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Department of Pomology, Estación Experimental de Aula Dei, Consejo Superior de Investigaciones Científicas (EEAD-CSIC), Zaragoza, Spain

Postharvest performance of 17 apple accessions grown in NE Spain after long-term cold storage

P. Mignard, R. Gimenéz, M.A. Moreno*

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Summary

In this work, 17 accessions from the Apple Germplasm Bank of the EEAD-CSIC were harvested and stored in a cold room for six months, at low temperature (1-1.5 °C) and controlled relative humidity (85-90%). The objective of this study was to evaluate differences among accessions at biochemical level between harvest and after long-term cold storage, defining their suitability for storage. Basic fruit quality parameters and antioxidant compounds were evaluated, and individual sugars and major organic acids were determined. A PCA was carried out explaining 70% of the total variability. 'Evasni' presented the best performance in postharvest after cold storage. The concentrations of organic acids and phenolic compounds, in general, decreased after long-term conservation. Triploid accessions with presence of russeting highlighted for their higher content in bioactive compounds even after cold storage. Nevertheless, the russeting seemed to be related to cell damages and increased chilling injuries such as browning or dehydration. Spanish accessions 'Reneta' and 'Solafuente' and other non-Spanish bred cultivars showed their suitability for conservation with no chilling injuries after long-term cold storage. The present work highlighted the interest of native accessions and non-Spanish cultivars to be considered in future breeding programs thanks to their best marketability and consumer acceptability.

Keywords: basic fruit quality, chilling injury, *Malus* × *domestica* Borkh, organic acids, phenolics, sugars

Introduction

Apple (*Malus* × *domestica* Borkh) has great importance on the market since it is among the most extensively consumed fresh fruits in human nutrition. Apple fruit is available on the marketplace all year round for consumers. Moreover, it is considered one of the major temperate fruit crops cultivated worldwide (Mushtaq et al., 2020). In 2021, more than 93 M tons of apples were produced globally and 615,830 tons were produced in Spain (FAOSTAT, 2023).

It is well known that fruits and vegetables represent a major source of general biomolecules for humans (Boeing et al., 2012). Apple fruit is also a source of micro-nutrients, sugars, organic acids, oligosaccharides, minerals, phytochemical and antioxidant compounds, and dietary fiber. Antioxidant compounds could improve the quality and the shelf life of vegetables and reduce the risk of postharvest diseases and/or chilling injuries (Davey et al., 2007; Singh et al., 2018). The content of the bioactive compounds differs considerably depending on genetics, environmental conditions, farming practices, ripening date or even postharvest storage conditions (Quiles et al., 2005; Mignard et al., 2021a; 2021b; Zhang et al., 2021). Biomolecules such as antioxidant compounds, sugars and organic acids are very vulnerable to degradation in fruits, mostly due to certain handling practices, chilling injuries, and other several stresses. These aspects can reduce the antioxidant capacity and, therefore, the nutritional and organoleptic quality of fresh apples (Villa-Rodriguez et al., 2015). The quality of fruits and vegetables after long-term cold storage has been traditionally well-defined in terms of sensorial qualities (freshness, color, and nonappearance of chilling injuries), texture (firmness, juiciness, and crispness) and care (pathogenic microorganisms) (Villa-Rodriguez et al., 2015). Nevertheless, most of the studies in this field have not focused on the effect of cold storage on nutritional quality. Nonetheless, fruits and vegetables are subject to qualitative and quantitative changes during long-term cold storage (Leja et al., 2003; Zhang et al., 2021).

The constantly growing demand for fresh fruit on the market highlights the critical need to maintain a high level of both aesthetic and organoleptic quality of fruits, even for a long time after harvest, in order to guarantee consumer satisfaction (Cainelli et al., 2019). Low-temperature storage reduces the metabolic activity and slows the deterioration of fresh fruits during postharvest and a delay of senescence as the apple fruit is considered a climacteric fruit (Leja et al., 2003; Mushtaq et al., 2020). Antioxidants metabolism is closely connected to chilling injuries in postharvest (Davey et al., 2007; Foyer and Noctor, 2005; Mushtaq et al., 2020). Indeed, a long period of cold storage could negatively influence the final apple quality and thus, its acceptability by consumers and marketability. Improper low temperatures often cause chilling injuries and result in an economic loss for the producers. Sub-optimal temperatures, depending on the accession, the ripening and the time of storage, could engender an excess of reactive oxygen species (ROS) in the fruit cells, resulting in the decrease of antioxidant substances such as ascorbic acid or total phenolics (Cainelli et al., 2019; Han et al., 2021; Khan et al., 2021).

Typical symptoms of chilling injuries in apple are browning (peel and/or flesh), dehydration or biomolecules degradation and thus, a decrease in fruit quality (Cebulj et al., 2021; Rasouli and Saba, 2018; Singh et al., 2018). Chilling injuries depend on the intensity of the abiotic stress caused by low temperatures, the genetic susceptibility of the accession to cold exposure, and the length of time of cold storage (Cainelli et al., 2019; Navarro et al., 2022). Therefore, to guarantee safe and good-looking apples with high organoleptic and functional quality, fresh apples must be stored in the appropriate environment during cold storage and transportation (Di Guardo et al., 2013).

This work focuses on evaluating the fruit quality for 17 accessions and observing the values of different bioactive compounds, such as antioxidants, individual sugars and major organic acids during long-term cold storage. The characterization of these compounds could contribute to understanding the performance of apple cultivars against their postharvest storage and shelf life (Leja et al., 2003). This work aims to improve our understanding of how different apple cultivars respond after six months of cold storage. The application of this knowledge to the fruit industry could offer different options for fruit management, achieving more health benefits and the best organoleptic quality for consumers.

* Corresponding author

Materials and methods

Plant material and field trial

The apple accessions [*Malus* × *domestica* Borkh] of the present study were obtained from the apple germplasm bank of the Experimental Station of Aula Dei (EEAD-CSIC, Zaragoza, NE Spain: 41° 43′ 42.7′′ N, 0° 48′ 44.1′′ W). A total of 17 accessions, consisting of five local Spanish accessions and 12 non-Spanish accessions were assessed (Tab. 1). Most of the non-Spanish accessions are commercial cultivars, while the Spanish accessions are traditional and/or autochthone cultivars. The 17 accessions were classified according to their skin color as bicolor or red (11 accessions), green (3), yellow (1) and brown (2), with brown corresponding to apples 100% russeted covered. They were also classified according to their ploidy level as diploids (13 accessions) and triploids (4 accessions).

Fruit sampling and storage

A representative sample of 40 fruits per accession was harvested when they exhibited the ground colour representative of each accession. Samples were washed and then stored for six months in a commercial cold room. The storage temperature was 1-1.5 °C and the humidity fixed at 85-90%. Regarding to the fruit sampling for each accession, after the six months of cold storage, 12 apples that were similar in size, with a uniform colour and with no pest damage or mechanical injuries were selected for the subsequent analyses. Fruit traits were measured in three replicates (four fruits per replication) and means for each accession were calculated.

Basic fruit quality and phytochemical traits

Soluble solids content (SSC) and titratable acidity (TA) were determined on flesh juice as described by Mignard et al. (2021a) after six months of cold storage. SSC was measured with a digital refractometer (Atago PR-101, Tokyo, Japan) and was expressed as ºBrix. TA was determined using an automatic titration system (EasyPlus Titrator, Mettler Toledo, US) with 0.1 N NaOH to a pH end point of 8.1 and expressed as g malic acid per liter. The Ripening Index (RI) was calculated based on the SSC/TA ratio.

For the analysis of total phenolics content (TPC), total flavonoids content (TFC), vitamin C (ascorbic acid – AsA) and the relative antioxidant capacity (RAC), three replicates per accession were sampled and prepared as described by Mignard et al. (2021a). Briefly, samples were homogenized in a polytron (T25D Ultra-Turrax, IKA Works Inc., Wilmington, NC, USA) after one night in 10 mL of extraction solution [methanol/Milli-Q water, 80% (v/v) for TPC, TFC and RAC, and metaphosphoric acid, 5% (w/v) for AsA]. Extracts were centrifuged at 20,000 g for 30 min at 4 °C, and the supernatant was analysed using a 96-well microplate spectrophotometer photodiode array detector (Asys UVM 340 microplate reader; Biochrom, Cambridge, UK). With some modifications, TPC was determined using the Folin-Ciocalteau method (Singleton et al., 1965), TFC was analysed using a colorimetric assay based on the method of Zhishen et al. (1999), AsA was determined as described by Zaharieva and Abadía (2003) and RAC was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method adapted from Brand-Williams et al. (1995).

Individual sugars and organic acids were analysed by HPLC as previously described by Font i Forcada et al. (2019) and Mignard et al. (2022). In summary, sugars were analyzed using an Aminex HPX-87C column (300 mm × 7.8 mm, Biorad) while for organic acids a Rezex™ ROA-Organic Acid H+ (8%) column (300 mm × 7.8 mm, Phenomenex) was used. The individual sugars (glucose, fructose, sucrose), the sugar-alcohol (sorbitol) and organic acids (citric, malic, succinic, shikimic and tartaric) were identified by their retention time characteristics using the adequate standards. Concentrations were expressed as g per kg of fresh weight (FW) as described by Mignard et al. (2022). All chemicals and standards were of analytical grade.

Chilling injury study

According to chilling injuries, browning of pulp and skin on one hand and dehydration of the entire fruit on the other hand were evaluated. Symptoms of external and internal chilling injury were analysed using 15 fruits of each accession after six months of cold storage at 1-1.5 °C and the humidity fixed at 85-90%. The chilling injuries were evaluated visually in accordance with the following scale: 1, no damage; 2, presence of damage.

Tab. 1: Basic information of the 17 apple accessions used on this study.

Accession	Nº	EEAD Code	Classification	Origin	Skin Color	Flesh Color	Ploidy
Averdal	1	882021	Non-Spanish	-	Red	Cream	2
Baujade	2	923284	Non-Spanish	France	Green	Greenish	2
Bossost - MRF 76	3	3627	Spanish	Spain	Brown	Cream	3
Cripps Pink	4	933540	Non-Spanish	UK	Bicolor	Cream	2
Cul de Cirio - MRF 39	5	3551	Spanish	Spain	Bicolor	Cream	2
Delcon	6	2896	Non-Spanish	France	Bicolor	White	2
Evasni - Scarlet Spur	7	933554	Non-Spanish	France	Bicolor	Cream	2
Florina	8	3633	Non-Spanish	France	Bicolor	White	2
Golden Paradise	9	3739	Non-Spanish	Spain	Yellow	Cream	2
Granny Smith	10	2614	Non-Spanish	Australia	Green	Greenish	2
Morro de Liebre	11	3256	Spanish	Spain	Bicolor	White	2
Red Delicious	12	3085	Non-Spanish	US	Bicolor	Cream	2
Red Elstar	13	882002	Non-Spanish	Netherlands	Bicolor	White	2
Reineta Blanca del Canadá	14	308	Non-Spanish	France	Green	Cream	3
Reineta Gris	15	2883	Non-Spanish	France	Brown	Cream	3
Reneta	16	3408	Spanish	Spain	Bicolor	White	2
Solafuente	17	3559	Spanish	Spain	Bicolor	White	3

EEAD, Experimental Station of Aula Dei; US, United States; UK, United Kingdom.

Data analysis

All statistical analyses were carried out using R language (R Development Core Team, 2019) and IBM SPSS 24.0 (United States) software. Data were reported as means ± standard deviation (SD) and standard error (SE). Minimums, maximums, means, SD and SE are shown in Tab. 2 for the 17 traits analyzed both at harvest and postharvest periods. Student's t tests were performed for each trait, respectively, to highlight any significant ($P < 0.05$) differences between harvest and postharvest traits' means (Tab. 2). A one-way analysis of variance (ANOVA) was run to determine whether there were any statistically significant differences between the means of the evaluated traits among accessions and the Tukey test ($P \leq 0.05$) was performed aiming to separate the accessions according to each trait (Tab. S1 and S2). Student's t test was performed to determine differences between the period of analysis (harvest time and after six months of cold storage) for each accession (Fig. 1 and 2). Student's t tests were performed for each group (suitable/unsuitable accession for long-term cold storage), respectively, to highlight any significant difference ($P < 0.05$) (Tab. 4). Finally, Pearson's correlations and Principal Component Analysis (PCA) were performed to understand how biochemical traits contribute to variability among accessions.

Chemicals

All chemicals were of analytical grade. The 3,4,5-trihydroxybenzoic acid (gallic acid) was purchased from PanReac Quimica SA (Barcelona, Spain). The cathequin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteau's reagent, metaphosphoric acid (HPO_3),

Tab. 2: Average values for basic quality and bioactive compounds traits over 17 accessions at harvest and postharvest: units, minimum, maximum, mean values, standard error (SE), and standard deviation (SD).

Trait	Units	Differences harvest/ postharvest	Period	Min	Max	Mean	SE	SD
SSC	ºBrix	*	Harvest	11.58	17.14	13.68	0.37	1.53
			Postharvest	9.83	24.23	15.85	0.91	3.77
TA	g malic acid/L	ns	Harvest	3.29	14.03	6.74	0.75	3.08
			Postharvest	2.22	10.07	5.18	0.61	2.52
RI	-	*	Harvest	1.28	4.51	2.55	0.25	1.03
			Postharvest	1.57	8.06	3.69	0.40	1.68
TPC	mg GAE/100 g FW	*	Harvest	18.48	57.04	33.06	2.83	11.67
			Postharvest	6.14	49.84	22.97	2.64	10.90
TFC	mg CE/100 g FW	ns	Harvest	6.87	32.86	16.62	2.09	8.63
			Postharvest	5.83	39.01	16.87	2.26	9.34
AsA	mg AsA/100 g FW	***	Harvest	1.71	3.64	2.61	0.12	0.50
			Postharvest	0.53	3.54	1.46	0.19	0.79
RAC	mg Trolox/100 g FW	ns	Harvest	7.13	21.21	13.87	1.10	4.55
			Postharvest	5.57	19.46	13.40	1.01	4.16
Sugars	g/kg FW	ns	Harvest	71.13	112.18	91.87	3.02	12.43
			Postharvest	63.77	138.72	89.74	5.09	20.99
Sucrose	g/kg FW	*	Harvest	11.47	42.14	30.10	2.34	9.66
			Postharvest	5.97	38.61	21.56	2.48	10.21
Glucose	g/kg FW	*	Harvest	6.38	18.99	12.51	0.93	3.85
			Postharvest	5.15	23.53	15.69	1.20	4.96
Fructose	g/kg FW	ns	Harvest	31.39	53.03	44.60	1.42	5.86
			Postharvest	26.04	65.20	46.59	2.32	9.57
Sorbitol	g/kg FW	ns	Harvest	2.10	11.43	4.66	0.68	2.82
			Postharvest	2.04	19.80	5.90	1.15	4.76
Acids	g/kg FW	***	Harvest	3.63	11.26	6.22	0.47	1.93
			Postharvest	1.52	7.76	3.40	0.45	1.86
Malic	g/kg FW	***	Harvest	2.78	10.13	5.68	0.47	1.95
			Postharvest	0.96	7.04	2.90	0.46	1.88
Citric	g/kg FW	ns	Harvest	0.02	0.11	0.05	0.01	0.02
			Postharvest	0.01	0.24	0.04	0.01	0.06
Tartaric	g/kg FW	ns	Harvest	0.03	0.05	0.04	0.00	0.01
			Postharvest	0.02	0.07	0.04	0.00	0.01
Succ+Shi	g/kg FW	ns	Harvest	0.19	0.86	0.45	0.04	0.17
			Postharvest	0.18	0.81	0.43	0.04	0.17

Significant differences at *: $P \leq 0.05$; ***: $P \leq 0.001$ between Harvest and Postharvest for the trait assessed according to the Student's t test. Abbreviations: FW, fresh weight; SE, standard error; SD, standard deviation; SSC, soluble solids content; TA, titratable acidity; RI, ripening index; TPC, total phenolics content; TFC, total flavonoids content; AsA, Ascorbic acid; RAC, relative antioxidant content; Sugars, total sugars; Acids, total organic acids; Succ+Shi, succinic + shikimic.

and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox) were purchased from Sigma-Aldrich (Saint Louis, MO, USA).
All sugar standards (sucrose, glucose, fructose, and sorbitol) and sulfuric acid were purchased from PanReac Química SA (Barcelona, Spain), organic acids standards (citric, malic, oxalic, quinic, succinic, shikimic, and tartaric) were purchased from Sigma-Aldrich (Saint Louis, MO, USA).

Results and Discussion

Basic fruit quality traits and antioxidant compounds

The ANOVA analysis (Tab. S1 and S2) showed significant differences at $P \leq 0.001$ among the 17 different apple accessions for all traits evaluated at harvest (Mignard et al., 2021b; 2022) and after six months of cold storage (Fig. 1 and 2) unless for the AsA values at harvest period ($P \leq 0.05$). According to the basic fruit quality traits, high variations were found in this study as reported in other apple studies at harvest and postharvest periods (Alhaj Alali et al., 2020; Castel et al., 2020; Mignard et al., 2021a; 2022; Moon et al., 2020; Yang et al., 2021). In fact, the SSC ranged among apple accessions in postharvest from 9.8 °Brix ('Florina') to 24.2 °Brix ('Reineta Gris'), compared to a less variation from 11.6 °Brix ('Morro de Liebre') to 17.1 °Brix ('Bossost – MRF 76') at harvest date. The TA postharvest values differed greatly ranging from 2.2 g ('Cul de Cirio – MRF 39') to 10.1 g malic acid per liter ('Reineta Blanca del Canadá'). Meanwhile, at harvest time, data ranged from 3.3 g (again for the 'Cul de Cirio – MRF 39' accession) to 14.0 g malic acid per liter ('Bossost – MRF 76'). Higher SSC and lower TA values after cold storage have been also reported in peaches (Navarro et al., 2022). The SSC increased and TA decreased during the storage due to the consumption of organic acids in respiration and fruit maturation (Navarro et al., 2022). Regarding the RI, values ranged from 1.6 ('Baujade') to 8.1 ('Cul de Cirio – MRF 39') after six months of cold storage, compared to a less variation from 1.3 ('Bossost – MRF 76') to 4.5 ('Cul de Cirio – MRF 39') at harvest, because metabolic pathways causing ripening of fruits continue during postharvest (Ehsani-Moghaddam et al., 2013).

The antioxidant compounds varied greatly in this study as previously reported by other studies (Tab. S1, Figure 1) (Castel et al., 2020; Kevers et al., 2011; Mignard et al., 2021a). The TPC varied significantly among apple accessions in postharvest, from 6.1 mg ('Red Elstar') to 49.8 mg gallic acid equivalents (GAE)/100 g FW ('Reineta Gris'), compared with a slightly lower variation at harvest, from 18.5 mg ('Evasni') to 57 mg GAE/100 g FW ('Reineta Gris'). Total phenolics thus decreased in average from 33.5 mg at harvest to 23 mg GAE/100 g FW after a long-term cold storage. This decrease is in good agreement with Navarro et al. (2022) who studied a large number of peach cultivars cold-stored for a month. Moreover, López et al. (1994) reported that the decrease of phenolics could be the result of their oxidation associated with browning. However, phenols could be also synthesized as antioxidant factors against abiotic stresses (Foyer and Noctor, 2005).

For the TFC, values varied significantly ranging in postharvest from 5.8 mg ('Delcon') to 39.0 mg catechin equivalents (CE)/100 g FW ('Reineta Gris') and at harvest from 6.7 mg ('Averdal') to 32.9 mg CE/100 g FW ('Reineta Gris'). Regarding the ascorbic acid (AsA – Vitamin C), values ranged from 0.5 mg ('Red Elstar') to 3.5 mg AsA/100 g FW ('Reineta Gris') in postharvest and from 1.7 mg ('Evasni') to 3.6 mg AsA/100 g FW (again, the 'Reineta Gris' accession) at harvest. Finally, RAC values ranged from 5.6 mg ('Red Elstar') to 19.5 mg trolox/100 g FW ('Reneta') in postharvest and from 7.1 mg ('Evasni') to 21.2 mg trolox/100 g FW ('Cul de Cirio – MRF 39') at postharvest.

For the different basic quality traits, biochemical compounds and relative antioxidant capacity, comparison of means for each accession between the harvest date and after six months of cold storage (Fig. 1) showed significant differences among accessions. Thus, the SSC values tended to be slightly increased after six months of cold storage, unless for the 'Florina' and the 'Cripps Pink' accessions. In contrast, the TA, when the differences were significant, decreased for all the accessions assessed probably due to the process of fruit ripening after harvest. Indeed, it is well known that when fruits are harvested, they continue their ripening during the postharvest storage (Pott et al., 2020). Changes in antioxidants, sugars and organic acids describe the ripe of the fruits as peel color or fruit flavour (Ehsani-Moghaddam et al., 2013; Singh et al., 2018). Moreover, as the SSC tended to increase while the TA tended to decrease, the RI increased in postharvest.

The TFC and the RAC did not show a general pattern in their increase or decrease but the differences observed depended more on the studied accession (Fig. 1, Tab. 2). However, in the case of phenols, the values decreased significantly after six months of cold storage as previously reported in other apple (Alhaj Alali et al., 2020; Kevers et al., 2007) and peach studies (Navarro et al., 2022). Phenolics are not stable and undergo a clear metabolic turnover during cold storage, while flavonoids do not seem to be affected in this way as their contents were more constant between harvest and postharvest values. Several studies have shown significant fluctuations in the content of antioxidants in apple at low temperatures (Alhaj Alali et al., 2020; Villa-Rodriguez et al., 2015). Increased levels of bioactive molecules, such as antioxidant compounds, should be considered as an important guarantee of the nutritional value of apples. Nevertheless, Kevers et al. (2011) reported that total phenolics content showed an increase after three months of storage at cold temperature, followed by a decrease. The decrease continued over the following months, explaining the lower values observed after six months of conservation (Kevers et al., 2011). Moreover, the relationship between polyphenol content and resistance to postharvest diseases caused by *Penicillium expansum* has also been described and resistant and susceptible apple genotypes could be discriminated based on polyphenol content (Sun et al., 2017). In the present work, the cultivar 'Evasni – Scarlet Spur' showed the best performance under a long-term cold storage because of its increase in TFC and RAC and as the TPC was not significantly different between harvest and postharvest periods.

Storage induces noteworthy metabolic changes in apples, such as a decrease in the ascorbic acid level (Kevers et al., 2011). The general loss of ascorbic acid observed in the present work (Tab. 2, $P \leq 0.001$) could be an indicator of oxidative stress during storage (Foyer and Noctor, 2005; Kevers et al., 2007) as AsA plays a major role in defense to biotic and abiotic stresses in plants against free radicals inducing peroxidation.

Individual sugars and organic acids profiles

The profiles of the individual sugars and organic acids for the 17 apple accessions studied after six months of cold storage compared with harvest time are shown in Fig. 2. Firstly, it is easily noticed the great variability found in this study for all the traits assessed among accessions (Tab. S2). According to the total sugars values (Sugars) and the major sugar for apple (fructose), great and significant variations were observed among accessions and harvest/postharvest periods as previously reported (Tab. 2, Fig. 2) (Aprea et al., 2017; Castel et al., 2020; Leja et al., 2003; Mignard et al., 2022; Zhang et al., 2021). Total sugars ranged from 63.8 g ('Florina') to 138.7 g/kg FW ('Reineta Gris') in postharvest and from 71.1 g ('Morro de Liebre') to 112.8 g/kg FW ('Reineta Blanca del Canadá') at harvest time. Moreover, fructose (Fru) values in postharvest varied considerably from 26.0 g ('Baujade') to 65.2 g/kg FW ('Reineta Gris'), while values at harvest date ranged from 31.4 g ('Baujade') to

Fig. 1: Changes in basic fruit quality and antioxidant traits between harvest and postharvest date for the 17 apple accessions assessed. * indicates significant difference at P≤0.05 according to the student's t test assessed for each accession between harvest and postharvest, FW: fresh weight.

Fig. 2: Changes in individual sugars and organic acids between harvest and postharvest date for the 17 apple accessions studied in this study. * indicates significant difference at P≤0.05 according to the student's t test assessed for each accession between harvest and postharvest, all the traits are expressed in g/kg of fresh weight.

53.0 g/kg FW ('Cul de Cirio – MRF 39').

Regarding the total acids (Acids) among accessions, levels were within the range previously reported (CASTEL et al., 2020; MIGNARD et al., 2022; VALLARINO and OSORIO, 2019; YANG et al., 2021). Indeed, values ranged from 1.5 g ('Florina') to 7.8 g/kg FW ('Reineta Blanca del Canadá') in postharvest, and 3.3 g ('Cul de Cirio – MRF 39') to 10.7 g/kg FW ('Bossost – MRF 76') at harvest time. Regarding the malic acid, which is the major acid in apple, values ranged considerably, compared with the other acids, from 1.0 g ('Florina') to 7.0 g/kg FW ('Reineta Blanca del Canadá') in postharvest and from 2.8 g ('Cul de Cirio – MRF 39') to 10.1 g/kg FW ('Bossost – MRF 76') at harvest.

According to the individual sugars (sucrose, glucose, and fructose) and the sugar-alcohol (sorbitol), the changes observed in postharvest were diverse. Sugars contents exhibited differential performance during postharvest, depending mainly on the species and storage conditions (POTT et al., 2020). Sucrose values drop significantly for all accessions and the major loss was observed for the 'Florina', 'Red Delicious' and 'Red Elstar' cultivars. Fructose values generally increased or maintained the harvest level except for 'Florina' which showed a loss of fructose. Sorbitol showed different performance after a long-term cold storage. It is noteworthy the great increase in sorbitol for the two Reinette accessions, 'Reineta Blanca del Canadá' and 'Reineta Gris'. Moreover, glucose values increased with greater intensity, especially in the case of 'Averdal', 'Evasni', 'Red Delicious', 'Red Elstar', 'Reineta Blanca del Canada', and 'Reineta Gris'. In other fruits, such as bananas or kiwis, carbohydrates levels increased as a consequence of starch hydrolysis during postharvest storage (YUAN et al., 2017). Additionally, the sum of the individual sugars showed different performances after six months of cold conservation. Indeed, for the 'Evasni' and 'Reineta Gris' accessions, the sum of individual sugars increased while for 'Florina' and 'Reneta', the total sugars decreased.

Citric, tartaric, succinic and shikimic acids showed more irregular profiles according to accessions, but malic, the main acid in apples, and total acids decreased considerably (Tab. 2, $P \leq 0.001$, and Fig. 2) as reported in other studies (VALLARINO and OSORIO, 2019). Moreover, TA decreased slightly in postharvest as before mentioned. Previous studies showed that organic acids or sourness of the fruit negatively correlated with sensory acceptability of professional panellists (KEENAN et al., 2012). However, the equilibrium of sweetness and sourness determine consumer satisfaction for apple flavor. All the bioactive molecules assessed in this study influence the organoleptic perception of sweetness, sourness, and aroma (APREA et al., 2017; YANG et al., 2021) and contribute to the quality of the fruit and its acceptance by consumers. Because of the decrease of acids and the increase of SSC and some sugars like glucose or sorbitol during cold storage, fruits of 'Averdal', 'Bossost', 'Cul de Cirio', 'Delcon', 'Evasni', 'Golden Paradise', 'Granny Smith', 'Morro de Liebre', 'Red Delicious', and 'Reineta Gris' accessions should have a better acceptability by consumers after long-term cold storage. It is also important to highlight several cultivars ('Cripps Pink', 'Cul de Cirio', 'Evasni', 'Florina', 'Reineta Blance del Canadá', and 'Reineta Gris') maintaining their fruit quality regarding total phenolics or flavonoids.

Pearson's correlations between traits at postharvest

Significant bilateral correlations between the traits assessed at postharvest (Fig. 3, Tab. S1) were found ($P \leq 0.01$). The relative antioxidant capacity (RAC) was greatly and positively correlated with TPC (r=0.79) and TFC (r=0.73). TPC was also significantly and highly positive correlated with TFC (r=0.96). AsA likewise presented a significant and positive correlation with TPC (r=0.88) and TFC (r=0.89). Previous studies reported similar high and positive correlations between the antioxidant compounds at harvest (MIGNARD et al., 2021a; WANG et al., 2015).

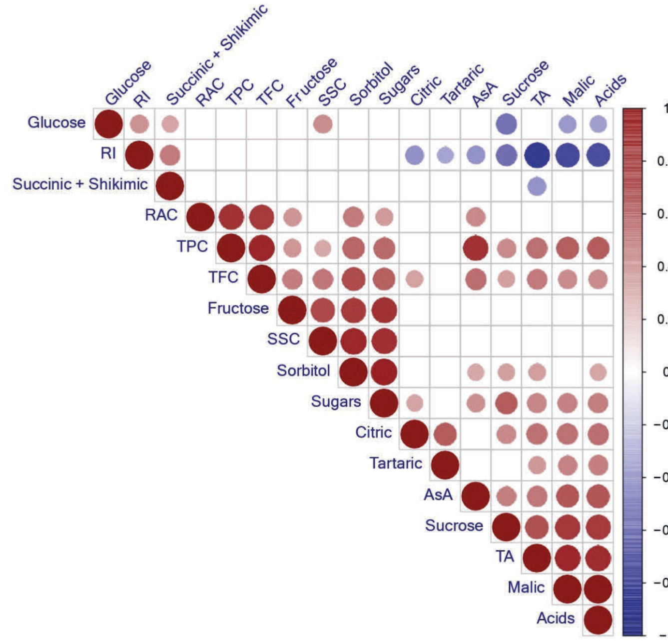

Fig. 3: Pearson's correlation coefficients for the traits studied for the 17 accessions assessed in this study. Abbreviations: SSC, soluble solids content; TA, titratable acidity; RI, ripening index; TPC, total phenolics content; TFC, total flavonoids content; AsA, Ascorbic acid; RAC, relative antioxidant content; Sugars, total sugars; Acids, total organic acid; Succ+Shi, succinic + shikimic. The size of the circle for each correlation and the color depicts the significance and the magnitude of the correlation coefficient, respectively.

Moreover, significant and positive correlations were found between TA and total acids (r=0.95) and between SSC and total sugars (r=0.92). SSC was also correlated with glucose (r=0.54), fructose (r=0.80) and sorbitol (r=0.87) while TA was also correlated with malic acid (r=0.96) and citric acid (r=0.57). These correlations highlight the common utilization of SSC and TA as approximate values for fruit sweetness and sourness, respectively (MIGNARD et al., 2022). Nevertheless, fruit sweetness or sourness cannot be defined only by SSC and TA but each biochemical traits of individual sugars and organic acids are really important (APREA et al., 2017).

Furthermore, metabolic pathways could explicate the negative correlation (Fig. 3, Tab. S1) between glucose and total organic acids (r=-0.34) and malic acid (r=-0.53) as organic acid degradation can occur through dicarboxylates decarboxylation (RUAN, 2014). This permits phosphoenolpyruvate (PEP) production, which plays a role in gluconeogenesis activation, resulting in increased glucose production and organic acids degradation (VALLARINO and OSORIO, 2019). As explained above, 15 accessions showed the tendency to show an increase in glucose after six months of cold storage, while 'Baujade' and 'Cripps Pink' decreased their values. Moreover, the sum of organic acids and malic acid, for all the 17 accessions assessed, decreased significantly while for almost all of them, glucose and fructose values remained or tended to increase. RYMENANTS et al. (2020) reported that the perceived sweetness by consumers was importantly and significantly influenced by the fruit acidity and vice versa. Indeed, APREA et al. (2017) reported a negative correlation between malic acid and the perceived sweetness of fruits. We could easily highlight the increase of sweetness sensation in apples after long-term cold storage than at harvest because of its significant decrease in malic acid for the more acid cultivars.

Principal components analysis

A principal component analysis (PCA) was carried out to appreciate how traits could influence the 17 different accessions assessed after a long-term cold storage (Fig. 4). The first two components, PC1 and PC2, accounted respectively for 46.2% and 23.8% of the total variability and thus, 70% of the total variance could be explained according to the first two components. Firstly, the PC1 loadings suggested that the separation of this component was principally due to acids and sugars parameters, TA and SSC for the basic fruit quality traits, and mostly all the antioxidant compounds. Moreover, the PC2 mostly contributed to the ripening index (RI), the succinic and shikimic acids and some sugars such as fructose or glucose, but with less significance. Accessions on the positive side of PC1 corresponding mainly to triploid accessions, with presence of russeting, induced in general, higher values of sugars, organic acids and antioxidant compounds as previously described (BUSATTO et al., 2019). Indeed, only five accessions were on the positive side of PC1 and three of them were triploids: 'Reineta Gris', 'Reineta Blanca del Canadá', and 'Bossost'. These three accessions showed higher values for total acids, total sugars and TPC (Fig. 1 and 2). However, most apple accessions exhibiting russeting are triploids (BUSATTO et al., 2019; REIG et al., 2015), and are less suitable in breeding programs, even though russeted apples could improve bioactive molecules in apple. In contrast, accessions on the negative side of PC1, correspond to most of the well-known foreign cultivars such as 'Granny Smith', 'Red Delicious' or 'Averdal', showed, in general, lower values for those biochemical compounds as previously reported (MIGNARD et al., 2021a).

The accession 'Cul de Cirio' was highlighted because of its high value for RI. This local Spanish accession showed a value of 4.5 for RI at harvest date and increased to 8.1 after six months of cold storage (Fig. 1). This increase was the major change between harvest and after six months of conservation that could be noticed in this study and we can see that the 'Cul de Cirio' accession maintained glucose, fructose and sorbitol values high after cold storage, while tartaric, citric and malic acids values were low. This means 'Cul de Cirio' could be a good accession for its marketability due to its low acidity and high sweetness after six months of storage. A panellist group should confirm the sensorial acceptance of this accession.

The observed values for the different fruit quality traits, antioxidants and both individual sugars and organic acids studied for 'Morro de Liebre' and 'Reneta' accessions, both originated from Spain, were similar to the well-known commercial cultivars or cultivars resulting from breeding programs such as 'Averdal', 'Delcon', 'Florina', 'Golden Paradise', 'Red Delicious' or 'Red Elstar'.

Visual symptoms for susceptibility to cold storage and relationship with fruit quality traits

Tab. 3 showed the susceptibility of the 17 accessions of this study to present chilling injury symptoms such as browning (skin or pulp) or dehydration of the fruits (Fig. 5 and 6) during long-term storage. After six months of conservation, nine accessions ('Baujade', 'Cripps Pink', 'Delcon', 'Evasni', 'Florina', 'Granny Smith', 'Red Delicious', 'Reneta', and 'Solafuente') showed no apparent cold damage (Tab. 3, Fig. 5). Out of these nine accessions characterized as suitable for long-term conservation, two were Spanish accessions ('Reneta' and 'Solafuente'). The other seven non-Spanish accessions were all well-known cultivars in the market and/or exhibited similar characteristics among them ('Baujade', 'Cripps Pink', 'Delcon', 'Evasni – Scarlet Spur', 'Florina', 'Granny Smith' and 'Red Delicious'). As a result of long time of breeding and selection, these accessions confirmed their suitability to permit the marketability of apples all year round.

Among the accessions assessed, eight ('Averdal', 'Bossost', 'Cul de Cirio', 'Golden Paradise', 'Morro de Liebre', 'Red Elstar', 'Reineta Blanca del Canadá', and 'Reineta Gris') were classified as unsuitable for long-term cold storage because of browning and/or dehydration after six months of cold storage (Tab. 3, Fig. 6). The modern organi-

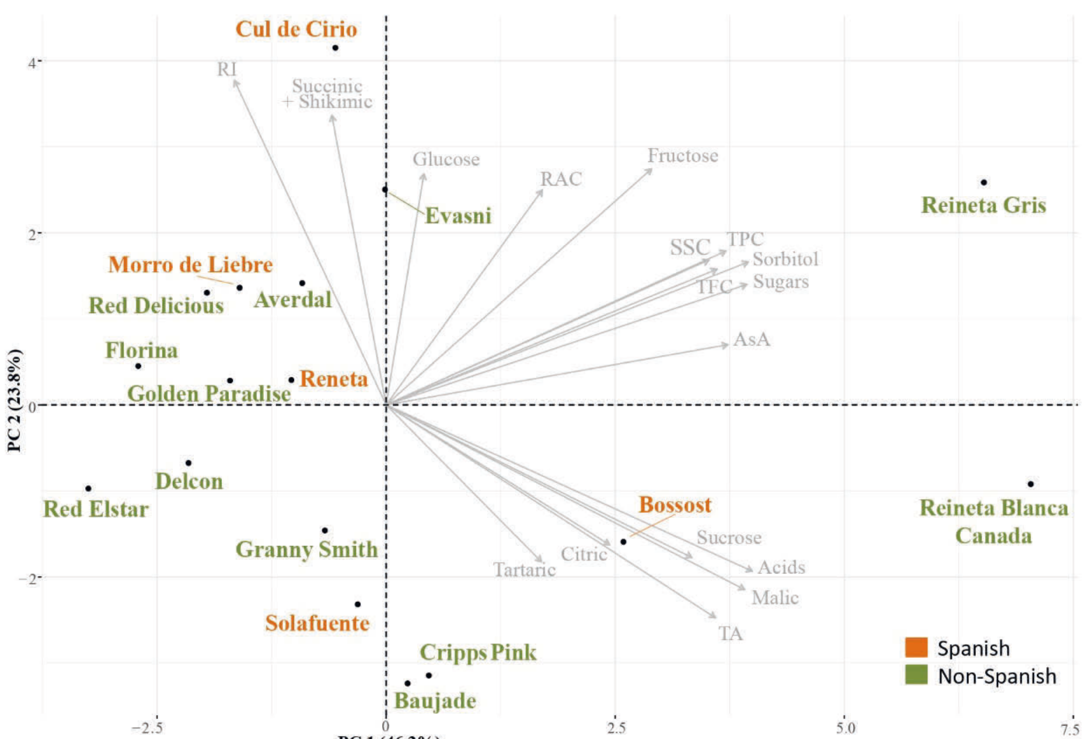

Fig. 4: Bidimensional distribution (PCA) showing the relation among the evaluated traits on the 17 apple accessions after six months of cold storage (post-harvest). Abbreviations: SSC, soluble solids content; TA, titratable acidity; RI, ripening index; TPC, total phenolics content; TFC, total flavonoids content; AsA, Ascorbic acid; RAC, relative antioxidant content; Sugars, total sugars; Acids, total organic acid.

Tab. 3: Susceptibility for the 17 apple accessions assessed following six months of cold storage.

Accession	N	General Appearance	Browning	Dehydration	Conservation
Averdal	1	Bad	2	2	Unsuitable
Baujade	2	Good	1	1	Suitable
Bossost - MRF 76	3	Bad	2	2	Unsuitable
Cripps Pink	4	Good	1	1	Suitable
Cul de Cirio - MRF 39	5	Good	2	1	Unsuitable
Delcon	6	Good	1	1	Suitable
Evasni - Scarlet Spur	7	Good	1	1	Suitable
Florina	8	Good	1	1	Suitable
Golden Paradise	9	Good	2	1	Unsuitable
Granny Smith	10	Good	1	1	Suitable
Morro de Liebre	11	Good	2	1	Unsuitable
Red Delicious	12	Good	1	1	Suitable
Red Elstar	13	Bad	2	1	Unsuitable
Reineta Blanca del Canadá	14	Bad	2	2	Unsuitable
Reineta Gris	15	Bad	2	2	Unsuitable
Reneta	16	Good	1	1	Suitable
Solafuente	17	Good	1	1	Suitable

1: inexistent; 2: presence of chilling injury symptoms.

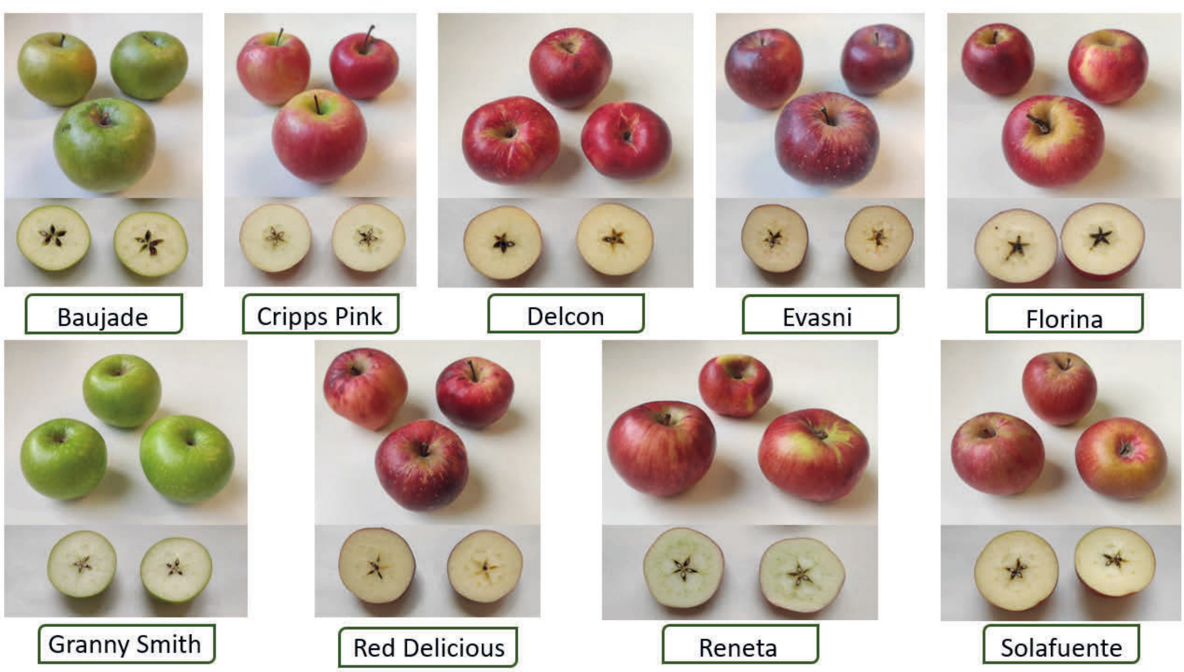

Fig. 5: Suitable accessions for long-term cold storage assessed in this study.

zation for fruit distribution and marketability aims to guarantee fruit quality also during storage, allowing high-quality standards to be maintained from the orchard to the consumer (Di Guardo et al., 2013). Indeed, browning is associated with lower fruit quality and freshness, and major deterioration. Its prevention by selecting tolerant cultivars has taken a great effort in horticultural and food research for years (Cebulj et al., 2021). It is well known that browning is caused by the oxidation of the phenolics compounds in damaged cells (Cebulj et al., 2021). Browning could thus occur by improper handling or processing, cutting, peeling or grinding. Nevertheless, cells could also be damaged by cold storage (Cebulj et al., 2021; Rasouli and Saba, 2018). The phenolics, when the cell is damaged enter in contact with the polyphenol oxidase enzyme (PPO) resulting into an oxidized phenol causing the appearing browning. In fact, in intact cells, PPO seems to have little activity toward phenolic compounds. PPO interacts with phenolics as substrates (Rasouli and Saba, 2018).

Tab. 4 showed the means and differences for the different traits assessed in this study when the accessions were affected by chilling injuries. Firstly, the increase in fruits presenting chilling injuries was significant for the main antioxidant compounds responsible for browning as TPC and TFC (Moon et al., 2020; Singh et al., 2018). Phenolic compounds can protect the fruit from oxidative stress at low temperatures, but they can also be responsible for greater browning (Singh et al., 2018). In general, TPC, TFC, AsA and RAC means were higher in accessions exhibiting chilling injuries (Tab. 4) as

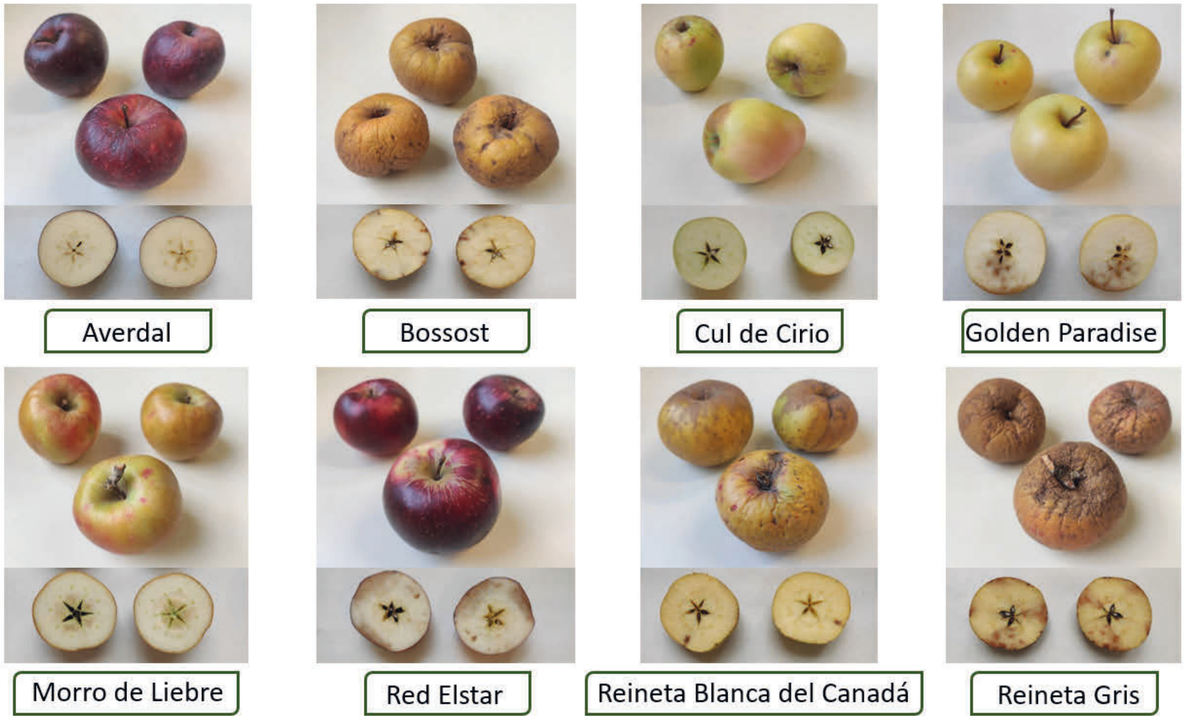

Fig. 6: Unsuitable accessions for long-term cold storage studied.

Tab. 4: Means for basic fruit quality and bioactive compounds traits over 17 accessions for both groups, suitable and unsuitable accessions for long-term cold storage.

Trait	Suitable accessions for storage	Unsuitable accessions for storage	Units	Significance
SSC	13.1	14.3	ºBrix	ns
TA	6.5	7.1	G malic acid/L	ns
RI	2.5	2.6	-	ns
TPC	27.5	39.4	mg GAE/100 g FW	*
TFC	13.6	20.0	mg CE/100 g FW	*
AsA	2.4	2.9	mg AsA/100 g FW	*
RAC	11.8	16.2	mg Trolox/100 g FW	*
Sucrose	30.6	29.9	g/kg FW	ns
Glucose	11.8	13.3	g/kg FW	ns
Fructose	41.6	47.9	g/kg FW	*
Sorbitol	3.2	6.3	g/kg FW	*
Sugars	87.3	97.0	g/kg FW	ns
Citric	0.05	0.05	g/kg FW	ns
Tartaric	0.04	0.04	g/kg FW	ns
Malic	5.55	5.82	g/kg FW	ns
Succ + Shi	0.39	0.51	g/kg FW	ns
Acids	6.03	6.43	g/kg FW	ns

Student's t test with significance at *: $P \leq 0.05$; ns: no significant.
Abbreviations: FW, fresh weight; SSC, soluble solids content; TA, titratable acidity; RI, ripening index; TPC, total phenolics content; TFC, total flavonoids content; AsA, Ascorbic acid; RAC, relative antioxidant content; Sugars, total sugars; Acids, total organic acids; Succ+Shi, succinic + shikimic.

'Bossost', 'Cul de Cirio', 'Reineta Blanca del Canada', and 'Reineta Gris') compared to 'Cripps Pink', 'Delcon', 'Red Delicious', and 'Solafuente' accessions, without symptoms. Moreover, KHANIZADEH et al. (2006) described that the lack of substrate for PPO enzyme may be the cause of non-browning in apples. In other species, such as in peach or pear fruits, it has been reported that the more phenolics content the more PPO activity could be found and thus, more browning would be observed (KHAN et al., 2021; SINGH et al., 2018).

There were also significant differences between groups for fructose and sorbitol. For the fruits exhibiting chilling injuries, the contents of fructose and sorbitol were higher than in sound fruits (Tab. 4). Nonetheless, the accessions exhibiting higher levels of fructose and sorbitol as well as antioxidant compounds, were the russeted apples. The accessions with russeting ('Bossost', 'Reineta Blanca del Canadá' and 'Reineta Gris'), visually appeared to be more dehydrated than the others. BUSATTO et al. (2019) reported that russeting is a genetic-controlled disorder resulting from the periderm coat consisting of a network of suberized cells straight above the skin. This disorder shows up as a brown and rough matrix deposition. Dehydration should be more conducive for a russeted apple due to this periderm disorder. Furthermore, the damage in cells making the possible contact between antioxidants and PPO enzyme causing browning (KHAN et al., 2021; MOON et al., 2020; SINGH et al., 2018) could explain the relationship between russeting, dehydration and thus, browning. Furthermore, dehydration could explain the increase in bioactive compounds as antioxidants, sugars, sorbitol or tartaric acid (Fig. 1 and 2) by their concentration (QUILES et al., 2005).

Conclusions

The quality traits of fruit (levels of sugars, organic acids and antioxidants) of the accessions studied showed different performances after the postharvest period.

This work showed the importance of the biochemical compounds in postharvest and the visual quality (dehydration or browning). Russeted accessions ('Bossost', 'Reineta Blanca del Canadá' and 'Reineta Gris'), all triploid accessions, showed more chilling injuries (browning and dehydration) than the other accessions. Additionally, 'Reneta' and 'Solafuente' were the Spanish accessions best conserved based on the visual appearance and fruit quality, while 'Evasni' seemed to be the less affected by long-term cold storage according to the antioxidant profile. These results highlighted the importance of the autochthone accessions ('Reneta' and 'Solafuente') for its use in future breeding programs. Moreover, other non-Spanish cultivars, resulting from long time selection processes, such as 'Baujade', 'Cripps Pink', 'Delcon', 'Evasni', 'Florina', 'Granny Smith', and 'Red Delicious', confirmed their suitability for long-term storage periods with no chilling injuries after six months of cold storage.

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Conflict of interest
No potential conflict of interest was reported by the authors.

References

ALHAJ ALALI, F., ASKARI SARCHESHMEH, M.A., BABALAR, M., 2020: Effect of ammonium content in nutrition solution on vitamin C, phenols, and antioxidant capacity of three apple cultivars during cold storage. DYSONA-Appl. Sci. 1(2), 64-72. DOI: 10.30493/DAS.2020.230629

APREA, E., CHARLES, M., ENDRIZZI, I., COROLLARO, M.L., BETTA, E., BIASIOLI, F., 2017: Sweet taste in apple: the role of sorbitol, individual sugars, organic acids and volatile compounds. Sci. Rep. 7(1), 1-10. DOI: 10.1038/srep44950

BRAND-WILLIAMS, W., CUVELIER, M.E., BERSET, C.L.W.T., 1995: Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol. 28(1), 25-30. DOI: 10.1016/s0023-6438(95)80008-5

BUSATTO, N., MATSUMOTO, D., TADIELLO, A., VRHOVSEK, U., COSTA, F., 2019: Multifaceted analyses disclose the role of fruit size and skin-russeting in the accumulation pattern of phenolic compounds in apple. PloS One 14(7), e0219354. DOI: 10.1371/journal.pone.0219354

CAINELLI, N., RUPERTI, B., 2019: Biochemistry and molecular biology in fruits during cold storage. Ann. Plant Rev. Online 2, 659-688. DOI: 10.1002/9781119312994.apr0694

CASTEL, L., PINA, A., IRISARRI, P., ERREA, P., 2020: Sugar content and organic acid profiles of local apple cultivars recovered from mountain zones. J. Appl. Bot. Food Qual. 93, 217-224. DOI: 10.5073/JABFQ.2020.093.026

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(7), 1402. DOI: 10.3390/plants10071402

DAVEY, M.W., AUWERKERKEN, A., KEULEMANS, J., 2007: Relationship of apple vitamin C and antioxidant contents to harvest date and postharvest pathogen infection. J. Sci. Food Agric. 87(5), 802-813. DOI: 10.1002/jsfa.2777

DI GUARDO, M., TADIELLO, A., FARNETI, B., LORENZ, G., MASUERO, D., VRHOVSEK, U., COSTA, F., 2013: A multidisciplinary approach providing new insight into fruit flesh browning physiology in apple (*Malus × domestica* Borkh.). PLos One 8(10), e78004. DOI: 10.1371/journal.pone.0078004

EHSANI-MOGHADDAM, B., DEELL, J.R., 2013: Relationships among postharvest ripening attributes and storage disorders in 'Honeycrisp' apple. Fruits 68(4), 323-332. DOI: 10.1051/fruits/2013078

FAOSTAT, 2023: Food and Agricultural Organization. http://www.fao.org/faostat/en/#compare/ (Accessed 21 of April of 2023).

FONT I FORCADA, C., REIG, G., GIMÉNEZ, R., MIGNARD, P., MESTRE, L., MORENO, M.A., 2019: Sugars and organic acids profile and antioxidant compounds of nectarine fruits influenced by different rootstocks. Sci. Hortic. 248, 145-153. DOI: 10.1016/j.scienta.2018.12.010

FOYER, C.H., NOCTOR, G., 2005: Oxidant and antioxidant signalling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. Plant Cell Environ. 28(8), 1056-1071. DOI: 10.1111/j.1365-3040.2005.01327.x

HAN, J.W., JI, Z.T., ZUO, M., YANG, X.T., 2021: Integral evaluation for intermittent cold storage of apples by using mathematical modeling. J. Food Process Eng. 44(11), e13882. DOI: 10.1111/jfpe.13882

KHAN, M.R., HUANG, C., DURRANI, Y., MUHAMMAD, A., 2021: Chemistry of enzymatic browning in longan fruit as a function of pericarp pH and dehydration and its prevention by essential oil, an alternative approach to SO_2 fumigation. PeerJ 9, e11539. DOI: 10.7717/peerj.11539

KHANIZADEH, S., GROLEAU, Y., LEVASSEUR, A., CHARLES, M.T., TSAO, R., YANG, R., TOIVONEN, P., 2006: SJCA38R6A74'(Eden). HortScience 41(6), 1513-1515.

KEENAN, D.F., VALVERDE, J., GORMLEY, R., BUTLER, F., BRUNTON, N.P., 2012: Selecting apple cultivars for use in ready-to-eat desserts based on multivariate analyses of physico-chemical properties. LWT-Food Sci. Technol. 48(2), 308-315. DOI: 10.1016/j.lwt.2012.04.005

KEVERS, C., FALKOWSKI, M., TABART, J., DEFRAIGNE, J.O., DOMMES, J., PINCEMAIL, J., 2007: Evolution of antioxidant capacity during storage of selected fruits and vegetables. J. Agric. Food Chem. 55(21), 8596-8603. DOI: 10.1021/jf071736j

KEVERS, C., PINCEMAIL, J., TABART, J., DEFRAIGNE, J.O., DOMMES, J., 2011: Influence of cultivar, harvest time, storage conditions, and peeling on the antioxidant capacity and phenolic and ascorbic acid contents of apples and pears. J. Agric. Food Chem. 59(11), 6165-6171. DOI: 10.1021/jf201013k

LEJA, M., MARECZEK, A., BEN, J., 2003: Antioxidant properties of two apple cultivars during long-term storage. Food Chem. 80(3), 303-307. DOI: 10.1016/S0308-8146(02)00263-7

MIGNARD, P., BEGUERÍA, S., REIG, G., FONT I FORCADA, C., MORENO, M.A., 2021a: Genetic origin and climate determine fruit quality and antioxidant traits on apple (*Malus × domestica* Borkh). Sci. Hortic. 285, 110142. DOI: 10.1016/j.scienta.2021.110142

MIGNARD, P., BEGUERÍA, S., REIG, G., FONT I FORCADA, C., MORENO, M.A., 2021b: Phenotypic analysis of fruit quality traits and effect of climate in an apple (*Malus × domestica* Borkh) germplasm bank of Aragón, Spain. Acta Hortic. 1307, 109-114. DOI: 10.17660/ActaHortic.2021.1307.17

MIGNARD, P., BEGUERÍA, S., GIMÉNEZ, R., FONT I FORCADA, C., REIG, G., MORENO, M.A., 2022: Effect of Genetics and Climate on Apple Sugars and Organic Acids Profiles. Agron. 12(4), 827. DOI: 10.3390/agronomy12040827

MOON, K.M., KWON, E.B., LEE, B., KIM, C.Y., 2020: Recent trends in controlling the enzymatic browning of fruit and vegetable products. Molecules 25(12), 2754. DOI: 10.3390/molecules25122754

MUSHTAQ, R., WANI, A.W., NAYIK, G.A., 2020: Apple. In: Nayik, G.A., Gull, A. (eds.), Antioxidants in fruits: properties and health benefits, 507-522. Springer Nature, Singapore. DOI: 10.1007/978-981-15-7285-2_26

NAVARRO, A., GIMÉNEZ, R., CANTÍN, C.M., MARTÍNEZ-GARCÍA, P.J., VAL, J., MORENO, M.A., 2022: Chilling injury in local and modern peach cultivars from a Spanish peach bank germplasm. Acta Hortic. 1352, 37-244. DOI: 10.17660/ActaHortic.2022.1352.32

POTT, D.M., VALLARINO, J.G., OSORIO, S., 2020: Metabolite changes during postharvest storage: Effects on fruit quality traits. Metabolites 10(5), 187. DOI: 10.3390/metabo10050187

QUILES, A., HERNANDO, I., PÉREZ-MUNUERA, I., LARREA, V., LLORCA, E., LLUCH, M.A., 2005: Polyphenoloxidase (PPO) activity and osmotic dehydration in Granny Smith apple. J. Sci. Food Agric. 85(6), 1017-1020. DOI: 10.1002/jsfa.2062

RASOULI, M., SABA, M.K., 2018: Pre-harvest zinc spray impact on enzymatic browning and fruit flesh color changes in two apple cultivars. Sci.

Hortic. 240, 318-325. DOI: 10.1016/j.scienta.2018.06.053

Reig, G., Blanco, A., Castillo, A.M., Gogorcena, Y., Moreno, M.A., 2015: Phenotypic diversity of Spanish apple (*Malus × domestica* Borkh) accessions grown at the vulnerable climatic conditions of the Ebro Valley, Spain. Sci. Hortic. 185, 200-210. DOI: 10.1016/j.scienta.2015.01.024

Ruan, Y.L., 2014: Sucrose metabolism: gateway to diverse carbon use and sugar signalling. Annu. Rev. Plant Biol. 65, 33-67. DOI: 10.1146/annurev-arplant-050213-040251

Rymenants, M., Van de Weg, E., Auwerkerken, A., De Wit, I., Czech, A., Nijland, B., Keulemans, W., 2020: Detection of QTL for apple fruit acidity and sweetness using sensorial evaluation in multiple pedigreed full-sib families. Tree Genet. Genomes 16(5), 1-16. DOI: 10.1007/s11295-020-01486-4

Singh, B., Suri, K., Shevkani, K., Kaur, A., Kaur, A., Singh, N., 2018: Enzymatic browning of fruit and vegetables: A review. Enzymes in Food Technology 63-78. DOI: 10.1007/978-981-13-1933-4_4

Singleton, V.L., Rossi, J.A., 1965: Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Viticu. 16(3), 144-158.

Sun, J., Janisiewicz, W. J., Nichols, B., Jurick II, W. M., Chen, P., 2017: Composition of phenolic compounds in wild apple with multiple resistance mechanisms against postharvest blue mold decay. Postharvest Biol. Technol. 127, 68-75. DOI: 10.1016/j.postharvbio.2017.01.006

Vallarino, J.G., Osorio, S., 2019: Organic acids. In: Postharvest physiology and biochemistry of fruits and vegetables, 207-224. Woodhead Publishing. DOI: 10.1016/B978-0-12-813278-4.00010-5

Villa-Rodriguez, J.A., Palafox-Carlos, H., Yahia, E.M., Ayala-Zavala, J.F., Gonzalez-Aguilar, G.A., 2015: Maintaining antioxidant potential of fresh fruits and vegetables after harvest. Crit. Rev. Food Sci. Nutr. 55(6), 806-822. DOI: 10.1080/10408398.2012.685631

Wang, X., Li, C., Liang, D., Zou, Y., Li, P., Ma, F., 2015: Phenolic compounds and antioxidant activity in red-fleshed apples. J. Funct. Food. 18, 1086-1094. DOI: 10.1016/j.jff.2014.06.013

Yang, S., Meng, Z., Li, Y., Chen, R., Yang, Y., Zhao, Z., 2021: Evaluation of Physiological Characteristics, soluble sugars, organic acids and volatile compounds in ‘Orin’ apples (*Malus domestica*) at different ripening stages. Molecules 26(4), 807. DOI: 10.3390/molecules26040807

Yuan, Y., Zhao, Y., Yang, J., Jiang, Y., Lu, F., Jia, Y., Yang, B., 2017: Metabolomic analyses of banana during postharvest senescence by 1H-high resolution-NMR. Food Chem. 218, 406-412. DOI: 10.1016/j.foodchem.2016.09.080

Zaharieva, T.B., Abadía, J., 2003: Iron deficiency enhances the levels of ascorbate, glutathione, and related enzymes in sugar beet roots. Protoplasma 221(3-4), 269-275. DOI: 10.1007/s00709-002-0051-6

Zhang, B., Zhang, M., Shen, M., Li, H., Zhang, Z., Zhang, H., Zhao, J., et al., 2021: Quality monitoring method for apples of different maturity under long-term cold storage. Infrared Phys. Technol. 112, 103580. DOI: 10.1016/j.infrared.2020.103580

Zhishen, J., Mengcheng, T., Jianming, W., 1999: The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64(4), 555-559. DOI: 10.1016/S0308-8146(98)00102-2

Address of the corresponding author:
M.A. Moreno, Department of Pomology, Estación Experimental de Aula Dei, Consejo Superior de Investigaciones Científicas (EEAD-CSIC), P.O. box 13034, 50080 Zaragoza, Spain
E-mail: mmoreno@eead.csic.es

Table S1. Means for the 17 accessions assessed and for the 17 traits evaluated at Harvest period.

Accession	SSC		TA		RI		TPC		TFC		AsA		RAC	
Units	ºBrix		g malic acid/ L		-		mg GAE/100 g FW		mg CE/100 g FW		mg AsA/100 g FW		mg Trolox/100 g FW	
Averdal	12.33	abc	4.00	a	3.33	ef	27.40	ab	6.70	a	2.76	ab	13.19	bc
Baujade	14.01	cde	9.52	de	1.55	abc	34.49	abc	17.23	cde	3.04	ab	13.49	bc
Bossost	17.14	g	14.03	f	1.28	a	53.10	de	31.08	f	2.44	ab	19.47	de
Cripps Pink	14.65	ef	6.83	c	2.15	abc	20.03	a	9.08	ab	2.13	ab	8.75	ab
Cul de Cirio	14.41	def	3.29	a	4.51	g	40.83	b-e	15.38	bcd	3.07	ab	21.21	e
Delcon	13.04	a-e	6.46	bc	2.17	bcd	20.93	a	8.75	ab	2.35	ab	9.30	ab
Evasni	12.46	abc	3.48	a	3.76	efg	18.48	a	6.87	a	1.71	a	7.13	a
Florina	14.23	def	6.94	c	2.40	cd	30.46	abc	18.44	de	2.36	ab	14.19	bcd
Golden Paradise	13.47	b-e	4.25	ab	3.47	ef	25.47	ab	10.74	abc	2.54	ab	9.30	ab
Granny Smith	12.33	ab	10.26	e	1.32	ab	34.44	abc	20.26	de	2.84	ab	13.03	bc
Morro de Liebre	11.58	a	3.97	a	3.05	de	39.83	bcd	19.49	de	2.69	ab	16.42	cde
Red Delicious	13.65	b-e	3.60	a	4.06	fg	21.75	a	8.92	ab	1.78	a	10.60	ab
Red Elstar	13.31	b-e	7.33	cd	1.91	abc	25.66	ab	13.21	a-d	2.74	ab	10.64	ab
Reineta Blanca del Canada	15.69	fg	9.97	e	1.62	abc	45.57	cde	30.31	f	3.28	ab	18.51	cde
Reineta Gris	16.56	g	9.66	e	1.71	abc	57.04	e	32.86	f	3.64	b	20.65	e
Reneta	12.97	a-d	3.98	a	3.41	ef	39.45	bcd	22.88	e	2.35	ab	18.56	cde
Solafuente	12.45	abc	7.32	cd	1.75	abc	24.53	ab	9.89	abc	2.53	ab	10.26	ab
Sig ANOVA	***		***		***		***		***		*		***	

Means with the same letter are not significantly different from each other (Significance of ANOVA at *: P≤0.05; ***: P≤0.001 followed by Tukey tests for each trait). Abbreviations: FW, fresh weight; SSC, soluble solids content; TA, titratable acidity; RI, ripening index; TPC, total phenolics content; TFC, total flavonoids content; AsA, Ascorbic acid; RAC, relative antioxidant content.

Table S1. Continued

Accession	Sucrose		Glucose		Fructose		Sorbitol		Total Sugars	
Units	g/kg FW		g/kg FW		g/kg FW		g/kg FW		g/kg FW	
Averdal	19.14	abc	14.08	def	42.03	a-d	4.62	abc	79.87	ab
Baujade	38.48	ef	16.47	ef	31.39	a	2.17	a	88.51	a-d
Bossost	40.37	ef	12.17	a-e	48.21	cd	11.43	e	112.18	de
Cripps Pink	42.14	ef	6.38	a	42.70	bcd	3.43	ab	94.65	a-e
Cul de Cirio	23.05	bc	18.99	f	53.03	d	8.06	cde	103.12	b-e
Delcon	20.48	abc	18.93	f	43.36	bcd	2.61	ab	85.38	abc
Evasni	18.27	ab	15.12	def	43.09	bcd	2.71	ab	79.18	ab
Florina	34.40	de	10.91	a-e	42.90	bcd	5.97	bcd	94.18	a-e
Golden Paradise	27.21	bcd	13.32	c-f	46.24	bcd	2.10	a	88.88	a-e
Granny Smith	23.46	bc	12.71	b-e	41.85	abc	1.87	a	79.89	ab
Morro de Liebre	11.47	a	16.34	ef	40.00	abc	3.32	ab	71.13	a
Red Delicious	23.93	bc	14.74	def	44.63	bcd	4.11	ab	87.40	abc
Red Elstar	35.81	def	7.55	abc	42.87	bcd	3.86	ab	90.09	a-e
Reineta Blanca del Canada	44.47	f	9.69	a-d	50.60	cd	8.04	cde	112.80	e
Reineta Gris	39.88	ef	10.38	a-e	50.33	cd	8.91	de	109.51	cde
Reneta	34.24	de	6.72	ab	50.32	cd	3.66	ab	94.94	a-e
Solafuente	28.46	cd	11.43	a-e	36.35	ab	2.49	ab	78.72	a
Sig ANOVA	***		***		***		***		***	

Means with the same letter are not significantly different from each other (Significance of ANOVA at *: P≤0.05; ***: P≤0.001 followed by Tukey tests for each trait). Abbreviations: FW, fresh weight; SSC, soluble solids content; TA, titratable acidity; RI, ripening index; TPC, total phenolics content; TFC, total flavonoids content; AsA, Ascorbic acid; RAC, relative antioxidant content.

Table S1. Continued

Accession	Citric acid		Tartaric acid		Malic acid		Succinic and Shikimic acids		Total Acids	
Units	g/kg FW		g/kg FW		g/kg FW		g/kg FW		g/kg FW	
Averdal	0.024	ab	0.043	abc	3.956	ab	0.680	i	4.703	abc
Baujade	0.048	b-e	0.033	a	5.836	de	0.233	ab	6.149	cd
Bossost	0.107	g	0.052	c	10.131	h	0.387	c-f	10.677	g
Cripps Pink	0.054	c-f	0.038	abc	5.864	e	0.232	ab	6.187	cd
Cul de Cirio	0.018	a	0.034	abc	2.781	a	0.515	efg	3.348	a
Delcon	0.047	bcd	0.045	abc	5.850	e	0.357	bcd	6.299	cd
Evasni	0.026	ab	0.048	abc	3.898	a	0.857	j	4.830	abc
Florina	0.056	c-f	0.040	abc	5.531	b-e	0.319	abc	5.945	bcd
Golden Paradise	0.040	abc	0.039	abc	4.256	a-d	0.533	gh	4.868	abc
Granny Smith	0.047	b-e	0.045	abc	6.428	ef	0.217	a	6.738	de
Morro de Liebre	0.024	ab	0.037	abc	4.114	abc	0.520	fg	4.695	abc
Red Delicious	0.027	ab	0.042	abc	3.635	a	0.661	hi	4.365	ab
Red Elstar	0.073	ef	0.044	abc	6.913	ef	0.429	c-g	7.460	de
Reineta Blanca del Canada	0.071	def	0.042	abc	7.765	fg	0.482	d-g	8.360	ef
Reineta Gris	0.076	f	0.051	bc	9.013	gh	0.563	ghi	9.702	fg
Reneta	0.039	abc	0.033	ab	3.960	ab	0.382	cde	4.414	ab
Solafuente	0.060	c-f	0.037	abc	5.672	cde	0.510	efg	6.281	cd
Sig ANOVA	***		***		***		***		***	

Means with the same letter are not significantly different from each other (Significance of ANOVA at *: P≤0.05; ***: P≤0.001 followed by Tukey tests for each trait). Abbreviations: FW, fresh weight; SSC, soluble solids content; TA, titratable acidity; RI, ripening index; TPC, total phenolics content; TFC, total flavonoids content; AsA, Ascorbic acid; RAC, relative antioxidant content.

Table S2. Means for the 17 accessions assessed and for the 17 traits evaluated at Postharvest period.

Accession	SSC		TA		RI		TPC		TFC		AsA		RAC	
Units	ºBrix		g malic acid/ L		-		mg GAE/100 g FW		mg CE/100 g FW		mg AsA/100 g FW		mg Trolox/100 g FW	
Averdal	17.50	cde	3.60	ab	4.87	de	16.35	abc	9.57	a	0.98	ab	10.62	a-d
Baujade	13.00	abc	8.33	e	1.57	a	22.14	a-d	15.55	abc	2.47	d	11.18	a-e
Bossost	19.90	def	8.97	ef	2.22	a-d	23.39	bcd	15.63	abc	1.36	abc	12.44	a-e
Cripps Pink	12.77	abc	6.30	cd	2.02	ab	17.11	a-d	12.72	ab	1.27	abc	11.26	a-e
Cul de Cirio	16.30	b-e	2.22	a	8.06	f	33.67	def	29.21	bcd	2.05	cd	18.64	cde
Delcon	16.47	b-e	4.82	bc	3.41	a-d	10.95	ab	5.83	a	0.78	ab	8.41	ab
Evasni	20.60	ef	3.36	ab	6.14	ef	22.71	a-d	15.35	abc	1.33	abc	14.22	b-e
Florina	9.83	a	2.34	a	4.25	b-e	21.84	a-d	16.40	abc	1.38	abc	17.27	cde
Golden Paradise	15.50	bcd	3.68	ab	4.21	a-e	15.45	abc	10.15	ab	0.74	ab	12.56	a-e
Granny Smith	14.93	bc	6.57	d	2.27	a-d	20.52	a-d	18.88	abc	1.48	bc	11.23	a-e
Morro de Liebre	13.50	abc	3.74	ab	3.61	a-e	29.38	cde	16.53	abc	1.07	ab	18.89	de
Red Delicious	14.90	bc	3.62	ab	4.14	a-e	15.23	abc	7.26	a	0.85	ab	10.65	a-d
Red Elstar	12.43	ab	2.65	a	4.71	cde	6.14	a	7.84	a	0.53	a	5.57	a
Reineta Blanca del Canada	21.07	ef	10.07	f	2.12	abc	42.12	ef	34.19	cd	2.65	de	16.35	b-e
Reineta Gris	24.23	f	7.92	de	3.09	a-d	49.84	f	39.01	d	3.54	e	18.85	de
Reneta	12.63	ab	3.14	a	4.05	a-e	25.20	bcd	20.29	a-d	1.32	abc	19.46	e
Solafuente	13.80	abc	6.68	d	2.07	abc	18.38	a-d	12.39	ab	0.99	ab	10.12	abc
Sig ANOVA	***		***		***		***		***		***		***	

Means with the same letter are not significantly different from each other (Significance of ANOVA at ***: $P \leq 0.001$ followed by Tukey tests for each trait). Abbreviations: FW, fresh weight; SSC, soluble solids content; TA, titratable acidity; RI, ripening index; TPC, total phenolics content; TFC, total flavonoids content; AsA, Ascorbic acid; RAC, relative antioxidant content.

Table S2. Continued

Accession	Sucrose		Glucose		Fructose		Sorbitol		Total Sugars	
Units	g/kg FW		g/kg FW		g/kg FW		g/kg FW		g/kg FW	
Averdal	16.57	abc	23.53	g	53.22	efg	6.67	bc	99.99	de
Baujade	27.77	cde	13.18	c	26.04	a	2.04	a	69.03	ab
Bossost	38.61	e	11.96	bc	47.79	c-f	10.38	cd	108.74	ef
Cripps Pink	38.01	e	5.15	a	44.10	b-e	4.08	ab	91.34	cde
Cul de Cirio	13.01	ab	18.99	d-g	51.69	def	5.76	ab	89.46	b-e
Delcon	11.53	ab	19.81	efg	43.91	b-e	3.22	ab	78.46	a-d
Evasni	20.94	bc	21.46	fg	55.16	fgh	7.74	bc	105.30	e
Florina	13.06	ab	11.81	bc	36.83	b	2.07	a	63.77	a
Golden Paradise	27.27	cde	13.99	cd	44.37	b-e	3.91	ab	89.54	b-e
Granny Smith	13.50	ab	16.18	cde	39.90	bc	2.04	a	71.62	abc
Morro de Liebre	5.97	a	16.52	c-f	45.16	b-f	5.57	ab	73.22	abc
Red Delicious	16.04	abc	20.21	efg	48.30	c-f	3.73	ab	88.28	b-e
Red Elstar	12.63	ab	14.23	cd	41.98	bcd	3.61	ab	72.46	abc
Reineta Blanca del Canada	35.52	e	16.85	c-f	63.16	gh	13.53	d	129.06	fg
Reineta Gris	33.57	de	20.14	efg	65.20	h	19.80	e	138.72	g
Reneta	19.59	bc	6.97	ab	48.21	c-f	4.12	ab	78.90	a-d
Solafuente	22.88	bcd	15.76	cde	36.96	b	2.09	a	77.69	abc
Sig ANOVA	***		***		***		***		***	

Means with the same letter are not significantly different from each other (Significance of ANOVA at ***: P≤0.001 followed by Tukey tests for each trait). Abbreviations: FW, fresh weight; SSC, soluble solids content; TA, titratable acidity; RI, ripening index; TPC, total phenolics content; TFC, total flavonoids content; AsA, Ascorbic acid; RAC, relative antioxidant content.

Table S2. Continued

Accession	Citric acid		Tartaric acid		Malic acid		Succinic and Shikimic acids		Total Acids	
Units	g/kg FW		g/kg FW		g/kg FW		g/kg FW		g/kg FW	
Averdal	0.013	a	0.044	bcd	1.953	a	0.536	fg	2.547	ab
Baujade	0.073	bc	0.037	abc	4.267	bc	0.219	ab	4.596	cd
Bossost	0.018	a	0.035	abc	5.493	cd	0.337	a-e	5.884	d
Cripps Pink	0.039	ab	0.037	abc	4.809	bc	0.175	a	5.059	cd
Cul de Cirio	0.010	a	0.023	ab	1.178	a	0.695	gh	1.908	a
Delcon	0.031	ab	0.047	bcd	1.411	a	0.304	abc	1.793	a
Evasni	0.027	a	0.034	abc	1.787	a	0.534	fg	2.380	ab
Florina	0.047	ab	0.024	ab	0.957	a	0.490	ef	1.519	a
Golden Paradise	0.041	ab	0.017	a	1.520	a	0.475	c-f	2.054	a
Granny Smith	0.014	a	0.026	ab	3.770	b	0.196	a	4.006	bc
Morro de Liebre	0.009	a	0.024	ab	1.479	a	0.452	c-f	1.964	a
Red Delicious	0.017	a	0.030	ab	1.782	a	0.811	h	2.640	ab
Red Elstar	0.017	a	0.048	bcd	1.370	a	0.313	a-d	1.749	a
Reineta Blanca del Canada	0.243	d	0.066	d	7.042	d	0.407	c-f	7.759	e
Reineta Gris	0.011	a	0.035	abc	5.207	bc	0.427	c-f	5.680	d
Reneta	0.022	a	0.043	a-d	1.491	a	0.380	b-f	1.936	a
Solafuente	0.102	c	0.060	cd	3.733	b	0.486	def	4.380	cd
Sig ANOVA	***		***		***		***		***	

Means with the same letter are not significantly different from each other (Significance of ANOVA at ***: P≤0.001 followed by Tukey tests for each trait). Abbreviations: FW, fresh weight; SSC, soluble solids content; TA, titratable acidity; RI, ripening index; TPC, total phenolics content; TFC, total flavonoids content; AsA, Ascorbic acid; RAC, relative antioxidant content.