 0.23 bc	64.50 $\pm$ 0.11 g	33.06 $\pm$ 0.03 a
	480	32.80 $\pm$ 0.98 d	11.60 $\pm$ 0.06 cd	23.85 $\pm$ 0.38 f	26.50 $\pm$ 0.40 d	64.00 $\pm$ 0.23 g	31.42 $\pm$ 0.68 ab
	0	66.25 $\pm$ 0.49 a	6.80 $\pm$ 0.17 e	35.25 $\pm$ 0.32 a	35.95 $\pm$ 0.38 a	79.00 $\pm$ 0.17 a	12.54 $\pm$ 0.14 e
	5	49.85 $\pm$ 0.95 b	6.55 $\pm$ 0.20 e	25.85 $\pm$ 1.07 d	26.70 $\pm$ 1.10 b	75.80 $\pm$ 0.12 b	14.38 $\pm$ 0.23 e
	10	45.40 $\pm$ 1.15 c	9.85 $\pm$ 0.03 d	26.25 $\pm$ 0.38 d	28.05 $\pm$ 0.38 b	69.40 $\pm$ 0.23c	20.85 $\pm$ 0.40 d
	15	40.15 $\pm$ 1.36 de	10.90 $\pm$ 0.81 c	25.70 $\pm$ 1.05 d	27.90 $\pm$ 1.34 b	67.10 $\pm$ 0.64 cd	25.05 $\pm$ 0.80c
	30	37.40 $\pm$ 0.98 e	12.25 $\pm$ 0.20 b	26.00 $\pm$ 0.75 d	28.70 $\pm$ 0.75 b	64.80 $\pm$ 0.23 d	29.36 $\pm$ 0.19 b

(continued on next page)

Table 6 (continued)

Cultivar	Time (min)	Color parameters					
		L*	a*	b*	C*	h°	BI
	60	40.10 ± 1.50 de	15.25 ± 0.72 a	29.90 ± 1.44 c	36.25 ± 0.03 a	63.20 ± 0.12 d	33.26 ± 0.33 ab
	120	42.10 ± 0.40 d	16.10 ± 0.40 a	32.70 ± 0.69 b	36.45 ± 0.78 a	63.80 ± 0.06 d	30.35 ± 0.49 b
	240	36.25 ± 0.09 e	15.55 ± 1.18 a	26.15 ± 0.03 d	30.25 ± 0.55 bc	60.65 ± 1.13 e	36.06 ± 2.13 a
	360	37.30 ± 0.01 e	14.55 ± 0.55 ab	29.70 ± 0.40 c	33.05 ± 0.61 ab	63.95 ± 0.55 d	34.46 ± 1.05 ab
	480	34.60 ± 0.52 f	11.75 ± 0.38 b	24.80 ± 0.75 de	27.45 ± 0.84 b	64.65 ± 0.03 d	33.75 ± 0.48 ab

Statistically significant differences ($p < 0.05$) are presented by different letters following the mean values within the same columns and for each cultivar.

driven by high POX activity, which degrades anthocyanins without causing browning unless PPO activity is also elevated (Espley et al., 2019). Additionally, high POX activity correlates with faster anthocyanin loss and decreased color intensity, but browning does not necessarily occur in the presence of ascorbic acid (Kader et al., 2006).

'Baya Marisa' displayed slight fluctuations in ascorbic acid over time, peaking at 15 min (0.23 mg/g FW) before steadily declining to its lowest level at 60 min (0.16 mg/g FW). Ascorbic acid levels in 'Idared' remained stable with minor late declines. 'Opal' showed minimal fluctuations, while 'Red Boskoop' maintained stable levels during the first 15 min followed by a slight decline to 0.11 mg/g FW at 480 min. 'Topaz' exhibited relatively stable ascorbic acid content, with slight decreases by the end of the experiment. Despite declines over time, 'Golden Delicious' consistently maintained the highest ascorbic acid levels at all time points. 'Crown Prince Rudolph' showed stable levels with minimal changes. Each cultivar showed distinct vitamin C decay patterns, with 'Golden Delicious' experiencing up to a 50 % reduction during air exposure, yet retaining the highest

4. Conclusion

The comparative analysis of enzymatic and biochemical attributes among seven apple cultivars reveals significant variability in browning potential, predominantly driven by PPO and POX activity, TPC, ascorbic acid and anthocyanins. 'Idared' exhibited the highest PPO activity and fastest browning, while 'Baya Marisa' PPO activity was the lowest, indicating absence or minimal browning. Color parameters and the browning index showed substantial changes in white-fleshed cultivars, indicative of browning. In contrast, browning index did not change for 'Baya Marisa'. The TPC significantly decreased in all studied cultivars from 0 to 480 min after air exposure, where 'Baya Marisa' had significantly higher TPC compared to white-fleshed cultivars at any given time point, due to high anthocyanin content in the flesh. Ascorbic acid degradation was noted in all cultivars across the time intervals. 'Golden Delicious' had significantly higher ascorbic acid content compared to other cultivars. The results underline the importance of cultivar selection based on enzymatic profiles and biochemical composition to optimize postharvest apple quality. Future studies should explore alternative methods to inhibit enzymatic browning in white-fleshed apples while leveraging the natural resistance traits of red-fleshed cultivars like 'Baya Marisa'.

CRedit authorship contribution statement

Grohar Mariana: Writing – review & editing, Supervision, Software, Investigation, Formal analysis. **Franci Stampar:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Simkova Kristyna:** Investigation. **Metka Hudina:** Writing – review & editing. **Jerneja Jakopic:** Writing – review & editing, Supervision, Conceptualization. **Aljaz Medic:** Software, Project administration, Methodology, Data curation, Conceptualization, Writing – review & editing, Validation, Supervision. **Jan Juhart:** Writing – original draft, Visualization, Software, Resources, Methodology, Investigation, Formal analysis, Data curation.

Originality

This manuscript is an original work and has not been published previously nor is it currently under consideration for publication elsewhere.

Approval of the manuscript

All authors have read and approved the final version of the manuscript and agree to its submission to the *Journal of Food Composition and Analysis*.

Ethical approval

This study does not involve any studies with human or animal subjects.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2025.108040.

Data availability

Data will be made available on request.

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