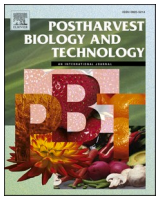
Postharvest Biology and Technology 233 (2026) 114066

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio



Development and implementation of an affordable high-throughput imaging system for phenotyping enzymatic browning in apples

Carlos Miranda [a,b,*], Patricia Irisarri [c,d], Sara Crespo-Martínez [b], Francisco Javier Bielsa [c,d], Nerea Iturmendi [a,e], Haizea Romeo [b], Jorge Urrestarazu [a,b], Ana Pina [c,d], Luis Gonzaga Santesteban [a,b], Lourdes Castel [c,d], Pilar Errea [c,d]

[a] Instituto de Investigación Multidisciplinar en Biología Aplicada (IMAB), Universidad Pública de Navarra (UPNA), Campus de Arrosadia, Pamplona 31006, Spain
[b] Departamento de Agronomía, Biotecnología y Alimentación, Universidad Pública de Navarra (UPNA), Campus de Arrosadia, Pamplona 31006, Spain
[c] Unidad de Hortofruticultura, Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA), Avda. Montañana 930, Zaragoza 50059, Spain
[d] Instituto Agroalimentario de Aragón (IA2), CITA-Universidad de Zaragoza, Zaragoza 50013, Spain
[e] Instituto de Innovación y sostenibilidad en Cadena Agroalimentaria (ISFOOD), Universidad Pública de Navarra (UPNA), Campus de Arrosadia, Pamplona 31006, Spain

ARTICLE INFO

Keywords:
Browning index
Genetic resources
Malus × *domestica* Borkh
Digital image analysis

ABSTRACT

Enzymatic browning (EB) substantially affects the visual quality and marketability of fresh-cut apples. This study aimed to develop an affordable high-throughput imaging system for phenotyping EB in apples. Browning was quantified using four CIELab-derived indices; a browning Index (BI), the difference in BI (ΔBI), a normalized CIE color difference (ΔE*); and a CIEDE2000 color difference (ΔE_{00}) at multiple time points post-cutting to evaluate browning speed (S_{EB}) and intensity (I_{EB}) in 142 apple cultivars, including commercial and traditional Spanish cultivars from germplasm collections. The image-based system has demonstrated high accuracy and practical relevance, overcoming limitations associated with traditional colorimeter-based approaches. A wide phenotypic range was observed, in which elite reference cultivars fell within a narrow band at the lower end of the range. Measurements taken at 30 min post-cutting were found to be nearly equivalent to those at 60 min, allowing to optimize the phenotyping protocol without compromising precision. EB has been shown to be an inherently stable trait, though different year effects were noted, particularly for BI and ΔBI. Among the indices evaluated, ΔE_{00} proved less effective for cultivar differentiation, whereas ΔBI showed the highest discriminant capacity and strongest correlation with visual browning, making it the most suitable index for phenotyping purposes. These findings provide a robust methodological basis for screening low-browning apple genotypes, establish a classification framework for EB expression levels, and highlight the potential of underutilized traditional cultivars in developing improved fresh-cut apple products.

1. Introduction

The demand for fresh-cut apples, sold as a snack or included in ready-to-eat salads, has increased in recent years due to shifts in consumer preferences, as there is a growing demand for fresh products that are convenient to consume and offer high nutritional value (Nicola et al., 2022). The development of this kind of fresh-cut or minimally processed fruit involves peeling, slicing, or chopping, which results in a range of degradative changes. These changes present additional challenges to the fresh-cut industry, as maintaining quality for an acceptable marketing period is crucial. One substantial factor that contributes to the deterioration of apple quality is the development of browning in cut surfaces, which results in unfavorable alterations in the visual appeal and organoleptic characteristics of the food. Browning negatively impacts the product's market value and, therefore, its exclusion from certain markets (Jaeger et al., 2018).

The browning observed in freshly cut apples has an enzymatic origin, and is mainly attributed to the action of two enzymes, namely polyphenol oxidase (PPO) and peroxidase (POD) (Zhu et al., 2023) that cause the oxidation of apple phenolics when they are released from the

* Corresponding author at: Instituto de Investigación Multidisciplinar en Biología Aplicada (IMAB), Universidad Pública de Navarra (UPNA), Campus de Arrosadia, Pamplona 31006, Spain.
E-mail address: carlos.miranda@unavarra.es (C. Miranda).

https://doi.org/10.1016/j.postharvbio.2025.114066
Received 16 September 2025; Received in revised form 31 October 2025; Accepted 12 November 2025
Available online 18 November 2025

vacuoles. These are released after the occurrence of physical stresses such as cutting, peeling, and other forms of mechanical damage, and interact with PPO (in the presence of oxygen) and POD (in the presence of H_2O_2), with subsequent reactions leading to the formation of melanin, the brown color pigment. The synthesis of polyphenols as a substrate of browning relies on the activity of phenylalanine ammonia lyase (PAL), whereas superoxide dismutase (SOD), a key enzyme in the cell membrane protection system, can limit the occurrence of browning reducing the accumulation of intracellular reactive oxygen species (ROS) (Wang et al., 2024).

Common strategies to prevent EB include processing and packaging under modified atmospheres and low temperatures, thermal processes, dipping the cuts in anti-browning agents such as organic acids (ascorbic or citric, mainly) and their derivatives, or applying edible coatings, among other methods (Altisent et al., 2014; Arnold and Gramza--Michałowska, 2022; Kumar et al., 2018; Pignata et al., 2018). An alternative approach to control EB is to use or breed apples which are low or non-browning, as apple cultivars show differences in their susceptibility to fresh-cut browning (Burke, 2010; Kalinowska et al., 2014; Toivonen, 2006). This approach prevents the drawbacks of anti-browning formulations and processes that are banned from commercialization for safety reasons or increase production costs in fresh-cut apple slices. The relevance of this issue is exemplified by the fact that even a few gene-modified (GM cultivars (Artic™ apples), which do not turn brown when cut, have been allowed to be marketed in both Canada and the USA (Xu, 2015). However, public concerns about the use of GMOs and the current legal constraints on their use, particularly in the European Union, make it not feasible to extend this approach to a broader context. As a result, alternative solutions are required. Among these potential solutions, exploitation of traditional cultivars can play a great role, through either the direct use of some cultivars or by their use in breeding programs, for the development of non-browning apple selections (Cebulj et al., 2023, 2021).

The physical characteristics of fruit browning are generally assessed by evaluating the color of the cut surface and the subsequent color change over time, typically in the CIELab 1976 space (Carter et al., 2018). This is achieved through the calculation of indices derived from the values of L* (lightness), a* (greenness to redness), and b* (yellowness to blueness) (Shimizu et al., 2021). The most commonly utilized indices in apples are the browning index (BI) or brown color purity, and ΔE* or normalized CIE color difference (Arnold and Gramza-Michałowska, 2022). However, other indices have also been proposed (Shimizu et al., 2021), including ΔBI or BI difference, ΔE_{00} or CIEDE2000 color difference (Sharma et al., 2005), which is an adjusted index of ΔE* that is more accurately calibrated to human perception, among others. Nevertheless, little information is available regarding the comparative performance or suitability of these indices for phenotyping and assessing variability in EB in apples, as few formal comparisons have been conducted. It has been demonstrated (Shimizu et al., 2021) that a BI enables a more precise evaluation of color tone, while the ΔBI is more closely associated with visual distinctions between cultivars than ΔE_{00}. Additionally, flesh tone (yellowish, reddish) may impact the capacity to assess variations in EB evolution over time in high-oxidation cultivars. In other studies, although no formal comparison was performed, the results suggest that different indices generally tend to agree but there may be more or less severe discrepancies in the relative ranking of cultivars (Serra et al., 2021). In any case, pre-existing research has been conducted with a limited number of cultivars, generally ranging from two to six (Arnold and Gramza-Michałowska, 2022; Shimizu et al., 2021), and, at most, between 14 and 17 (Burke, 2010; Serra et al., 2021). These studies have predominantly emphasized major elite table cultivars, representing a narrow spectrum of browning potential, often biased towards those with low oxidation. Consequently, the actual range of quantifiable variation in EB in apples remains undetermined, and there is currently no established scale or guideline for determining the relative browning potential of a given variety. Additionally, there is a lack of pertinent information regarding phenotyping, such as the extent to which the recorded values and the ranking of cultivars may vary across different years or even among fruits from the same season.

In the majority of studies (as reviewed by Arnold and Gramza--Michałowska, 2022), EB is characterized through the use of colorimeters, which facilitate precise control over the illumination of the surface under examination and yield highly accurate results. To obtain accurate phenotyping of EB, it is essential to measure the entire surface of the fruit portion. This is because PPO and phenolics are distributed unevenly in the flesh, which causes browning patterns to form (Duangmal et al., 2017; Quevedo et al., 2014). In this regard, colorimeters present a significant limitation, as the measurement area is restricted (circles of 8–11 mm in diameter), requiring the acquisition of multiple measurements per fruit with an appropriate sampling strategy (Burke, 2010). This also constrains the speed and volume of samples that can be processed per session, rendering the cost-effective phenotyping of large batches challenging (Shimizu et al., 2021). In this regard, the combination of a digital camera or flatbed scanner with image processing software has been demonstrated to offer a cost-effective and highly versatile alternative for the phenotyping of traits related to flesh color (Bouillon et al., 2024; Shimizu et al., 2021; Subhashree et al., 2017). Nevertheless, these systems have yet to advance beyond the proof-of-concept stage, and, to the best of our knowledge, have not been employed for the high throughput (HT) phenotyping of EB in apple collections.

The objective of this study was to apply an affordable, high-throughput image analysis system to digitally phenotype apple browning in a large and diverse set of traditional Spanish and reference cultivars. The specific aims were to quantify the range of expression of enzymatic browning in fresh-cut apple halves using the most common EB indices, and to compare the performance of these indices in terms of uniformity of measurements, and classification and discriminant capabilities.

2. Materials and methods

2.1. Plant material

The study has been conducted in 2020 and 2021 on 142 apple cultivars from 104 genotypes (Table S1), including international references (15 cvs.), and traditional Spanish cultivars from the germplasm collections in Universidad Pública de Navarra (UPNA) (67 cvs.), and Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA) (60 cvs.). The germplasm collections are located, respectively, in Pamplona, Navarra (42.79038, −1.63036) and in Zaragoza, Aragón (41.72345, −0.80986) and Bescós de Garcipollera, Aragón (42.62526, −0.49778). The set contains *M. domestica* cultivars including cider, dessert, processing, and heritage cultivars that are part of a core collection which optimizes the representativeness of the genetic variation of the Spanish germplasm collections (Miranda et al., 2018). In order to determine EB related traits, cultivars were harvested at full ripening stage. The optimal harvest time was determined by monitoring the ripeness level of all cultivars on a weekly basis. This monitoring process involved the use of several maturity indicators, combined with expert knowledge. Fruits were cut in half to check the browning of seeds and that the starch iodine index exceeded value 6, according to (CTIFL, 2002) scale. Changes in background skin color and dropped apples were additional indicators of ripeness (Watkins, 2003). The sample size for determining the ripening stage was five apples, uniform in size and appearance, and positioned at mid-height in the outer part of the canopy. The process of monitoring and harvesting was particularly challenging due to the broad range of maturity dates, and commenced in early July, concluding in early November for the later ripening cultivars. At harvest, a sample of ten fruits per cultivar was collected, employing the same sampling criteria established for monitoring ripeness, and stored at 5ºC for a week before phenotyping.

2.2. Phenotyping of enzymatic browning (EB)

2.2.1. Image acquisition

The determination of the EB was made by image analysis from photographs taken under standardized conditions using an affordable approach. The image acquisition system (Fig. 1) consisted of an Olympus OM-D MKII (Olympus, Tokyo, Japan) digital camera with a Zuiko ED 30 mm 1:3.5 macro lens (Olympus). Batches of 10 lower halves of fruit were placed in matte-black plastic packing alveoli trays to prevent them from tumbling over and placed under the camera. The batches were illuminated with two NanGuang CN-576C LED panels (Guangdong NanGuang Ltd, Shantou, China) set at 5500 K and arranged to provide uniform illumination and avoid shadows in a room without any external light. A grey check card (Kaavie GC-2 Pocket) was used to determine optimum exposure and white balance settings, and it was included in each photograph to facilitate white balance and color corrections. The following parameters were adjusted in the camera: sRBG color space, RAW mode, exposure mode, manual; white balance, preset manual; metering, ESP matrix; focal length, 30 mm; ISO speed, ISO-200; aperture, *f*4.5; exposure time, 1/100 s.

For each batch, the first measurement was taken within 1 min after the start of the preparation, and then photographs were taken approximately at 5, 10, 15, 20, 25, 30, 45, and 60 min after the start of batch preparation. The exact time after cutting corresponding to each photograph was obtained from their timestamps and used for subsequent calculations.

2.2.2. Image processing and browning index calculation

Images were processed using a custom color evaluation script (Miranda, 2024a) for the open-source image analysis software Fiji (Schindelin et al., 2012), available at GitHub (https://github.com/Carm1r/Pheno_ImageJ). The script automated the process of obtaining the average color of the entire fruit surface (Fig. 2). Firstly, the script performs background extraction and identification of individual fruit halves. Then, it transforms the original RGB image into three images containing each L*, a*, and b* color values, and lastly obtains the average of the CIEL*a*b* color values of the entire fruit surface.

Browning for each fruit at each photography acquisition time was evaluated from the values obtained for L*, a* and b* through four indices:

- Browning Index (BI), according to the method of Palou et al. (1999):

$$BI = [100(x - 0.310)]/0.172 \quad (1)$$

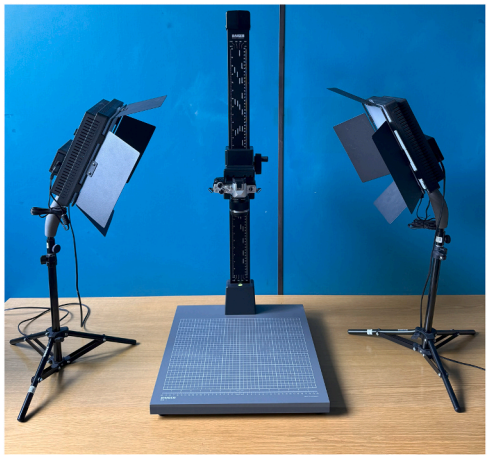


Fig. 1. Depiction of the image acquisition system used in this study consisting of a digital DLSR camera mounted in an adjustable camera arm and two LED panels. Photographs were taken with the LED panels as the sole light source in the room.

$$\text{where} x = (a^* + 1.75L^*)/(5.645L^* + a^* - 3.012b^*) \quad (2)$$

- Difference in BI (ΔBI) from the time of cutting as

$$\Delta BI = BI_0 - BI_t \quad (3)$$

- Normalized CIE color difference (ΔE*) from the time of cutting as

$$\Delta E^* = \sqrt{(L_t^* - L_0^*)^2 + (a_t^* - a_0^*)^2 + (b_t^* - b_0^*)^2} \quad (4)$$

- CIEDE2000 (Luo et al., 2001) color difference (ΔE_{00}) from the time of cutting defined as

$$\Delta E_{00} = \sqrt{\left(\frac{\Delta L'}{k_L S_L}\right)^2 + \left(\frac{\Delta C'}{k_C S_C}\right)^2 + \left(\frac{\Delta H'}{k_H S_H}\right)^2 + R_T\left(\frac{\Delta C'}{k_C S_C}\right)\left(\frac{\Delta H'}{k_H S_H}\right)} \quad (5)$$

and calculated according to expressions described in Sharma et al. (2005), by means of the package *ColorNameR* (Sanchez-Beekman, 2021).

For each fruit analyzed, logarithmic regressions were fitted with the time after cutting as the independent variable and the values of each of the four EB indices (BI, ΔBI, ΔE* and ΔE_{00}) as the dependent variable, in order to obtain the fitted EB values for each index at 5, 30 and 60 min after fruit cutting. The values obtained were then used to characterize the dynamics of each browning index in terms of speed and intensity, as follows:

- *Initial browning speed (S_{EB})*: Defined as the angle (rad) formed by the slope of the line connecting the EB values at 0 min (EB_0) and 5 min (EB_5) after cutting:

$$S_{EB} = \tan^{-1}((EB_5 - EB_0)/5) \quad (6)$$

- *Browning intensity (I_{EB})*: Defined as the EB values at 60 min after cutting (I_{60}). Additionally, the EB values at 30 min (I_{30}) were also evaluated to assess if they could accelerate the phenotyping data collection process without significant loss of precision.

2.3. Evaluation of index performance

2.3.1. Evaluation of the uniformity of the measurements

The uniformity of the measurements was evaluated within and between years; within the same year by the coefficient of variation (CV) of the 10-fruit values measured per cultivar and EB index, while for the between-years, one-way analysis of variance with year as a factor was conducted on the EB values for each index, for each cultivar and for all cultivars pooled.

2.3.2. Consistency of classifications in EB levels

To assess the consistency of cultivar classifications in EB levels across the indices, Pearson's pairwise correlation coefficients were calculated with the R package GGally (Schloerke et al., 2024), as well as the proportion of cultivars classified into the same EB level. To that end, cultivars were classified in S_{EB} and I_{EB} levels at 30 min and 60 min after cutting using the methodology described in Miranda et al. (2017) and Royo et al. (2017), which allows defining the states of expression for a phenotypical trait in a clear and unambiguous way. Briefly, the number of expression states is determined according to the range and the variability (standard deviation, SD) of the fitted S_{EB} and I_{EB} values observed between and within the cultivars. Consequently, a wider expression range between cultivars and lower variability within them would result in a greater number of states. Thus, the discrimination unit (DU), used to define the size of the trait states was calculated as:

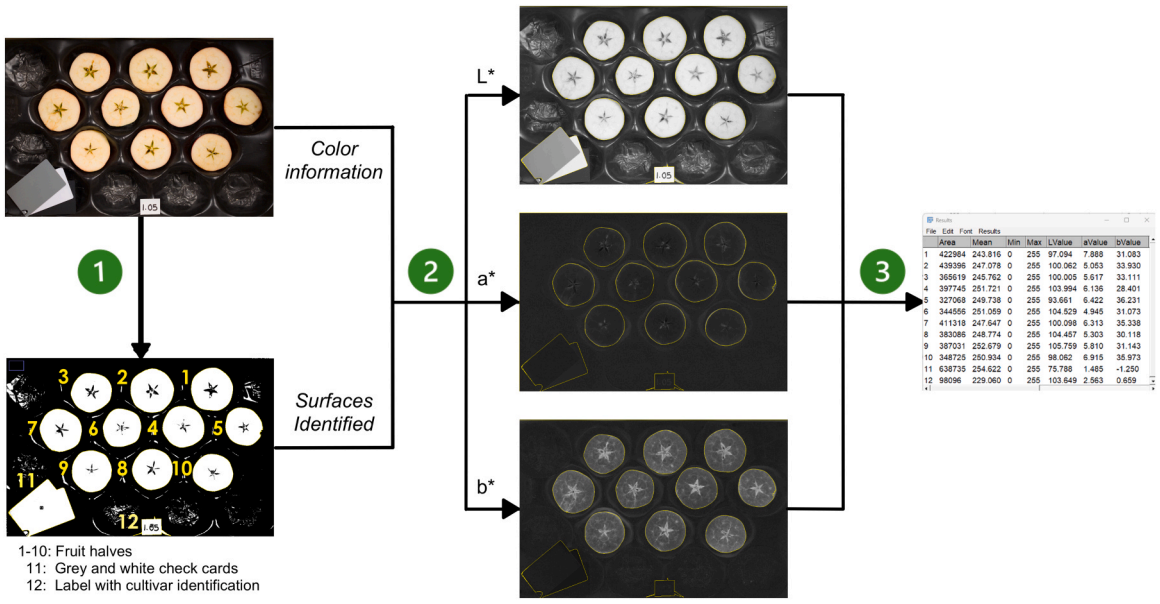


Fig. 2. Image processing workflow followed the custom script *Pheno_ImageJ*. 1: The script extracts the background of the image and identifies and numbers (highlighted in yellow) individual surfaces greater than a user-defined size. 2: The original image is transformed into three images in which each pixel contains only the L*, a* or b* color information, and the boundaries of the surfaces are superimposed. 3: The script obtains the average value of L*, a* and b*, respectively, of the pixels enclosed on each surface.

$$DU = Rg_{set} + SD_{set} \quad (7)$$

where Rg_{set} is the mean intra-cultivar range (that is, the difference between the max and min trait values within each cultivar) and SD_{set} is the SD of Rg_{set} in the set of cultivars. Once the DU was obtained, the number of expression levels for the trait was calculated using the frequency distribution of trait values measured on the set of cultivars. The central level was centered on the median of the distribution and the rest of levels placed at increases/decreases of 1 DU with respect to the central level. The R package *phenoclass* (Miranda, 2024b), available at GitHub (https://github.com/Carm1r/phenoclass) was created to facilitate the definition process of DUs and states of expression.

2.3.3. Discriminant ability of the EB indices

The discriminating ability of each EB index was evaluated using the discriminating ratio (DR) according to Levy et al. (1999) and Browning et al. (2004). Briefly, for each EB index, the mean SD of the measurements obtained for each cultivar (SD_W) and the SD of the mean values measured from different cultivars (SD_B) were calculated. SD_B was corrected using SD_W to estimate underlying SD (SD_U, $SD_U = \sqrt{SD_B^2 - SD_W^2/k}$), which represents an unbiased estimate of the SD, where k is the number of measurements per cultivar ($k = 10$). Finally, DR is calculated as SD_U/SD_W. In addition, confidence intervals were calculated using non-central F distributions for each DR, and the statistical independence of the DRs obtained for the four EB index, in S_{EB}, I_{30} and I_{60}, was evaluated using Q-statistic, according to Levy et al. (1999).

2.4. Data curation, handling and analysis

Data curation, handling and analysis were performed in RStudio 2023.06.0 (RStudio Team, 2020) environment of R 4.4.0 (R Core Team, 2022). Graphical display of EB values, correlations and repeatability of the measurements was performed using the R package *ggplot2* (Wickham, 2016).

3. Results and discussion

3.1. Browning patterns in apple germplasm collections after fresh cutting

Apple halves are typically browned rapidly during the first 5–20 mins after cutting, the increase slowing significantly after that. Fig. 3 shows a selection of representative EB patterns found within the set of cultivars. Those patterns have been adequately fitted by logarithmic equations in order to obtain the EB values at the precise desired moments. The patterns found among the cultivars and illustrated in Fig. 3 closely match previous reports (Burke, 2010; Shimizu et al., 2021; Subhashree et al., 2017), in which the most rapid initial EB typically occurs within the first 10–20 mins but in highly sensitive cultivars may occur even in the first 5 mins (Burke, 2010; Shimizu et al., 2021). The rate of browning has been reported to decline exponentially so that most of the browning potential is achieved within 60 mins (López-Nicolás et al., 2007; Lozano et al., 1994; Shimizu et al., 2021; Subhashree et al., 2017). In this study, only the first 60 mins after cutting have been investigated for practical reasons, as it allows to phenotype large sets in reasonable periods of time. Although the EB process may continue for at least 24 h after cutting (Serra et al., 2021; Shimizu et al., 2021).

3.2. Range of value differences between cultivars

A wide range of EB intensities were observed in the cultivars (Fig. 4a, e), with up to 6-fold (ΔBI), 15-fold (ΔE*, ΔE_{00}) or even 60-fold (BI) differences between the cultivars with the lowest EB and those with highest. However, as shown by the interquartile range in the boxplots, most cultivars were typically within a 2-fold range regardless of the index used. Most of the I_{EB} occurred in the first 30 mins after the fruit was cut, as typically I_{EB} values increased up to 30 % afterwards, but the extent of the increase was different depending on the index used. Thus, I_{EB} for BI at 30 mins was very close to the final one (average increase of 9 %±3 %), whereas ΔBI showed the highest increases (23 %±9 %). Finally, ΔE* and ΔE_{00} showed similar increases and intermediate to the other two (17 %±7 %). Concerning S_{EB} (Fig. 4e), the range of values was also very wide (between 5- and 130-fold), although, as was also the case in I_{EB}, the accessions were generally within a much narrower range

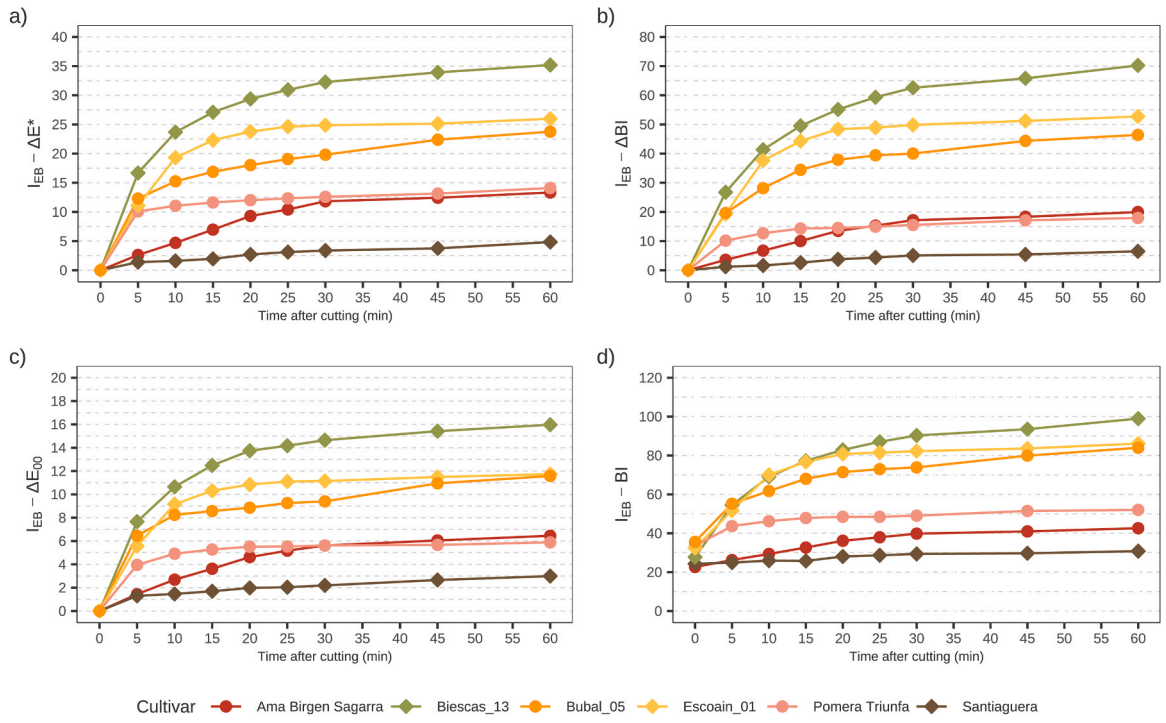


Fig. 3. Selection of representative patterns of enzymatic browning found within the cultivars evaluated in this study, evaluated by four indices. Each dot corresponds to the average value for ten bottom halves of apples, cut at the maximum diameter.

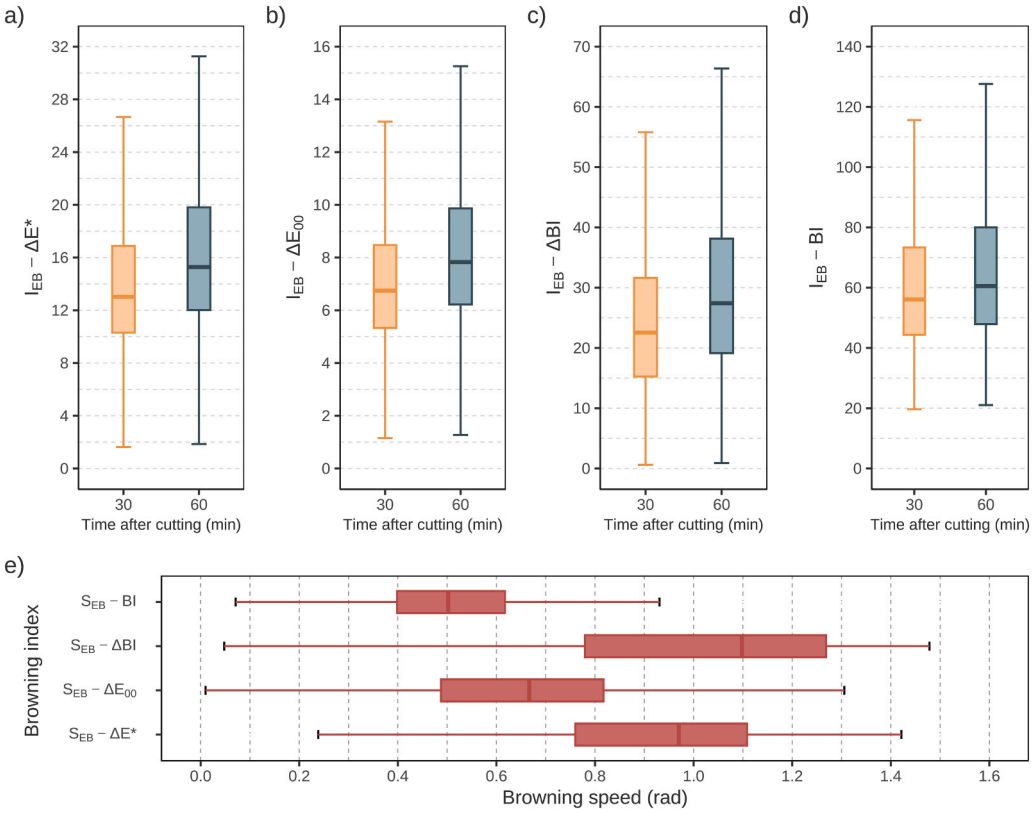


Fig. 4. Range of values for the enzymatic browning intensity found in the set of 142 traditional and reference cultivars, using the indices a) normalized CIE color difference (ΔE*), b) CIEDE2000 color difference (ΔE00), c) difference in browning index (ΔBI) and d) browning index (BI) at 30 min and 60 min after cutting; and e) range of values for the initial browning speed using the same indices.

Table 1
Enzymatic browning (EB) values for intensity (I_{EB}) and speed (S_{EB}) found in the reference cultivars using four indices over the two years of study. EB intensity has been evaluated at 30 (T30) and 60 (T60) min after cutting.

		Intensity of EB (I_{EB}) values						Speed of EB (S_{EB}) values					
		ΔE*		ΔE_{00}		ΔBI		BI					
Type	Cultivar	T30	T60	T30	T60	T30	T60	T30	T60	ΔE*	ΔE_{00}	ΔBI	BI
Gala group	Gala	8.3	10.3	4.5	5.4	13.9	17.4	52.1	56.8	0.71	0.38	0.85	0.50
	Mondial Gala	8.3	9.6	4.4	4.9	10.4	12.3	56.2	58.3	0.77	0.55	0.57	0.22
	Royal Gala	8.6	10.6	4.5	5.6	13.4	17.5	55.2	57.5	0.57	0.34	0.72	0.39
	Galaxy	9.5	12.4	4.9	6.1	17.2	23.3	63.3	68.8	0.34	0.28	0.07	0.44
	Gala Must	7.9	9.8	4.3	4.5	14.6	18.2	47.7	52.3	0.65	0.41	0.94	0.39
Fuji		11.3	13.2	5.4	5.9	16.1	19.9	50.1	52.1	0.97	0.64	0.98	0.51
Golden Supreme		4.4	5.4	2.2	2.7	5.2	6.8	27.6	29.4	0.35	0.20	0.21	0.15
Golden group	Golden Delicious	8.6	11.3	4.1	5.8	12.2	17.1	49.8	56.6	0.64	0.41	0.29	0.26
	Pinkgolden	17.5	19.9	6.2	6.9	25.5	30.7	49.1	53.3	1.11	0.81	1.15	0.58
Cripps Pink		10.9	12.6	4.6	5.3	12.2	14.6	42.5	45.2	0.93	0.55	0.92	0.47
Pinova		11.6	13.4	5.1	5.8	17.9	21.6	53.7	57.8	0.94	0.59	1.05	0.55
Traditional	Esperiega	10.8	12.0	6.1	6.3	12.7	14.1	32.4	33.9	1.02	0.73	1.05	0.42
	Reinette Blanche	11.5	13.5	6.2	7.4	17.9	21.1	50.0	53.1	0.88	0.56	1.07	0.53
	Reinette Gris	14.7	17.3	7.4	8.7	20.9	25.0	39.6	44.6	1.01	0.69	1.11	0.53
	Verde Doncella	7.7	9.3	4.2	5.2	9.2	12.1	37.4	39.5	0.68	0.48	0.61	0.35

(1.5x).

The reference cultivars (Table 1) showed mostly low levels of I_{EB} and were placed mostly around or below the first quartile (ΔE*, ΔE_{00}) or between the first quartile and the median (BI, ΔBI), thereby resulting in the remaining range of expression populated almost exclusively by the traditional cultivars. The relative order among cultivars varied depending on the index used, but in general 'Golden Supreme' and 'Verde Doncella' were the least sensitive, while both 'Reinettes' and 'Pinkgold' were the most sensitive. The 'Gala' cultivars evaluated showed low sensitivity to browning (except for BI) and were similar to each other. In contrast, within the 'Golden' group, 'Golden Delicious' displayed a similar browning tendency comparable to that of 'Gala', whereas 'Pinkgold', ranked among the most sensitive cultivars tested. In terms of S_{EB}, the reference cultivars showed moderate or high browning speeds, mostly falling within the first quartile (BI) or the interquartile range (ΔE*, ΔE_{00}, ΔBI).

The values and ranking orders obtained in this study for I_{EB} and S_{EB} in the reference cultivars agree with findings reported in the available literature using either colorimeters (Arnold and Gramza-Michałowska, 2022; Burke, 2010; Putnik et al., 2017; Serra et al., 2021) or similar photography booth setups (Shimizu et al., 2021; Subhashree et al., 2017). Until now, research in EB has been focused on a few major dessert cultivars (Arnold and Gramza-Michalowska, 2022; Burke, 2010; Putnik et al., 2017), which represent a narrow fraction of the genetic diversity within the species. Given that consumer satisfaction with fresh cultivars is strongly influenced by visual aspects (Jaeger et al., 2018; Musacchi and Serra, 2018), it is not surprising that those elite cultivars fall within a narrow band at the lower end of the total EB variability when analyzed alongside a core collection that maximizes the genetic diversity of the species, which includes cider, table, processing, and heritage cultivars.

3.3. Uniformity of EB measurements within fruits in the same season and between years

The uniformity of measurements was assessed both within and between years. For within-year uniformity, the differences in EB within the 10 fruits measured per cultivar and year were evaluated by the CV. This is a statistic often used as a measure of experimental quality, considering that lower CV values indicate greater accuracy measurements. CV values vary according to the type of trial, the crop and, in particular, the trait under study (Ferreira et al., 2016) but, in agronomy, is quite frequent the general classification proposed by Pimentel-Gomes (2009): low (<10 %), average (10 %-20 %), high (20 %-30 %) and very high (>30 %). In this work, the uniformity in I_{EB} (Fig. 5a) was similar at 30 and 60 mins after cutting. I_{EB} proved to be a fundamentally stable character, i.e., with low levels of CV, which were classified as typically moderate-low (CV<16 % in ΔE*, ΔE_{00} and BI) or moderate (CV<21 % in ΔBI) and within the 2-fold range. For S_{EB} (Fig. 5b), similar differences between indices were observed, but with broader differences between cultivars (typically up to 4-fold) and in the moderate-high CV levels (typically up to 32 %).

For between-year uniformity (Fig. 6, Table S.2), differences in I_{EB} were observed in all indices, although the intensity of the effect differed according to the index. The mean variations for ΔE* and ΔE_{00} were moderate, ranging from 20 % to 30 %. In contrast, the variations for ΔBI and BI were considerably more pronounced, reaching 80 % and 95 %, respectively. Indeed, the year effect was significant for both ΔBI and BI in most cultivars (90 %), whereas it was only significant for ΔE* and ΔE_{00} in approximately half of them. Similar results were observed for S_{EB} (Fig. 6e), although the intensity of the year effect was considerably lower, ranging from 2 % (non significant) of ΔE* to 15 % of BI. The impact of the time after cutting on I_{EB} was also assessed. No differences in uniformity were observed between measurements taken at 30 and 60 min for any of the indices.

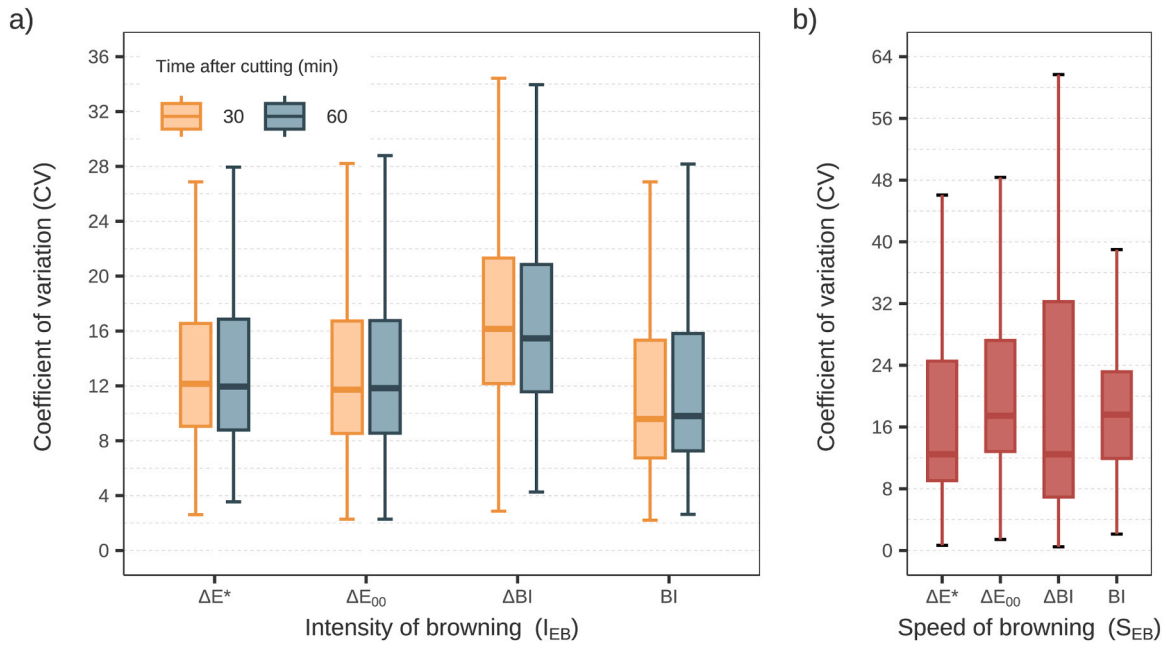


Fig. 5. Within-year uniformity of measurements by means of the coefficient of variation (CV,%) within the 10-fruit samples taken on each cultivar for a) browning intensity and b) browning speed found in the142 traditional and reference cultivars, using the indices normalized CIE color difference (ΔE*), CIEDE2000 color difference (ΔE00), difference in browning index (ΔBI) and browning index (BI) at 30 min and 60 min after cutting.

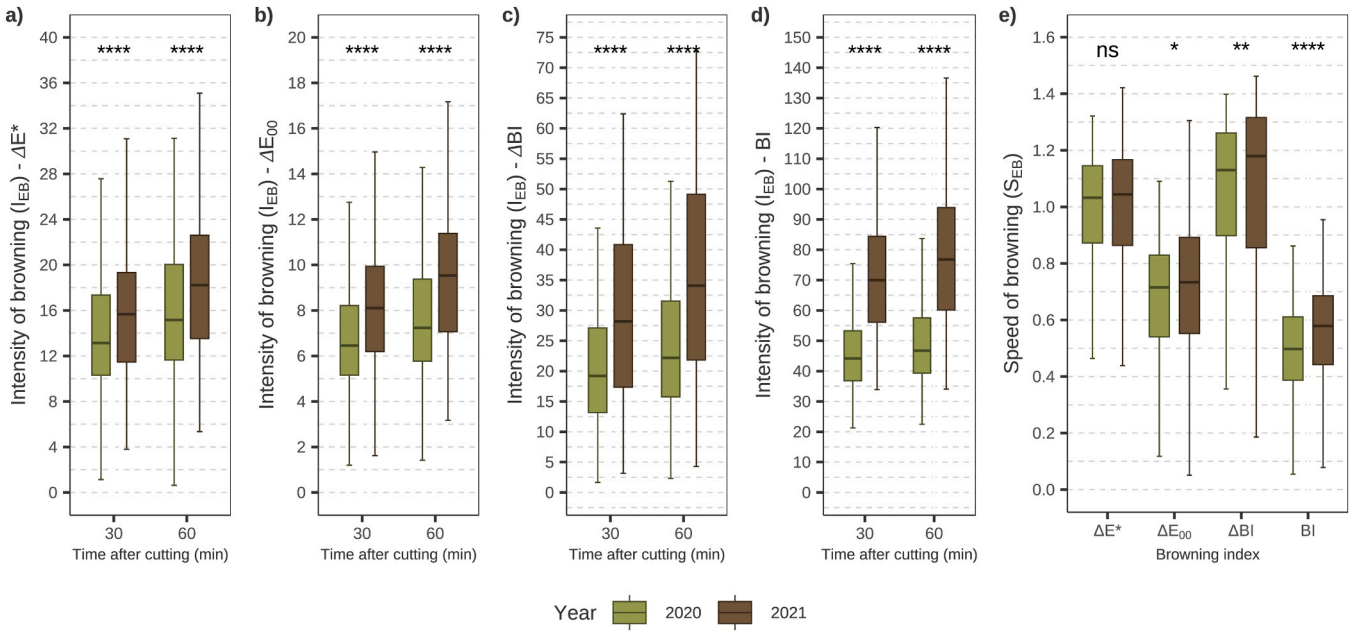


Fig. 6. Between-years uniformity of the measurements of browning intensity (a,d) and speed (e) in a pooled subset of 39 traditional and reference cultivars, using the indices: normalized CIE color difference (ΔE*), CIEDE2000 color difference (ΔE00), difference in browning index (ΔBI) and browning index (BI) at 30 min and 60 min after cutting. Significances correspond to the results of ANOVA analysis (ns, non significant; *, $p < 0.05$; **, $p < 0.01$; **** $p < 0.0001$).

Little information is available in the literature on the uniformity of EB measurements. To the best of our knowledge, only Burke (2010) has reported this kind of information on EB performed with a colorimeter, focused on sample size (n), and measuring a pool of 17 commercial cultivars and advanced breeding selections. In that research, I_{EB} was found to be a relatively uniform trait, with a pooled variance of $s^2 = 4.43$. This indicated that using n = 15 fruits per variety allowed to detect differences of d= 2.5 units in ΔE* at 60 min for a significance level of α= 0.05 and a power (1-β) = 0.9 using the Snedecor and Cochran (1989) approximation for sample size. It is noteworthy that this value falls within the range of color differences that the untrained eye can distinguish between 2.5 and 3.0 units of ΔE* (Arnold and Gramza-Michalowska, 2022). That sample size was considered enough to separate the high, intermediate and low browning fruits from one another (the range of EB values evaluated was narrower as the most sensitive variety showed maximum I_{EB} values of ΔE*≈12, below the 1st quartile in our study). Using Burke's approach to evaluate uniformity, virtually the same results were obtained in this study, as pooled variance was $s^2 = 4.04$, which translates into the same sample sizes and differences detected (d≈3 units in ΔE* at 60 min for the 10-fruit sample size).

All in all, our results expressed as CVs or sample sizes confirm that EB is generally a uniform trait in apples.

3.4. Classification of cultivars in EB levels

The wide range of values observed permitted the delineation of five distinct categories of I_{EB} for the four indices and two times after cutting (30 and 60 min) evaluated (Table 2). The majority (≈95 %) of cultivars were always classified within the lower three categories (low, moderate and high), while only some cultivars were included in the upper two (very high and extremely high).

The reference cultivars were consistently classified at the "low" or "moderate" levels, except 'Pinkgold,' which was classified as "high" using ΔE*. Only three reference cultivars (Golden Supreme', 'Reinette Blanche' and 'Verde Doncella') were consistently classified at the same level. The remaining reference cultivars tended to be classified similarly in terms of ΔE*, ΔE_{00}, and ΔBI, while displaying a different level of classification in BI. About traditional cultivars, it is noteworthy that approximately 15–25 belonged to the "low" level of I_{EB}, depending on the index utilized. Moreover, the I_{EB} levels from "high" and above were exclusively comprised of traditional cultivars.

In the case of S_{EB}, it was possible to delineate four categories (Table 3) for each of the four indices; in contrast to what was observed in I_{EB}, a majority of cultivars (50 %-60 %) were classified in the "high" level, except for BI, where they were mainly in the "moderate" level. The rest of the cultivars were distributed relatively evenly among the other categories. 'Golden Supreme' was the only reference cultivar that exhibited a "low" browning speed. 'Golden Delicious', 'Verde Doncella' and the five 'Gala' cultivars were classified as "moderate", whereas the rest were generally "high speed" browning cultivars. As with I_{EB}, the reference cultivars were ranked similarly across all indices, except for BI, where most references were classified as "moderate" and the rest as "low".

3.5. Relationships among EB indices

As indicated in the references, cultivars were not classified and ordered uniformly for the indices and times after cutting evaluated. Fig. 7 illustrates the bivariate correlations between them, demonstrating a high degree of correspondence ($p < 0.001$) between the two post-cutting periods 30 and 60 min (correlation coefficient r between 0.984 and 0.986), which means that approximately 90 % of the accessions were classified in the same level, while the changes were largely due to the proximity of their I_{EB} values to the boundaries (lower or upper) of the level assigned at that time. Nevertheless, the discrepancies between the indices were more pronounced, with the r ranging from 0.399 to 0.941 (always significant). The indices that exhibited the strongest correlation with one another were the ΔE*-ΔE_{00} (0.941) and ΔBI-BI (0.889) pairs. Conversely, the weakest relationships (<0.500) were observed between BI and both ΔE* and ΔE_{00}. Consequently, the proportion of cultivars classified at the same level ranged from 40 % (ΔE*-BI) to 94 % (ΔE*-ΔE_{00}). These discrepancies in classification were predominantly not due to values being proximate to level limits. Moreover, in up to 6 % of cases (ΔE*-BI), the differences spanned two levels. With regard to S_{EB}, analogous considerations may be drawn, although the correlation and similarity of classifications were, in general, notably lower.

The relationship between S_{EB} and I_{EB} was subsequently investigated, finding that, although statistically significant, the correlations ranged from moderate ($r \approx 0.8$ for ΔE_{00}) to low ($r \approx 0.4$ for BI). An examination of the shape of the relationships (Fig. S1) reveals that, except for BI, where there is considerable variability, the relationship is of a logarithmic type with a markedly steep slope. This implies that while "low" I_{EB} cultivars generally exhibit a "low" rate of oxidation, from "moderate" I_{EB} up, the browning speed is consistently at least "high".

The discriminant capacity of the EB indices was ultimately assessed through their DR values (Fig. 8). For I_{EB} at 60 min after cutting (Fig. 8a), the differences between indices in DR were moderate (7–13 %) and statistically significant ($p < 0.05$), with ΔBI demonstrating the highest discriminant capacity, followed by ΔE* and BI, which exhibited similar DRs. The results for 30 min after cutting exhibited a similar trend, albeit with diminished differentiation between indices (relative differences of 4–10 %). Overall, no difference was identified in evaluating I_{EB} at either 30 or 60 min after cutting with any of the indices. In contrast, for S_{EB} (Fig. 8b), the discrepancies in index performance were more pronounced (8–25 %), with ΔBI once again exhibiting the highest DR, while ΔE_{00} (along with BI) demonstrated the lowest discriminatory capability.

There is a lack of consensus in the literature regarding the index to be employed when evaluating EB by fruit surface color change. The most commonly utilized are derived from CIELAB space, such as ΔE* and BI (Arnold and Gramza-Michalowska, 2022). Nevertheless, other indices, such as ΔC, ΔBI, and ΔE_{00}, are also employed with some frequency. The measurement of PPO activity and the evaluation of surface color are the most commonly used methods for quantifying EB in apples. Color is generally evaluated using indices derived from the CIELab space, which can be obtained directly using colorimeters or by converting RGB images from cameras. The most frequently utilized indices are ΔE* and BI (Arnold and Gramza-Michalowska, 2022), although other indices, such as ΔC, ΔBI, and ΔE_{00}, are also commonly employed. Information is scarce regarding the suitability of these indices or formal comparisons of their relative efficiency. This is due to a tendency to select a single index, and when multiple indices are employed, the number of cultivars analyzed is typically limited (between three and five in the majority of studies). Subhashree et al. (2017) characterized three cultivars with BI and ΔE*, identifying notable ordination differences in susceptibility to EB contingent on the index. In a study conducted by Serra et al. (2021), the authors assessed ΔD and ΔE* in 14 cultivars. Their findings revealed that, while both indices demonstrated a strong correlation in general, there were slight discrepancies in the ordination of some cultivars. Finally, Shimizu et al. (2021) compared ΔE_{00}, ΔBI, and BI in six yellow-fleshed cultivars, finding that the sorting by ΔE_{00} and ΔBI was highly similar, but that by BI differed significantly. They also found notable sorting differences between the three indices when evaluating red-fleshed cultivars. They concluded that BI allowed for a more appropriate evaluation of color tone and that ΔBI was better related to

Table 2
Enzymatic browning levels for the intensity of browning (EB_I) using the indices normalized CIE color difference (ΔE*), CIEDE2000 color difference (ΔE_{00}), difference in browning index (ΔBI) and browning index (BI) at 30 min and 60 min after cutting, and the number of cultivars classified on each level.

	Time (min)	Threshold values for EB_I level					N° of accessions on each level				
Index		**Low (L)**	**Moderate (M)**	**High (H)**	**Very high (VH)**	**Extremely High (EH)**	**L**	**M**	**H**	**VH**	**EH**
ΔE*	30	< 9.7	9.7–15.5	15.5–21.4	21.4–27.2	> 27.2	25	79	29	8	1
	60	< 11.3	11.3–18.2	18.2–25.0	25.0–31.9	> 31.9	21	81	31	8	1
ΔE_{00}	30	< 5.1	5.1–8.3	8.3–11.4	11.4–14.5	> 14.5	29	84	25	3	1
	60	< 6.1	6.1–9.7	9.7–13.2	13.2–16.8	> 16.8	36	75	27	3	1
ΔBI	30	< 16.0	16.1–30.3	30.3–44.6	44.6–58.8	> 58.8	33	71	31	4	3
	60	< 19.7	19.7–36.4	36.4–53.1	53.1–69.8	> 69.8	33	66	34	6	3
BI	30	< 42.8	42.8–67.5	67.5–92.2	92.2–117.0	> 117.0	25	70	39	5	3
	60	< 46.4	46.4–73.0	73.0–99.7	99.7–126.3	> 126.3	28	67	38	6	3

Table 3
Enzymatic browning levels for the speed of browning (EB_S) using the indices normalized CIE color difference (ΔE*), CIEDE2000 color difference (ΔE_{00}), difference in browning index (ΔBI) and browning index (BI), and the number of cultivars classified on each level.

	Threshold values for EB_S level				N° of accessions on each level			
Index	**Low (L)**	**Moderate (M)**	**High (H)**	**Very high (VH)**	**L**	**M**	**H**	**VH**
ΔE*	< 0.47	0.47–0.79	0.79–1.11	> 1.11	15	24	80	23
ΔE_{00}	< 0.20	0.20–0.50	0.50–0.80	> 0.80	5	35	71	31
ΔBI	< 0.48	0.48–0.89	0.89–1.30	> 1.30	21	18	85	18
BI	< 0.40	0.40–0.60	0.60–0.80	> 0.80	24	89	26	3

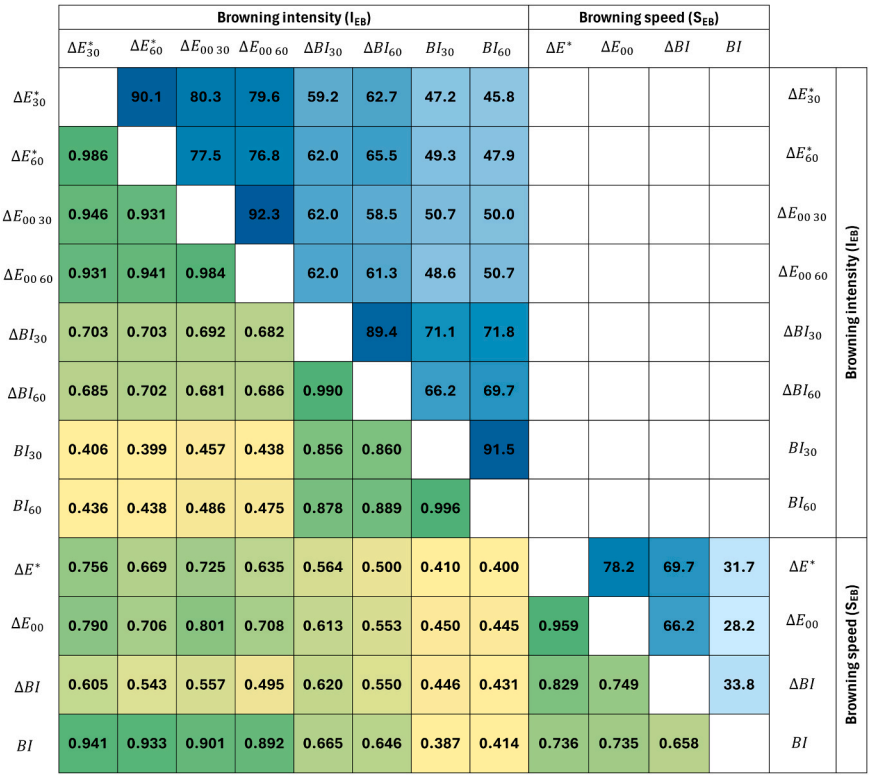


Fig. 7. Pairwise relationships among enzymatic browning (EB) indices. Below the diagonal (green tones): Pearson correlation matrix of the values obtained for the indices in the set of cultivars. All correlations are significant ($p < 0.001$). Above the diagonal (blue tones): proportion (%) of accessions that were classified in the same EB level for the pair of indices.

visual differences between cultivars than ΔE_{00}. Additionally, the latter index was deemed unsuitable for evaluating differences in EB evolution in cultivars with a high degree of browning. In this study, the correlation between ΔBI and ΔE_{00} (Fig.S1) shows a logarithmic pattern flattening at moderate values of ΔBI, agreeing with the findings of Shimizu et al. (2021).

In our study, a formal evaluation of the efficiency of the most commonly used EB indices has been carried out for the first time through correlation and discriminant capacity analyses. It was determined that, despite the high correlations found, each index produces a distinct ranking and classification, rendering them essentially non-interchangeable. Furthermore, the discriminant capacity was evaluated using the DR. This is an instrument developed in the field of clinical research and widely used in the comparison of indices or measurement scales. In agronomy, it has been used to compare the efficiency of water potential measurements (Cole and Pagay, 2015; Santesteban et al., 2019, 2011) as well as fruit sensory preference scales (Yeung et al., 2021). The results of this formal analysis, conducted on a wide dataset, corroborate the findings of prior studies performed more limited datasets. They confirm the superiority of ΔBI for detecting visual differences concerning other indices, particularly ΔE_{00}, as highlighted by Shimizu et al. (2021). ΔE_{00} is an improved index representing color differences that approximate human vision, and its values are adjusted to the visual experience, such that the deeper the color, the lower the difference perception (Luo et al., 2001), therefore rendering ΔE_{00} unsuitable for phenotyping purposes. Consequently, in light of the aforementioned results, ΔBI would be the most recommendable index.

4. Concluding remarks

A phenotyping system developed to determine enzymatic browning in large fruit samples (~10–15 fruit halves) has been successfully applied for the first time on a large scale. This method adequately accounted for any surface heterogeneity typical of EB. The procedure is fast, and the time required to prepare each batch of 10 fruits could be considered negligible, since an operator with some training needed less than 1 min to cut by knife and arrange the fruits on the alveoli trays. This makes it possible to adequately evaluate EB even in cultivars with a high oxidation rate. Moreover, it has been shown that the phenotyping process could be substantially shortened, given that the differences between the determinations at 30 and 60 min after cutting are negligible.

The system therefore shows the advantages already referred to by previous researchers in terms of allowing high-throughput, high precision and affordable phenotyping (Bouillon et al., 2024; Shimizu et al.,

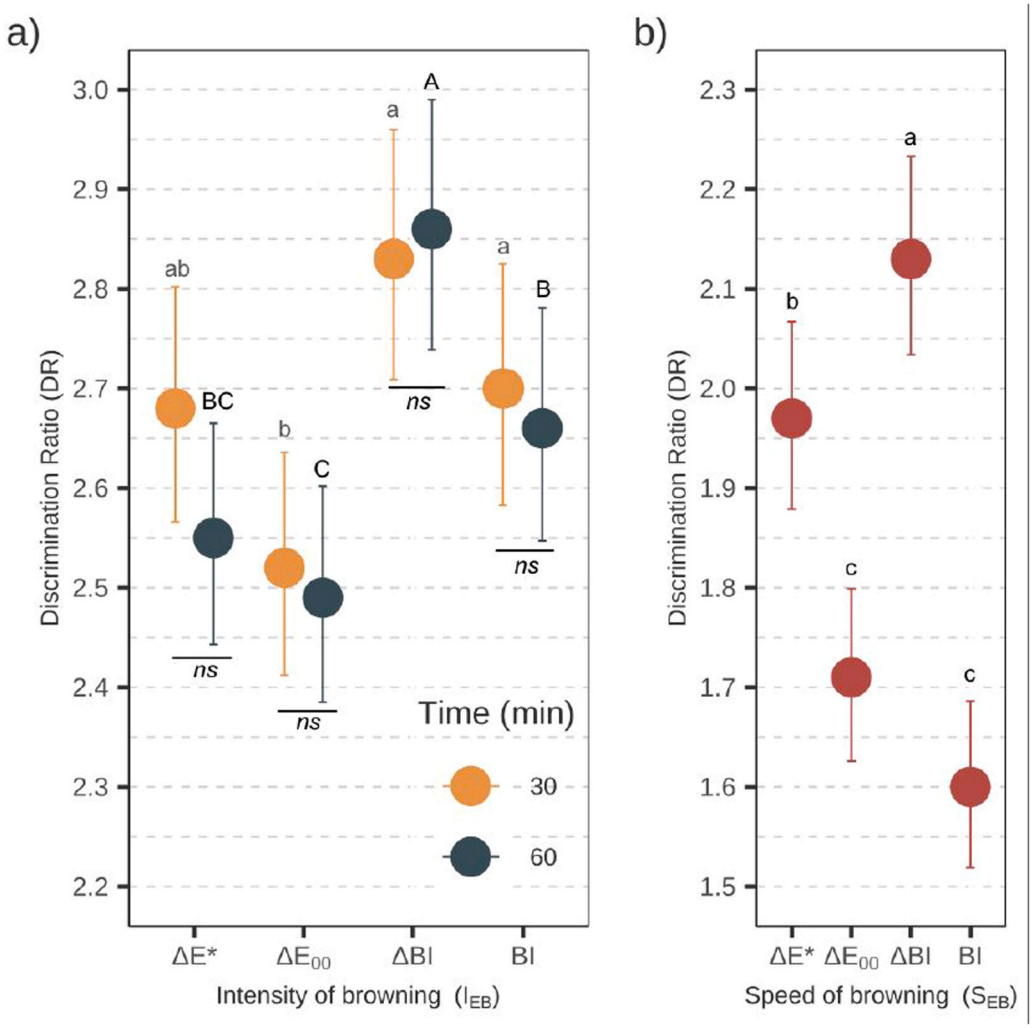


Fig. 8. Discrimination ratios (mean and 95 % confidence interval) for the enzymatic browning (EB) indices used. a) Values for the intensity of browning at 30 min and 60 min after cutting. Significant statistical independence of EB indices at 30 min (lowercase letters) or 60 min (uppercase letters) by Q-statistics is indicated above the values, whereas below is indicated the independence of times after cutting for the same index. b) Values for the speed of browning, with statistical independence calculated by Q-statistics indicated by different letters above the values.

2021; Subhashree et al., 2017). The work scale that can be achieved with the methodology developed has made possible to go beyond a proof-of-concept phase and apply it on a large scale to a very diverse collection of 142 apple cultivars including commercial (cider, dessert, processing), and heritage cultivars. From a practical point of view, the methodology developed can be also easily applied to the evaluation of fruit pieces identical to those of final products (slices, strips, etc.) as it can handle irregular shapes, avoiding the sampling problems inherent with the use of colorimeters.

The considerable number of samples used in the study has allowed us to demonstrate that EB is an inherently stable trait for a given cultivar in each year of observation, although notable differences between years can be observed. This can be associated to the fact it is a feature influenced by weather and management circumstances. Thus, EB phenotyping of a cultivar requires determinations over a sufficient number of seasons, which adds value to the low-cost high-throughput imaging system developed.

The indices evaluated in this study have demonstrated a comparable efficacy for the classification and ranking of cultivars according to their EB speed and intensity. However, the differences in performance among them are substantial enough to render some more suitable for phenotyping. Thus, ΔBI showed a stronger correlation with visual inspection and demonstrates a higher discriminant capacity than the others, making it the most appropriate index for this type of assessment.

All in all, the findings of this study provide a robust methodological basis that can be easily implemented germplasm collection and breeding programs for screening low-browning genotypes, highlighting the potential of underutilized traditional cultivars in developing improved fresh-cut apple products.

CRediT authorship contribution statement

Sara Crespo-Martínez: Writing – review & editing, Visualization, Investigation. **Pilar Errea:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition. **Lourdes Castel:** Investigation, Data curation. **Patricia Irisarri:** Writing – review & editing, Investigation, Data curation. **Carlos Miranda:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Software, Project administration, Funding acquisition, Formal analysis, Conceptualization. **Jorge Urrestarazu:** Writing – review & editing, Investigation, Data curation. **Haizea Romeo:** Investigation, Data curation. **Luis Gonzaga Santesteban:** Writing – review & editing, Visualization, Methodology, Investigation. **Ana Pina:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization. **Nerea Iturmendi:** Writing – review & editing, Investigation. **Bielsa Francisco:** Writing – review & editing,

Investigation, Data curation.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Carlos Miranda reports financial support was provided by Spain Ministry of Science and Innovation - State Investigation Agency. Ana Pina reports financial support was provided by European Social Fund. Pilar Errea reports financial support was provided by European Social Fund. Pilar Errea reports financial support was provided by Spain Ministry of Science and Innovation - State Investigation Agency. Ana Pina reports financial support was provided by Spain Ministry of Science and Innovation - State Investigation Agency.

Acknowledgement

This work has been funded by the projects PID2019-108081RR-C21-C22, and PID2022–141847OR-C31-C32 funded by MCIN/AEI 10.13039/501100011033 ERDF, UE and the consolidated group A12 of Gobierno de Aragón – European social fund of the European Union. Open access funding provided by Universidad Pública de Navarra.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.postharvbio.2025.114066.

Data availability

Data will be made available on request.

References

Altisent, R., Plaza, L., Alegre, I., Viñas, I., Abadias, M., 2014. Comparative study of improved vs. traditional apple cultivars and their aptitude to be minimally processed as “ready to eat” apple wedges. LWT Food Sci. Technol. 58, 541–549. https://doi.org/10.1016/j.lwt.2014.03.019.

Arnold, M., Gramza-Michałowska, A., 2022. Enzymatic browning in apple products and its inhibition treatments: a comprehensive review. Compr. Rev. Food Sci. Food Saf. 21, 5038–5076. https://doi.org/10.1111/1541-4337.13059.

Bouillon, P., Fanciullino, A.-L., Belin, E., Bréard, D., Boisard, S., Bonnet, B., Hanteville, S., Bernard, F., Celton, J.-M., 2024. Image analysis and polyphenol profiling unveil red-flesh apple phenotype complexity. Plant Methods 20, 71. https://doi.org/10.1186/s13007-024-01196-1.

Browning, L.M., Krebs, J.D., Jebb, S.A., 2004. Discrimination ratio analysis of inflammatory markers: implications for the study of inflammation in chronic disease. Metabolism 53, 899–903. https://doi.org/10.1016/j.metabol.2004.01.013.

Burke, A.E., 2010. Quantifying flesh browning, polyphenoloxidase, total phenolic content and vitamin C in select apple varieties and progeny (Doctoral dissertation). Cornell University.

Carter, E.C., Schanda, J.D., Hirschler, R., Jost, S., Luo, M.R., Melgosa, M., Ohno, Y., Pointer, M.R., Rich, D.C., Viénot, F., others, 2018. CIE 015: 2018 Colorimetry. Int. Comm. Illum. Vienna, Austria.

Cebulj, A., Populin, F., Masuero, D., Vrhovsek, U., Angeli, L., Morozova, K., Scampicchio, M., Costa, F., Busatto, N., 2023. A multifaced approach sheds light on the molecular details underlaying the mechanism preventing enzymatic browning in ‘Majda’ apple cultivar (*Malus domestica* Borkh.). Sci. Hortic. 318, 112137. https://doi.org/10.1016/j.scienta.2023.112137.

Cebulj, A., Vanzo, A., Hladnik, J., Kastelec, D., Vrhovsek, U., 2021. Apple (Malus domestica Borkh.) cultivar ‘majda’, a naturally non-browning Cultivar: an assessment of its qualities. Plants 10, 1402. https://doi.org/10.3390/plants10071402.

Cole, J., Pagay, V., 2015. Usefulness of early morning stem water potential as a sensitive indicator of water status of deficit-irrigated grapevines (*Vitis vinifera* L.). Sci. Hortic. 191, 10–14. https://doi.org/10.1016/j.scienta.2015.04.034.

CTIFL, 2002. Pomme. Code amidon, aide à la décision de récolte. Centre technique interprofessionnel des fruits et légumes, Paris, France.

Duangmal, K., Worapotpisut, C., Romposa, N., Katemake, P., 2017. Uneven enzymatic browning on fresh-cut apple and its measurement. Acta Hortic. 1179, 69–76. https://doi.org/10.17660/ActaHortic.2017.1179.11.

Ferreira, J.P., Schmildt, E.R., Schmildt, O., Cattaneo, L.F., Alexandre, R.S., Cruz, C.D., 2016. Comparison of methods for classification of the coefficient of variation in papaya. Rev. Ceres 63, 138–144. https://doi.org/10.1590/0034-737X201663020004.

Jaeger, S.R., Machín, L., Aschemann-Witzel, J., Antúnez, L., Harker, F.R., Ares, G., 2018. Buy, eat or discard? A case study with apples to explore fruit quality perception and food waste. Food Qual. Prefer 69, 10–20. https://doi.org/10.1016/j.foodqual.2018.05.004.

Kalinowska, M., Bielawska, A., Lewandowska-Siwkiewicz, H., Priebe, W., Lewandowski, W.W., 2014. Apples: Content of phenolic compounds vs. variety, part of apple and cultivation model, extraction of phenolic compounds, biological properties. Plant Physiol. Biochem. 84. https://doi.org/10.1016/j.plaphy.2014.09.006.

Kumar, P., Sethi, S., Sharma, R.R., Singh, S., Varghese, E., 2018. Improving the shelf life of fresh-cut ‘Royal Delicious’ apple with edible coatings and anti-browning agents. J. Food Sci. Technol. 55, 3767–3778. https://doi.org/10.1007/s13197-018-3308-6.

Levy, J., Morris, R., Hammersley, M., Turner, R., 1999. Discrimination, adjusted correlation, and equivalence of imprecise tests: application to glucose tolerance. Am. J. Physiol. Endocrinol. Metab. 276, E365–E375. https://doi.org/10.1152/ajpendo.1999.276.2.E365.

López-Nicolás, J.M., Pérez-López, A.J., Carbonell-Barrachina, Á., García-Carmona, F., 2007. Kinetic Study of the Activation of Banana Juice Enzymatic Browning by the Addition of Maltosyl-β-cyclodextrin. J. Agric. Food Chem. 55, 9655–9662. https://doi.org/10.1021/jf0713399.

Lozano, J.E., Drudis-Biscarri, R., Ibarz-Ribas, A., 1994. Enzymatic browning in apple pulps. J. Food Sci. 59, 564–567.

Luo, M.R., Cui, G., Rigg, B., 2001. The development of the CIE 2000 colour-difference formula: CIEDE2000. Color Res. Appl. 26, 340–350. https://doi.org/10.1002/col.1049.

Miranda, C., 2024a. Carm1r/Pheno_ImageJ: Phenotyping tools and scripts for ImageJ. Release V1.0.0 (v1.0.0). Zenodo. https://doi.org/10.5281/zenodo.10663947.

Miranda, C., 2024b. Carm1r/phenoclass: Tools for setting objective quantitative levels of expression in characterizations in R. Updated version (v1.0.0). Zenodo. https://doi.org/10.5281/zenodo.10663963.

Miranda, C., Dapena, E., Urbina, V., Pereira-Lorenzo, S., Errea, P., Moreno, M.A., Urrestarazu, J., Fernandez, M., Ramos-Cabrer, A.M., Diaz-Hernandez, M.B., Pina, A., Santesteban, L.G., Laquidain, M.J., Dalmases, J., Espiau, M.T., Reig, G., Gogorcena, Y., Ascasibar, J., Royo, J.B., 2017. Development of a standardized methodology for phenotypical characterizations in apple. Acta Hortic. 367–370. https://doi.org/10.17660/ActaHortic.2017.1172.69.

Miranda, C., Errea, P., Urrestarazu, J., Pina, A., Dapena, E., 2018. Definición del núcleo optimizado de la colección de conservación del manzano españ. Ol. Actas Hortic. 80, 67–71.

Musacchi, S., Serra, S., 2018. Apple fruit quality: overview on pre-harvest factors. Sci. Hortic. 234, 409–430. https://doi.org/10.1016/j.scienta.2017.12.057.

Nicola, S., Cocetta, G., Ferrante, A., Ertani, A., 2022. Chapter 7 - Fresh-cut produce quality: implications for postharvest, in: Florkowski, W.J., Banks, N.H., Shewfelt, R. L., Prussia, S.E. (Eds.), Postharvest Handling (Fourth Edition). Academic Press, San Diego, pp. 187–250. https://doi.org/10.1016/B978-0-12-822845-6.00007-5.

Palou, E., López-Malo, A., Barbosa-Cánovas, G.V., Welti-Chanes, J., Swanson, B.G., 1999. Polyphenoloxidase activity and color of blanched and high hydrostatic pressure treated banana puree. J. Food Sci. 64, 42–45. https://doi.org/10.1111/j.1365-2621.1999.tb09857.x.

Pignata, G., Tibaldi, G., Gaino, W., Nicola, S., 2018. Mixing and dipping fresh-cut “Gala Brookfield” and “Granny Smith” apples. Acta Hortic. (1209), 409–416. https://doi.org/10.17660/ActaHortic.2018.1209.60.

Pimentel-Gomes, F., 2009. Curso De Estatística Experimental, 15th ed, Fifteenth ed. FEALQ, Piracicaba, Brasil.

Putnik, P., Bursać Kovačević, D., Herceg, K., Levaj, B., 2017. Influence of Cultivar, Anti-Browning Solutions, Packaging Gasses, and Advanced Technology on Browning in Fresh-Cut Apples During Storage. J. Food Process Eng. 40, e12400. https://doi.org/10.1111/jfpe.12400.

Quevedo, R., Valencia, E., López, P., Gunckel, E., Pedreschi, F., Bastías, J., 2014. Characterizing the variability of enzymatic browning in fresh-cut apple slices. Food Bioprocess Technol. 7, 1526–1532. https://doi.org/10.1007/s11947-013-1226-1.

Core Team, R., 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ⟨https://www.R-project.org/⟩.

Royo, J., Miranda, C., Santesteban, L., Laquidain, M., Urrestarazu, J., Urbina, V., Dalmases, J., Dapena, E., Fernandez, M., Blázquez, D., Pereira-Lorenzo, S., Diaz, M., Ramos-Cabrer, A.M., Ascasibar, J., Errea, P., Sanzol, J., Pina, A., Espiau, M., Moreno, A., Gogorcena, Y., Blanco, A., 2017. Harmonized methodology for the pomological characterization of apple (*Malus x domestica* Borkh). Inst. Nac. De. Invest. óN. Y. Tecnol. ía Agrar. Madr.

Team, R.Studio, 2020. RStudio: Integrated Development Environment for R. Rstudio Software. PBC, Boston, MA. ⟨http://www.posit.co/⟩.

Sanchez-Beekman, M., 2021. ColorNameR: Give Colors a Name. R package version 0.1.0.

Santesteban, L.G., Miranda, C., Marín, D., Sesma, B., Intrigliolo, D.S., Mirás-Avalos, J.M., Escalona, J.M., Montoro, A., de Herralde, F., Baeza, P., Romero, P., Yuste, J., Uriarte, D., Martínez-Gascueña, J., Cancela, J.J., Pinillos, V., Loidi, M., Urrestarazu, J., Royo, J.B., 2019. Discrimination ability of leaf and stem water potential at different times of the day through a meta-analysis in grapevine (*Vitis vinifera* L.). Agric. Water Manag. 221, 202–210. https://doi.org/10.1016/j.agwat.2019.04.020.

Santesteban, L.G., Miranda, C., Royo, J.B., 2011. Suitability of pre-dawn and stem water potential as indicators of vineyard water status in cv. Tempranillo. Aust. J. Grape Wine Res. 17, 43–51. https://doi.org/10.1111/j.1755-0238.2010.00116.x.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P., Cardona, A., 2012. Fiji: an open-source

platform for biological-image analysis. Nat. Methods 9, 676–682. https://doi.org/10.1038/nmeth.2019.
Schloerke, B., Cook, D., Larmarange, J., Briatte, F., Marbach, M., Thoen, E., Elberg, A., Crowley, 2024. GGally: Extension to "ggplot2". R package.
Serra, S., Anthony, B., Boscolo Sesillo, F., Masia, A., Musacchi, S., 2021. Determination of post-harvest biochemical composition, enzymatic activities, and oxidative browning in 14 apple cultivars. Foods 10, 186. https://doi.org/10.3390/foods10010186.
Sharma, G., Wu, W., Dalal, E.N., 2005. The CIEDE2000 color-difference formula: implementation notes, supplementary test data, and mathematical observations. Color Res. Appl. 30, 21–30. https://doi.org/10.1002/col.20070.
Shimizu, T., Okada, K., Moriya, S., Komori, S., Abe, K., 2021. A high-throughput color measurement system for evaluating flesh browning in apples. J. Am. Soc. Hortic. Sci. 146, 241–251. https://doi.org/10.21273/JASHS05027-20.
Snedecor, G., Cochran, W., 1989. Statistical Methods, Eighth ed. Iowa State University Press.
Subhashree, S.N., Sunoj, S., Xue, J., Bora, G.C., 2017. Quantification of browning in apples using colour and textural features by image analysis. Food Qual. Saf. 1, 221–226. https://doi.org/10.1093/fqsafe/fyx021.
Toivonen, P.M.A., 2006. Fresh-cut apples: challenges and opportunities for multi-disciplinary research. Can. J. Plant Sci. 86, 1361–1368.
Wang, W.-Y., Bi, J.-F., Hu, J.-X., Li, X., 2024. Metabolomics comparison of four varieties apple with different browning characters in response to pretreatment during pulp processing. Food Res. Int. 190, 114600. https://doi.org/10.1016/j.foodres.2024.114600.
Watkins, C.B., 2003. Principles and Practices of Postharvest Handling and Stress, in. Apples: Botany, Production and Uses. CABI Publishing, pp. 585–614.
Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.
Xu, K., 2015. Arctic apples: a look back and forward. New Y. Fruit. Quaterly 23, 21–24.
Yeung, H.F., Homwongpanich, K., Michniuk, E., Rovai, D., Migliore, M., Lammert, A., Lahne, J., 2021. A tale of 3 scales: how do the 9-pt, Labeled Affective Magnitude, and unstructured Visual Analog scales differentiate real product sets of fresh berries? Food Qual. Prefer 88, 104109. https://doi.org/10.1016/j.foodqual.2020.104109.
Zhu, Y., Zhang, M., Mujumdar, A.S., Liu, Y., 2023. Application advantages of new non-thermal technology in juice browning control: a comprehensive review. Food Rev. Int. 39, 4102–4123. https://doi.org/10.1080/87559129.2021.2021419.