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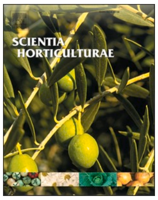

Research Paper

From valley to mountain: Organoleptic and nutritional properties of apple accessions from Northeast Spain

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ABSTRACT

Local apple accessions are vital for their adaptability to specific climates and unique organoleptic characteristics, playing a key role in biodiversity conservation and agricultural resilience. This study examined 36 apple genotypes from the CITA collection, including 25 diploid and 11 triploid varieties distributed across five altitudes in northeastern (NE) Spain. The collection encompassed both autochthonous landraces and non-Spanish commercial cultivars. The analysis focused on physicochemical attributes such as fruit firmness, total phenolic content (TPC), and individual sugars. Among these six genotypes (five autochthonous landraces and one commercial cultivar used as reference [‘Gala’]), were present at two different altitudes within the CITA collection and were further evaluated to assess the influence of altitude elevation on these traits. Significant variability in fruit quality was observed, with notable correlations between physicochemical traits. Principal component analysis (PCA) explained 50.2% of the total variance, highlighting genetic variability among the accessions and its impact on fruit traits. The results showed that ploidy (diploid/triploid) and origin (autochthonous/commercial - non-Spanish) are key factors shaping apple characteristics. Higher altitudes were associated with increased TPC in specific accessions, likely due to cooler temperatures and greater UV and sun exposure enhancing phenolic biosynthesis. Fructose content also increased with altitude, reflecting slower fruit development and ripening, while variations in glucose and sucrose indicated the complexity of metabolic responses to both altitude and genetic factors. These findings demonstrate the high biodiversity of apples from NE Spain, particularly in terms of their organoleptic and nutritional qualities. Preserving autochthonous apple resources is essential, and breeding programs could benefit from selecting accessions with desirable biochemical traits for local climatic conditions. Additional research is needed to explore how environmental factors influence the health benefits of apples, especially their bioactive compounds, as well as their agronomic performance.

1. Introduction

Apple (*Malus* x *domestica* Borkh, family *Rosaceae*) is the world's leading production temperate fruit crop. In fact, global production exceeded 95.8 M tons in 2022, with Spain contributing 496 thousand tons (FAOSTAT, 2024). Although over 10,000 apple cultivars are currently documented (Pereira-Lorenzo et al., 2017), apple production is dominated by a few widely known and consumers-accepted cultivars such as ‘Delicious’, ‘Fuji’, ‘Gala’, ‘Golden’, and ‘Granny Smith’. This trend toward commercial cultivars monoculture is negatively impacting the farming of local autochthonous apple accessions, which are generally better adapted to local climatic growing conditions (Mignard et al., 2021a). As a result, this situation is leading to a dramatic loss of phytogenetic diversity and may, additionally, slow down breeding programs (Swarup et al., 2021).

Apple fruit is easily accessible and widely consumed worldwide, with year-round availability in the market thanks to their shelf-life extension applying controlled atmospheres, among other postharvest technologies. It serves as an important dietary source of nutrients such as carbohydrates, organic compounds, proteins, and vitamins (Mignard et al., 2023a). In fact, apple is one of the most widely recognized fruits, offering several well-documented benefits for human health (Calvo-Castro

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et al., 2022; Gibney et al., 2019; Ho et al., 2020; Millán-Laleona et al., 2023). Apple is also a significant source of non-nutritive bioactive molecules for humans (Asma et al., 2023). Among the bioactive compounds naturally present in vegetables and fruits, the polyphenols such as phenols, phenolic acids, flavonoids, anthocyanins, and tannins stand out (Ho et al., 2020; Lattanzio, 2013; Michalska and Łysiak, 2015). In addition to their antioxidant and anti-inflammatory properties, polyphenol compounds are also important for their role in the sensory quality of apple, as they influence fruit color, basic fruit quality indicators and maturity (Butkeviciute et al., 2022).

In recent years, climate change has been reducing apple production in low-altitude regions and is expected to have an even greater impact in the future due to the decreasing number of chilling hours in traditional apple-producing areas of Spain, such as the Ebro Valley (Rodríguez et al., 2021). However, these climatic shifts have also created new opportunities for apple cultivation at higher altitudes, where conditions, such as sufficient chilling hours, increased total annual rainfall, and more suitable mean surface temperatures during the growing season, are becoming increasingly favorable for apple growth. Hence, in different areas of the world such as Italy, France, Spain, New Zealand and India, optimum apple growing conditions have been shifting and farmers have been relocating their orchards to higher elevations (Cantín and Gracia, 2022; Iglesias et al., 2016; Sahu et al., 2020). It is important to highlight the significance of South Tyrol (Italy) as a key mountainous apple-producing region in Europe. With approximately 19,000 hectares under cultivation, it is the largest high-altitude apple-growing area in Europe and accounts for around 50 % of Italy’s total apple production (Meyer, 2014). In the context of this study, it is particularly relevant to emphasize the growing development of high-altitude apple cultivation in Spain, where changing climatic conditions are encouraging the expansion of orchards into mountainous areas (Iglesias et al., 2018) that were previously less suitable for this crop. On one hand, orchard management and environmental factors such as altitude, have been demonstrated to have a clear impact on the concentration of different nutrients and biochemical compounds of apple fruits, including antioxidants, organic acids or carbohydrates (Charles et al., 2018; Li et al., 2021a, 2021b; Stewart and Ahmed, 2020). On the other hand, the effect of growing altitude on several apple sensory attributes such as juiciness, flavour, sweetness, sourness or skin ground- and over-color has been also demonstrated (Cantín and Gracia, 2022; Charles et al., 2018; Fadanelli et al., 2005; Gracia and Cantín, 2022). Scientific models forecast a high risk that agro-ecosystems will be critically endangered in the foreseeable future, and adaptation strategies must be implemented promptly. In this context, there is a need to enhance our understanding of how the growing region can affect fruit quality and metabolite profiles (Boudichevskaia et al., 2020; Parajuli et al., 2019). Germplasm banks, therefore, provide an appropriate platform to address this concern (Mignard et al., 2023a; Swarup et al., 2021).

Mountainous areas such as the Pyrenees and the Iberian System in northeastern Spain, are considered remarkable biodiversity areas (Amblar-Francés et al., 2020; Pironon et al., 2022) with soil and climate variability that can influence the persistence of certain materials with specific adaptation characteristics (Pironon et al., 2022). The Central Ebro Basin growing area features an arid or semi-arid climate with warm and dry summers, high radiation, and significant day-night temperature variations (Mignard et al., 2021a). In contrast, the Central Pyrenees exhibit diverse precipitation and temperature patterns due to their varied topography (500–3404 m - Aneto), influenced by both Atlantic and Mediterranean climates, two distinct continental climate patterns. Annual rainfall in the mountains often exceeds 2000 mm, mostly in spring and autumn (Amblar-Francés et al., 2020).

In this scenario of climatic diversity, generations of farmers developed agriculture practices adapted to the socio-environmental conditions over decades, giving rise to a wide diversity of high-quality fruit plant material that constitutes an extraordinary genetic heritage. However, the severe population decline that occurred from the second half of the last century, especially in mountainous areas, led to the abandonment of most of the traditional growing areas (García-Ruiz et al., 1996). In response, survey missions were undertaken to avoid the loss of this diversity. During these missions, local apple tree accessions from these mountain areas were collected and recovered, their identity and genetic variability were analyzed (Pina et al., 2014), and they were established in the germplasm collection of the Center for Agri-Food Research and Technology of Aragon (Centro de Investigación y Tecnología Agroalimentaria de Aragón - CITA) for the study and valorization of this genetic diversity. In the context of global warming and climate change, understanding, characterizing, and preserving phytogenetic diversity in these mountainous areas has become increasingly urgent (Parry, 2019), making these germplasm banks central to the development of new strategies for agronomical activities (Mignard et al., 2021b; Swarup et al., 2021).

In this regard, the goal of this study is to better characterize Spanish mountainous autochthonous apple accessions in terms of organoleptic and nutritional qualities. To this end, biomolecular characterization was performed on 36 apple genotypes (set of 27 accessions and 9 commercial cultivars), to explore the relationships and correlations between these parameters. Finally, the influence of the growing altitude on apple fruit quality, including its impact on the fruit metabolite profile, was examined for five autochthonous apple accessions and compared with one reference and commercial cultivar, ‘Gala’.

2. Material and methods

2.1. Plant material

A total of 36 apple genotypes [*Malus* x *domestica* Borkh], including 25 diploid and 11 triploid accessions, were studied. These comprised 27 mountainous autochthonous landraces and nine non-Spanish commercial cultivars (Table 1). The plant material analyzed in this study belongs to the apple collection of the CITA which is distributed across five different growing sites at varying altitudes in northeastern Spain: Montañana (202 m); Ligüerre (483 m); Aínsa (569 m); Bescós de Garcipollera (929 m); and Albarracín (1182 m). These sites were classified as ‘Low’, ‘Medium’, ‘High’ or ‘Very High’ according to their altitude. Six of the previous mentioned 36 genotypes (‘Allueva’, ‘Esperiega de Ademuz’, ‘Gala’, ‘Pomera del País’, ‘Torres Albarra_01′, and ‘Vadiello_03’) were present at two different growing sites, and therefore at two different elevations, within the CITA collections (Fig. 1; Table 1).

For all the growing sites included in the CITA collection, apple orchards were established with a planting spacing of 4 m x 5 m. For all the accessions, trees were grafted onto MM-106 rootstock to enhance vigor (semi-dwarfing rootstock) and disease resistance. All trees included in the present study were planted between 5 and 10 years prior to sampling. The training system employed was the open vase method, promoting optimal light penetration and air circulation. No support structure was used for the trees throughout the study period. Irrigation was supplied via a drip irrigation system, ensuring efficient water delivery directly to the root zone. The soils at the experimental areas are characterized as slightly alkaline, non-calcareous sandy loam, with a pH from 7.2 to 8.1, organic matter content from 6.9 % to 7.9 %, and nutrient levels adequate and in the ranges published before for apple cultivation, as confirmed by prior soil analysis. Orchards management practices, including fertilization, irrigation and winter pruning, were carried out as in a commercial plantation.

The mountainous autochthonous landraces were collected during various prospecting missions to mountainous areas of Aragón since 2004. They were rescued from old orchards and abandoned lands in the Pyrenees and the Iberian System from NE Spain. After being confirmed as unique genotypes using SSR markers (Pereira-Lorenzo et al., 2017; Pina et al., 2014), they were vegetatively propagated for their incorporation in the CITA collection.

Data on maximum and minimum temperatures (°C), solar radiation

Table 1
Descriptive information of the 36 apple genotypes (27 accessions and nine commercial cultivars) assessed in this study.

N°	Accession Name	Origin	Growing Site	Growing Altitude	Harvest Date[a]	Ploidy[b]
1	Agorreta_03	Autochthone/Aragón	Garcipollera	High	290	3
2	Ainsa_09	Autochthone/Aragón	Garcipollera	High	269	2
3	Allueva [*]	Autochthone/Aragón	CITA/Albarracín	Low/Very High	295/299	2
4	Badami Golden	Commercial	Albarracín	Very High	276	2
5	Biescas_03	Autochthone/Aragón	Garcipollera	High	290	3
6	Biescas_10	Autochthone/Aragón	Garcipollera	High	297	2
7	Camuesa Castellano	Autochthone/Aragón	Ligüerre	Medium	297	2
8	Esperiega de Ademuz [*]	Autochthone/Aragón	CITA/Ligüerre	Low/Medium	298/258	2
9	Fuji	Commercial	CITA	Low	260	2
10	Gala [*]	Commercial	CITA/Albarracín	Low/ Very High	248/276	2
11	Gala Must	Commercial	CITA	Low	233	2
12	Golden Smoothee	Commercial	Albarracín	Very High	276	2
13	Granny Smith	Commercial	CITA	Low	277	2
14	Isarre_01	Autochthone/Aragón	Garcipollera	High	269	2
15	Javierre del Obispo_01	Autochthone/Aragón	Ligüerre	Medium	269	3
16	Moscardón_01	Autochthone/Aragón	Albarracín	Very High	299	3
17	Moscardón_03	Autochthone/Aragón	Albarracín	Very High	299	3
18	Normando	Autochthone/Aragón	CITA	Low	283	2
19	Pinova	Commercial	Albarracín	Very High	289	2
20	Pomera de la Gaya	Autochthone/Aragón	Garcipollera	High	290	2
21	Pomera del Pais [*]	Autochthone/Aragón	Ligüerre/Garcipollera	Medium/High	270/290	2
22	Reineta	Commercial	CITA	Low	247	3
23	Reineta de Calomarde	Autochthone/Aragón	CITA	Low	233	3
24	Reineto Calomarde	Autochthone/Aragón	Albarracín	Very High	299	3
25	Rojo	Autochthone/Aragón	Aínsa	Medium	261	2
26	Royal Gala	Commercial	CITA	Low	233	2
27	Royuela_01	Autochthone/Aragón	CITA	Low	233	3
28	San Juan de Toledo	Autochthone/Aragón	Aínsa	Medium	297	2
29	Santa Eulalia_01	Autochthone/Aragón	CITA	Low	263	2
30	Torres Albarra_01 [*]	Autochthone/Aragón	Ligüerre/Garcipollera	Medium/High	276/290	2
31	Torres Albarra_02	Autochthone/Aragón	Garcipollera	High	290	2
32	Tramacastilla_03	Autochthone/Aragón	Ligüerre	Medium	297	2
33	Vadiello_03 [*]	Autochthone/Aragón	CITA/Garcipollera	Low/High	283/283	2
34	Verde Doncella Troteras	Autochthone/Aragón	Aínsa	Medium	279	2
35	Yosa de Sobremonte_02	Autochthone/Aragón	Garcipollera	High	269	3
36	Yosa de Sobremonte_04	Autochthone/Aragón	Ligüerre	Medium	276	3

[*] Accessions assessed for the altitude study. Abbreviations: CITA, Centro de Investigación y Tecnología Agroalimentaria de Aragón.
[a] Harvest date was expressed in Julian Days;.
[b] Level of ploidy: 2, diploid; 3, triploid. Growing altitude: Low: < 300 m; Medium: 300–600 m; High: 600–1000 m; Very High: >1000 m.

(MJ/m^2), and precipitation (mm) from three meteorological stations ('Santa Cilia de Jaca', 'Montañana', and 'Teruel') near the study areas are provided in Supplementary File 1. All the meteorological data were obtained from the database provided by the Ministry of Agriculture, Fisheries, and Food using the weather station network of the Agroclimatic Information System for Irrigation (Sistema de Información Agroclimática para el Regadío - SiAR).

2.2. *Fruit sampling*

In 2018, thirty fruits were selected and harvested from each genotype at each growing site for further analyses. Fruits were hand-picked at commercial maturity, which was determined through sensory evaluation (color, firmness, aroma, and taste) and confirmed by the starch-iodine test using the CTIFL scale determination (Planton, 1995), with a minimum maturity index of 6. The harvest date was recorded for each genotype, ranging from August to late October 2018, depending on the accession and the growing site. After harvest, fruits were immediately stored at the recommended temperature for apples (1 °C to 2 °C) (Porritt, 1964) and processed at CITA within 2 weeks.

2.3. *Fruit quality traits evaluation*

Harvest date and flesh firmness were recorded for each accession immediately after harvest according to UPOV (1974), IBPGR (Watkins and Smith, 1983) and Royo-Díaz et al. (2017) guidelines. Firmness was measured twice at the equatorial part of the fruit, on opposite cheeks, using a digital fruit firmness analyzer (TR Turoni, Forlí, Italy) with an 11 mm plunger tip and the results were expressed in Newtons (N). Soluble solids content (SSC), titratable acidity (TA) and pH were determined from apple juice. Ten pieces of fruit from each accession were liquefied and filtered through a paper funnel (filter paper circles, folded, ø 185 mm, Chmlab group, Barcelona, Spain). SSC was measured at 20 °C using a refractometer (Pocket Refractometer Pal-1, Atago, Tokyo, Japan) and expressed as °Brix. TA and pH were determined at 20 °C by titration with 0.1 N NaOH to a pH end point of 8.1 (760-Sample Change, Metrohm, Herisau, Switzerland). Results were expressed as g malic acid per liter. Ripening index (RI) was determined based on the SSC/TA ratio.

2.4. *Fruit total phenolics analyses*

Total phenolics were analyzed in both fruit flesh and peel. A fresh sample composite of 5 g of flesh or 2 g of peel per accession and replicate was prepared from five fruits. Three replicates per accession of each tissue (flesh and peel) were sampled. Two replicates were frozen in liquid nitrogen and kept at −20 °C until the extraction of the total phenolic content (TPC), while the third replicate was incubated at 65 °C for five days. The percentage of dry matter content (%) for each accession and tissue samples were calculated by gravimetrically weighing each sample before and after five days of stove drying at 65 °C and used for calculating the results. The results were thus expressed in mg per 100 g of fresh weight (FW) and dry weight (DW).

For the total phenolics extraction, both flesh and peel samples (two repetitions per accession for each tissue) were homogenized in a T25D Ultra-Turrax (IKA-Werke GmbH, Staufen, Germany) with 10 mL of extraction solution [methanol/Milli-Q water, 80 % (v/v) + sodium

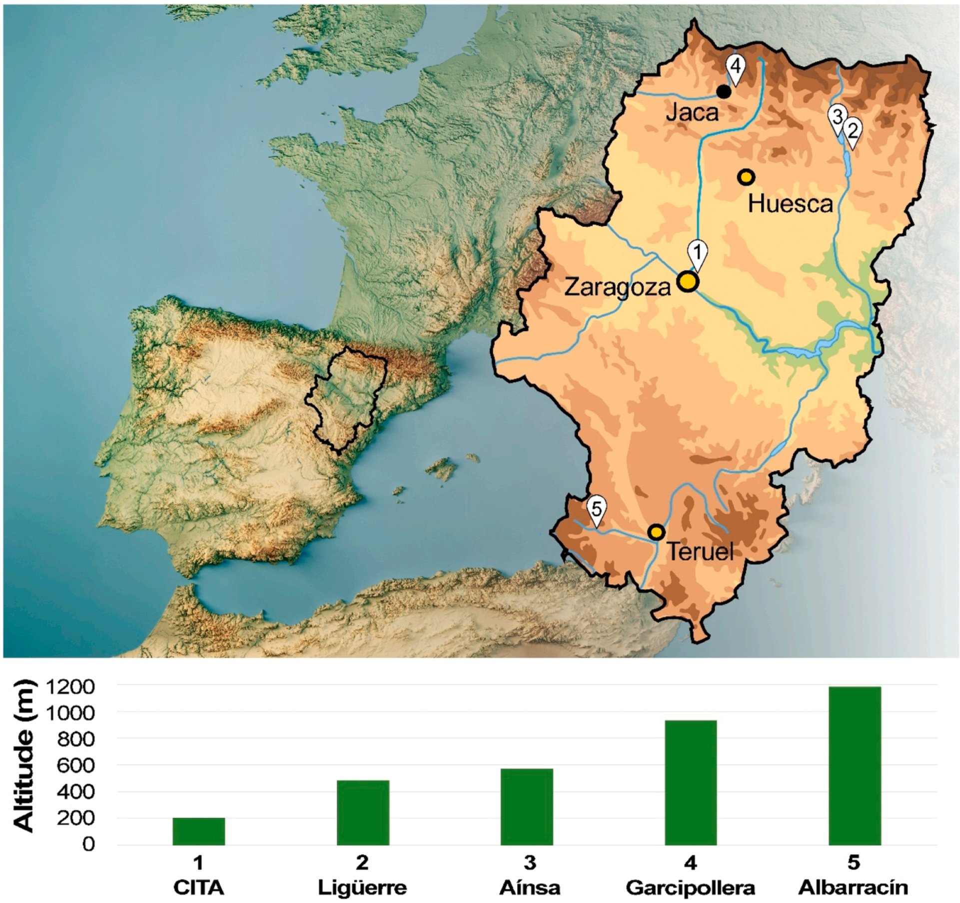

Fig. 1. Altitude (in m above sea level) of the seven growing sites assessed in this study and classification used accordingly. 1: Montañana, CITA (202 m); 2: Ligüerre (483 m); 3: Aínsa (569 m); 4: Bescós de Garcipollera (929 m); and 5: Albarracín (1182 m).

fluoride (NaF) at 2 mM]. Samples were centrifuged at 10,000 × *g* for 10 min at 4 °C, and the supernatant was collected and stored in darkness at −80 °C, while a second extraction was performed using the precipitate from the first extraction. Both supernatants were combined and stored until further analyses at −80 °C.

TPC was assessed using the Folin-Ciocalteau method (Singleton and Rossi, 1965) with modifications. Standard calibration curves were daily prepared and eight different concentrations were used. A volume of 0.5 mL of the extraction sample was mixed with 0.5 mL of Folin-Ciocalteau reagent (0.25 N). The sample was incubated for five minutes at room temperature before adding 0.5 mL of sodium carbonate [NaCO3, 7.5 % (w/v)] and 7 mL of Milli-Q water. The samples were then stored in the dark for one hour at room temperature. Absorbance was measured at 760 nm, and the results were expressed in milligrams of gallic acid equivalents (GAE) per 100 *g* of fresh weight (FW) and per 100 *g* of dry weight (DW).

2.5. *Fruit sugars analysis*

Individual sugars were analyzed by UPLC as reported by Castel et al. (2020). Briefly, five grams of fresh apple flesh were crushed and homogenized with 20 mL of Milli-Q water using a T25D Ultra-Turrax (IKA-Werke GmbH, Staufen, Germany) at room temperature. Then, samples were centrifuged at 10,000 × *g* for 10 min at 4 °C, and the supernatant was collected. A second extraction was performed with the precipitate of the first extraction adding 10 mL of Milli-Q water. Finally, the supernatants resulting from both extractions were filtered through a 0.20 μm polyester (PET) syringe filter and stored until further analyses at −80 °C.

Sugars were assessed using a Waters SUGAR-PAK I column (300 mm × 6.0 mm, Waters Chromatography, Cerdanyola del Vallés, Spain) with a mobile phase of 50 mg/L Na_2Ca EDTA at 90 °C and a flow rate of 0.44 mL/min. Individual sugars (glucose, fructose, sucrose, and the sugar-alcohol sorbitol) were identified by their characteristic retention times, using the appropriate standards. Total sugars were calculated as the sum of the four individual sugars identified by UPLC. Concentrations were expressed as grams per kilogram of fresh weight (FW).

2.6. *Chemicals*

All chemicals were of analytical grade. The sugar standards (sucrose, glucose, fructose, and sorbitol), Folin-Ciocalteau reagent, and sodium hydroxide (NaOH) were obtained from PanReac Química SA (Barcelona, Spain). Methanol (CH_3OH) was purchased from Chem-Lab Analytical (Zedelgem, Belgium), while sodium carbonate (Na_2CO_3), sodium fluoride (NaF), and Na_2Ca-EDTA were sourced from Sigma-Aldrich (Saint Louis, MO, USA).

2.7. *Data analysis*

Statistical analyses of the collected data for all accessions and commercial cultivars were carried out using R language (R Core Team, 2019) and SPSS Statistics software version 21.0 (IBM, New York, U.S.A.). The R packages used for statistical analysis included ggplot2; factoextra; FactoMineR; ggpubr; dplyr; Hmisc; and corrplot. The normality of the data for each trait was assessed using the Shapiro-Wilk test, which showed that glucose, total sugars, TA, and RI did not follow a normal distribution. Therefore, a logarithmic transformation was applied to these four parameters prior to the one-way analysis of variance analysis (ANOVA) (Supplementary File 2).

A one-way ANOVA was conducted to determine whether there were any statistically significant differences between the means of the evaluated traits. Additionally, the effects of accession, ploidy (diploid / triploid), and origin of the accession (local - autochthonous / non-Spanish - commercial) were assessed. Pearson's correlations and a principal component analysis (PCA) were then performed to better understand how the studied traits contribute to the overall variability among the 36 sample genotypes. t-tests were also conducted to investigate the effect of altitude on fruit biochemical traits in five different accessions ('Allueva', 'Esperiega de Ademuz', 'Pomera del Pais', 'Torres Albarra_01′, and 'Vadiello_03′) and one reference cultivar ('Gala'), in order to determine if altitude had a significant impact on the fruit traits assessed.

3. Results

3.1. Fruit quality traits

All the fruit quality attributes analyzed in this study exhibited considerable variability among the 36 genotypes assessed (Table 2; Supplementary File 3) both within and between each growing site. The pH of the fruit varied from 3.09 (in 'Royal Gala' grown at CITA) to 5.94 (in 'San Juan de Toledo' at Aínsa), while the titratable acidity (TA) ranged from 1.42 g malic acid/L (in 'Verde Doncella Troteras' at Aínsa) to 11.62 g malic acid/L (in 'Agorreta_03′ at Bescós de Garcipollera). Flesh firmness at ripening varied from 39.69 N (in 'Gala' at CITA) to 118.97 N (in 'Reineta de Calomarde' at CITA). Similarly, the soluble solids content (SSC) ranged from 11.10°Brix (in 'Royuela_01′ at CITA) to 18.40°Brix (in 'Allueva' at Albarracín), while ripening index (RI=SSC/TA) ranged from 1.43 (in 'Royuela_01′at CITA) to 9.23 (in 'Verde Doncella Troteras' at Aínsa).

3.2. Fruit total phenolic content

The total phenolic content (TPC) in the peel varied greatly among apple accessions (Table 2; Supplementary File 3), ranging from 52.04 mg GAE/100 g FW ('Torres Albarra_02′ grown at Bescós de Garcipollera) to 289.15 mg GAE/100 g FW ('Biescas_03′ at Bescós de Garcipollera), and from 153.04 mg GAE/100 g DW ('Torres Albarra_02′ at Bescós de Garcipollera) to 963.26 mg GAE/100 g DW ('Moscardon_01′ at Albarracín) (Table 2). Similarly, the TPC in the fruit flesh ranged from 6.31 mg GAE/100 g FW (in 'Gala Must' at CITA) to 114.62 mg GAE/100 g FW (in 'Torres Albarra_01′ at Bescós de Garcipollera), and from 45.06 mg GAE/100 g DW (in 'Gala Must' at CITA) to 628.62 mg GAE/100 g DW (in 'Moscardon_03′ at Albarracín). The average ratio between Peel TPC and Flesh TPC when expressed in FW basis was around 4, while the average ratio between Peel TPC and Flesh TPC was around 2 in DW basis since the peel dry matter content (%) almost doubles flesh dry matter content (%).

3.3. Fruit sugars composition

Regarding the individual sugars (Table 2; Supplementary File 3), fructose was the most abundant individual sugar in the fruit in g/kg FW (70.30 ± 15.62), followed by sucrose (52.25 ± 15.71), glucose (20.11 ± 9.48) and finally sorbitol (11.09 ± 5.71). The sucrose content varied considerably among different accessions and cultivars, ranging from 24.73 g/kg FW (in 'Torres Albarra_01′ grown at Bescós de Garcipollera) to 78.09 g/kg FW (in 'Badami Golden' at Albarracín) (Table 2). Glucose levels ranged from 5.54 g/kg FW ('San Juan de Toledo' at Aínsa) to 44.15 g/kg FW (in 'Torres Albarra_01′ at Bescós de Garcipollera), while fructose levels ranged from 42.91 g/kg FW ('Moscardón_01′ at Albarracín) to 101.60 g/kg FW ('Badami Golden' at Albarracín). Finally, the sugar-alcohol sorbitol varied from 2.22 g/kg FW (in 'Gala Must' at CITA) to 27.29 g/kg FW (in 'Esperiega de Ademuz' at Ligüerre). Total sugars values, which corresponded to the sum of the four individual sugars assessed (sucrose, glucose, fructose and sorbitol) varied significantly among samples, ranging from 111.57 g/kg FW (in 'Royuela_01′ at CITA) to 217.09 g/kg FW (in 'Golden Smoothee' at Albarracín).

3.4. Factors influencing apple fruit quality

The ANOVA analysis revealed significant differences ($P \leq 0.001$) among all the apple accessions assessed in the present study for each trait evaluated (Table 2; Supplementary File 3). Additionally, both ploidy (diploid/triploid) and the origin of the accession (local autochthonous landraces/non-Spanish commercial and reference cultivars) had significant effects on some of the parameters assessed ($P \leq 0.5$; $P \leq 0.001$) (Table 2). Specifically, ploidy played a crucial role in pH, TA, RI, TPC in the flesh, and sucrose levels (Fig. 2). Sucrose, TPC and TA values were higher in triploid accessions such as 'Reineta', 'Biescas_03′, 'Moscardon_01′, 'Moscardon_03′, or 'Agorreta_03′. In contrast, triploid accessions exhibited lower pH and RI values. Regarding to the origin effect, Spanish autochthonous accessions, such as 'Moscardon_03′, 'Yosa de Sobremonte_04′, 'Allueva', 'Camuesa Castellano', and 'Torres Albarra_01′, among others, displayed higher TPC levels and reached

Table 2
Basic statistics for the fruit quality traits for the 36 genotypes assessed. Units, mean values, minimum, maximum, standard deviation (SD), and one-way ANOVA results for the sources of variation in each attribute are shown.

	Units	Mean	Minimum	Maximum	SD	Source of variation		
						Accession	Ploidy	Origin
Harvest Date	Julian days	276	233	299	20.03	*	ns	*
Firmness	Newtons	79.77	39.69	118.97	14.41	***	ns	ns
pH	-	4.37	3.09	5.94	0.67	***	*	ns
TA	g/L	4.96	1.42	11.62	2.50	***	***	ns
SSC	°Brix	15.03	11.10	18.40	1.58	***	ns	ns
RI	-	3.95	1.43	9.23	2.13	***	***	ns
Peel TPC/FW	mg GAE/100 g FW	157.42	52.04	289.15	62.47	***	ns	ns
Peel TPC/DW	mg GAE/100 g DW	533.12	153.04	963.26	218.98	***	ns	ns
Flesh TPC/FW	mg GAE/100 g FW	47.53	6.31	114.62	25.17	***	*	***
Flesh TPC/DW	mg GAE/100 g DW	284.51	45.06	628.62	146.53	***	***	***
Sucrose	g/kg	52.25	24.73	78.09	15.71	***	***	ns
Glucose	g/kg	20.11	5.54	44.15	9.48	***	ns	ns
Fructose	g/kg	70.30	42.91	101.60	15.62	***	ns	ns
Sorbitol	g/kg	11.09	2.22	27.29	5.71	***	ns	ns
Total Sugars	g/kg	153.75	111.57	217.09	28.22	***	ns	ns

Statistical significance at ***: $P \leq 0.001$; *: $P \leq 0.05$; ns: not significant. Abbreviations: TPC, total phenolics content; GAE, Gallic acid equivalent; FW, Fresh weight; DW, Dry weight; SSC, soluble solids content; TA, titratable acidity; RI, ripening index.

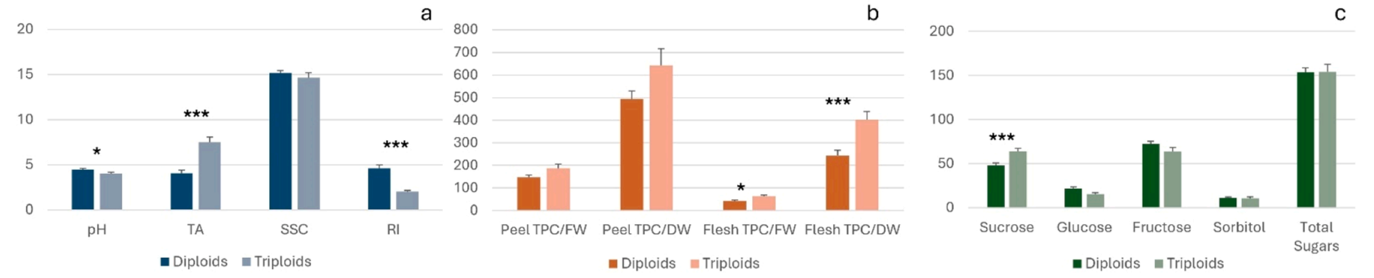

Fig. 2. Effect of apple ploidy on a) fruit basic quality traits; b) total phenolic content (TPC) of fruit flesh and peel; and c) individual and total sugar concentrations for the 36 genotypes assessed in this study. For each attribute mean ± standard error are shown. Statistical significance according to one-way ANOVA for ploidy effects at ***: $P \leq 0.001$ and *: $P \leq 0.05$. Abbreviations: TA, titratable acidity; TPC, total phenolic content; SSC, soluble solids content; RI, ripening index; FW, fresh weight; DW, dry weight. Units: TA, g/L; SSC,°Brix; TPC, mg GAE/100 g; Sugars, g/kg.

maturity (harvest date) later compared to non-Spanish commercial cultivars, such as ‘Gala Must’, ‘Gala’, ‘Royal Gala’, ‘Fuji’, and ‘Granny Smith’.

3.5. *Correlations between quality parameters and principal component analysis (PCA)*

Statistical correlation analyses were performed combining the results obtained from the fruit quality, total phenolic content, and individual sugar profiles. Significant correlations ($P \leq 0.01$ and $P \leq 0.05$) were found between different physicochemical traits assessed in this study (Fig. 3; Supplementary File 4). As expected, total sugars were positively correlated with individual sugars ($r = 0.536$ for sucrose, $r = 0.437$ for glucose, $r = 0.832$ for fructose and, $r = 0.465$ for sorbitol), with fructose showing the highest correlation coefficient, thus contributing most to the total sugars content. Similarly, glucose was positively correlated with fructose ($r = 0.518$) and negatively correlated with sucrose ($r =$ -0.412).

The flesh TPC (both in fresh and dry weight) was positively correlated with TPC in the fruit peel (FW: $r = 0.451$; DW: $r = 0.453$). As expected, TPC values for both peel and flesh, when expressed on a fresh weight basis, were highly correlated with their corresponding expressed on a dry weight basis ($r = 0.946$ and $r = 0.942$, respectively). Additionally, TA was negatively correlated with pH ($r =$ - 0.402) but positively associated with peel TPC (FW: $r = 0.410$; DW: $r = 0.448$). Furthermore, RI was negatively correlated with peel TPC ($r =$ - 0.392 in FW basis and $r =$ - 0.413 in DW basis).

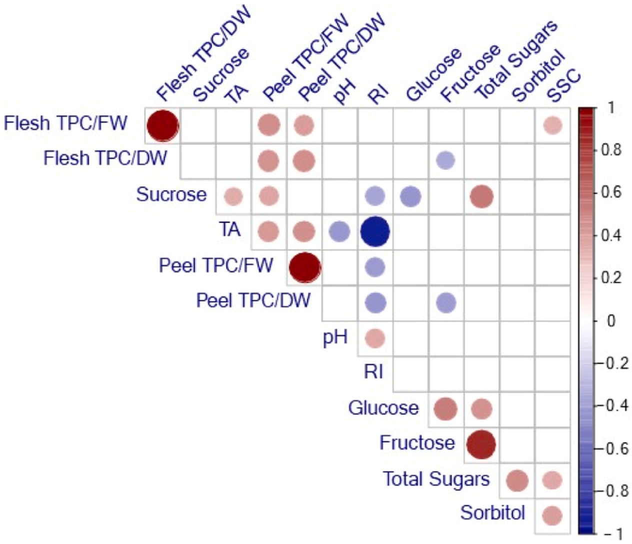

Fig. 3. Pearson’s correlation coefficients between fruit quality traits in the 36 apple genotypes assessed in this study. The size of the circle for each correlation depicts the magnitude of the Pearson’s correlation coefficient whereas the color depicts its direction (positive or negative). Only significant coefficients were drawn. Abbreviations: TPC, total phenolic content; FW, fresh weight; DW, dry weight; TA, titratable acidity; SSC, soluble solids content; RI, ripening index.

In addition, a principal component analysis (PCA) was performed to assess the distances between all studied accessions and cultivars based on the measured fruit attributes and to understand how these traits contribute to the separation of the apple accessions and cultivars (Fig. 4). The first two PCs, PC1 and PC2, accounted for 31.9 % and 18.3 % of the total variability, respectively. Together, the first two PCs, explained 50.2 % of the total variance of the dataset.

Interestingly, the loadings of PC1 indicated that the separation along this component was primarily influenced by biochemical traits, such as peel and flesh TPC and TA. Samples on the positive side of PC1, which mainly included commercial non-Spanish and diploid cultivars such as ‘Granny Smith’, ‘Gala’, and ‘Fuji’, generally exhibited lower TPC and TA values compared to autochthonous accessions. In contrast, accessions on the negative side of PC1, which predominantly consisted of local mountainous autochthonous landraces such as ‘Allueva’, ‘Yosa de Sobremonte_04′, ‘Moscardon_01′, and ‘Moscardon_03′, typically showed higher TPC values in both peel and flesh. The PCA also revealed that triploid accessions clustered on the negative side of the PC1, indicating a tendency toward higher TPC levels. Only two triploid accessions, ‘Javierre del Obispo_01′ and ‘Yosa de Sobremonte_02’ were plotted on the positive side of PC1.

Furthermore, PC2 was primarily explained by sugar-related parameters (SSC, sorbitol, glucose, and RI). Accordingly, accessions plotted on the positive side of PC2, exhibited higher SSC and glucose values, including ‘Torres Albarra_01′ and ‘Vadiello_03′. On the other hand, a group of non-Spanish commercial cultivars (‘Gala’, ‘Gala Must’, ‘Royal Gala’, ‘Granny Smith’, ‘Pinova’, ‘Fuji’, ‘Golden Smoothee’, and ‘Badami Golden’) with similar organoleptic fruit quality profiles clustered together in the PCA (Fig. 4). Notably, ‘Reineta’ was the only accession that separated from the rest of the non-Spanish commercial cultivars included in the study. Nevertheless, it is important to note that ‘Reineta’ is the only triploid non-Spanish accession in this research. The PCA results suggested that both origin and ploidy play a major role in the segregation of accessions based on their fruit quality profiles.

3.6. *Altitude effect on apple fruit quality*

The effect of altitude on physical and biochemical attributes of five apple accessions and one commercial cultivar included in the present study is summarized in Table 3 and Fig. 5. A significant altitude effect on fruit firmness was observed for the reference cultivar ‘Gala’ and the ‘Pomera del Pais’ accession, while no significant effect was found for the other four accessions. In both cases, fruit firmness was higher for fruit grown at higher altitudes. For the commercial cultivar ‘Gala’, firmness ranged from 39.69 N at CITA (202 m) to 67.33 N at Albarracín (1182 m), whereas for the autochthonous local accession ‘Pomera del Pais’ fruit

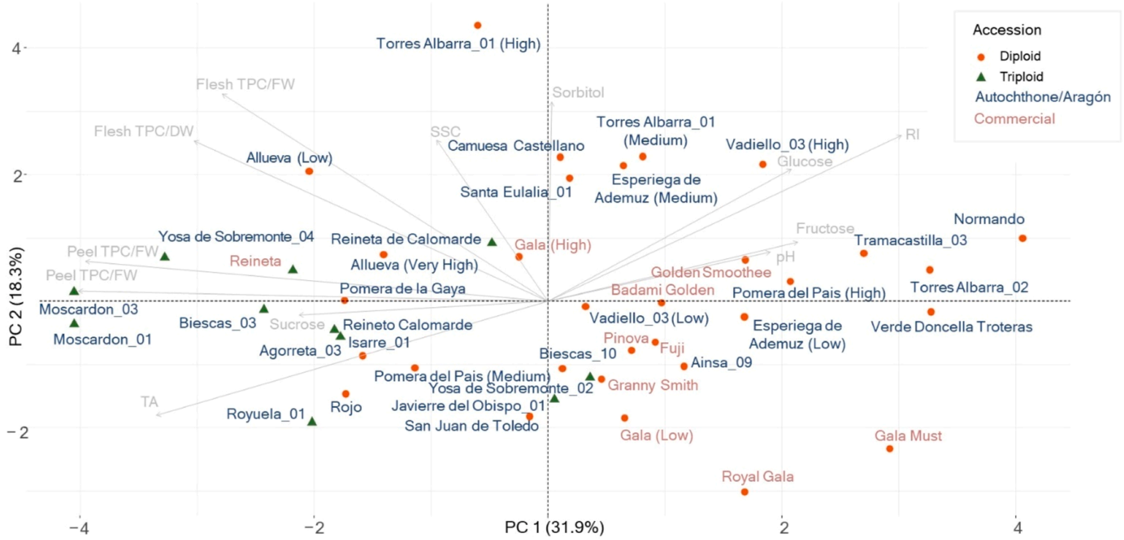

Fig. 4. Principal component analysis of eleven fruit quality traits assessed in the 36 apple genotypes evaluated in this study. The different colors of the accession's labels represent their origin: mountainous autochthonous accessions, such as those from the Pyrenees, and commercial non-Spanish cultivars. Point shape indicates accesions ploidy (diploid or triploid). According to the six genotypes included in the altitude study, the PCA incorporates data from both altitudes for each accession, with samples labelled as corresponding to 'low', 'medium', 'high', and 'very high' altitude respectively. Abbreviations: TPC, total phenolics content; FW, fresh weight; DW, dry weight; TA, titratable acidity; SSC, soluble solids content; RI, ripening index.

Table 3
Effect of growing altitude (low, medium, high or very high) on fruit physical and biochemical quality attributes for five apple accessions and one commercial cultivar used as reference.

			Firmness	Peel TPC/ FW	Peel TPC/ DW	Flesh TPC/ FW	Flesh TPC/ DW	Sucrose	Glucose	Fructose	Sorbitol	Total Sugars
Accession name	**Growing Altitude**		N	mg GAE/ 100 g FW	mg GAE/ 100 g DW	mg GAE/ 100 g FW	mg GAE/ 100 g DW	g/kg	g/kg	g/kg	g/kg	g/kg
Allueva	Low	Mean	66.74	181.40	566.87	98.92	494.61	53.65	8.12	46.42	22.09	130.28
		SD	23.28	21.32	66.61	0.62	3.10	0.12	0.57	0.14	0.03	0.80
	Very High	Mean	82.91	181.23	584.62	61.20	359.97	48.12	16.18	50.54	11.69	126.53
		SD	13.74	13.85	44.68	12.65	74.41	0.57	0.16	0.22	0.19	0.01
	Sig.		ns	ns	ns	ns	ns	*	*	*	*	ns
Esperiega de Ademuz	Low	Mean	79.38	101.62	406.48	41.71	278.06	30.67	23.30	68.52	7.86	130.35
		SD	8.39	25.91	103.65	6.14	40.91	0.78	0.98	0.62	0.08	0.75
	Medium	Mean	84.57	149.20	552.58	53.92	385.16	40.57	14.01	48.57	27.29	130.44
		SD	6.68	6.57	24.33	3.67	26.20	2.25	0.05	0.27	0.10	2.58
	Sig.		ns	ns	ns	ns	ns	ns	*	*	*	ns
Gala	Low	Mean	39.69	137.27	549.06	14.61	104.36	46.44	14.40	60.44	3.36	124.64
		SD	4.67	16.55	66.21	1.15	8.20	0.03	0.55	0.33	0.02	0.84
	Very High	Mean	67.33	157.54	543.25	58.07	322.59	62.12	10.90	79.09	11.94	164.05
		SD	22.73	16.32	56.26	4.36	24.21	0.30	0.16	0.28	0.03	0.45
	Sig.		*	ns	ns	*	*	*	ns	*	*	*
Pomera del Pais	Medium	Mean	74.68	188.83	726.27	37.14	232.11	41.98	12.44	71.88	6.68	132.98
		SD	1.01	34.87	134.13	17.71	110.72	0.70	1.14	0.48	0.01	2.33
	High	Mean	86.63	90.60	266.46	41.57	188.96	47.75	14.58	81.85	8.71	152.89
		SD	9.94	0.56	1.66	4.02	18.27	0.40	0.04	0.58	0.10	0.93
	Sig.		*	ns	ns	ns	ns	*	ns	*	*	*
Torres Albarra_01	Medium	Mean	63.41	146.55	488.48	66.60	369.98	30.85	29.93	70.94	13.44	145.16
		SD	10.21	13.97	46.57	7.37	40.94	0.17	0.21	0.16	0.03	0.17
	High	Mean	62.23	232.75	646.52	114.62	545.79	24.73	44.15	88.77	15.85	173.50
		SD	3.31	8.19	22.76	2.83	13.49	1.27	1.77	2.80	0.50	6.34
	Sig.		ns	*	ns	*	ns	ns	ns	ns	ns	ns
Vadiello_03	Low	Mean	75.95	184.06	541.36	46.99	223.76	63.43	13.93	63.51	7.76	148.63
		SD	8.25	6.98	20.53	4.48	21.34	1.51	0.02	0.15	0.04	1.67
	High	Mean	70.56	133.90	478.20	67.96	357.66	36.40	36.22	76.26	10.82	159.70
		SD	2.92	10.56	37.73	2.09	10.98	1.29	2.98	0.34	0.04	4.65
	Sig.		ns	*	ns	ns	*	*	ns	*	*	ns

Statistical significance at *: $P \leq 0.05$; ns: not significant. Abbreviations: SD, Standard deviation; Sig., signification according to the t-Tests; TPC, total phenolics content; GAE, Gallic acid equivalent; FW, Fresh weight; DW, Dry weight; SSC, soluble solids content; TA, titratable acidity; RI, ripening index.

firmness varied from 74.68 N at Ligüerre (483 m) to 86.63 N at Bescós de Garcipollera (929 m).

Regarding the TPC in fruit peel expressed on a fruit dry weight basis, no significant effect of growing altitude was observed for any of the studied accessions. However, for the autochthonous accessions 'Torres Albarra_01′ and 'Vadiello_03′, a significant altitude effect was observed on TPC in the fruit peel when expressed in fresh weight basis, but in opposite directions for both accessions. The TPC concentration in the

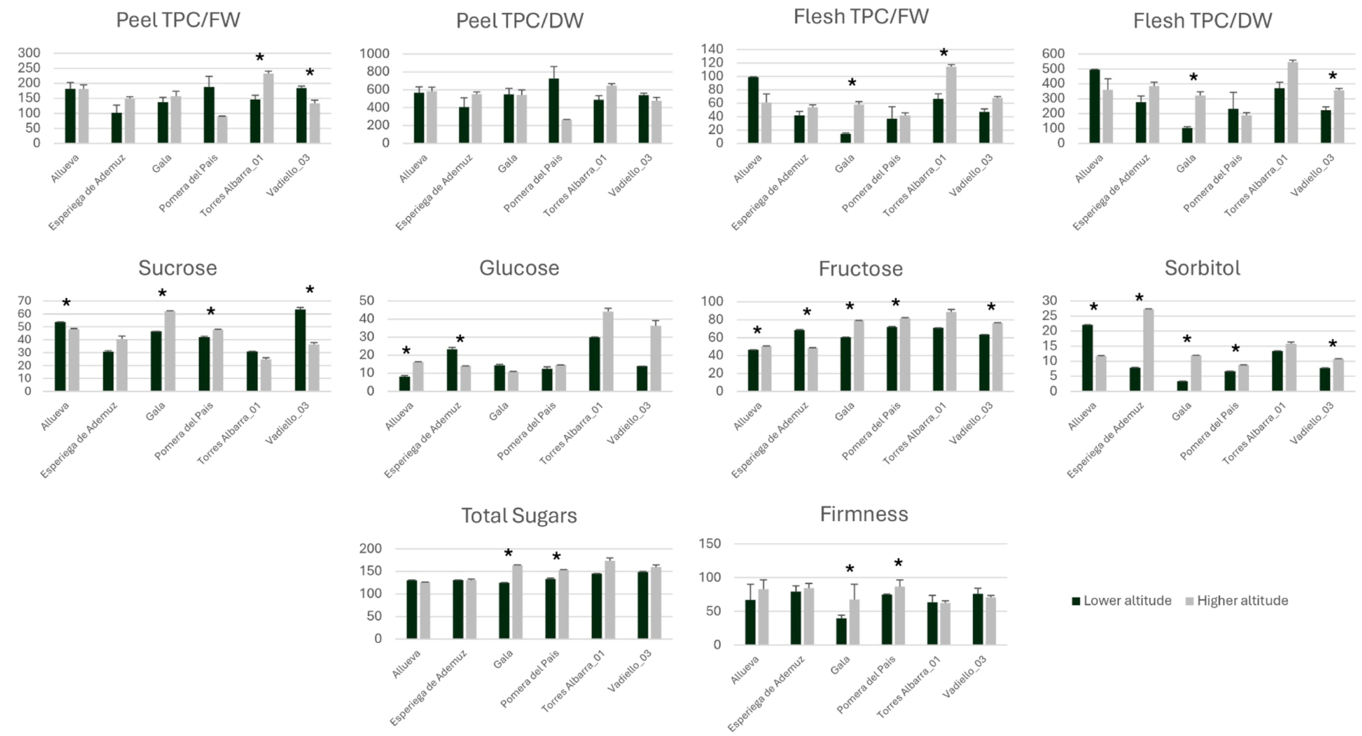

Fig. 5. Altitude-effect on fruit quality traits for five apple accessions and one commercial cultivar 'Gala'. Statistical significance according to the t-Tests at *: $P \leq 0.05$. Abbreviations: TPC, total phenolics content; FW, fresh weight; DW, dry weight; TA, titratable acidity; RI, ripening index. Units: TPC, mg GAE/100 g sample; Sugars, g/kg; Firmness, Newtons.

peel of 'Torres Albarra_01′ increased from 146.55 at Ligüerre (483 m) to 232.75 mg GAE/100 g FW at Bescós de Garcipollera (929 m), whereas for 'Vadiello_03', a decrease in TPC was observed at higher altitude, from 184.06 at CITA (202 m) to 133.90 mg GAE/100 g FW at Bescós de Garcipollera (929 m). No significant altitude effect on peel TPC/FW was observed in the other four accessions.

In relation to flesh TPC, both 'Gala' and 'Torres Albarra_01′ accessions showed significantly lower TPC values in the fruit grown at lower altitudes compared to those fruits grown at higher altitudes when measured in fresh weight. For 'Gala', flesh TPC increased from 14.61 at CITA (202 m) to 58.07 mg GAE/100 g FW at Albarracín (1182 m), while for 'Torres Albara_01′, TPC increased from 66.60 at Ligüerre to 114.62 mg GAE/100 g FW at Bescós de Garcipollera. No significant altitude effect was observed in the other four accessions for flesh TPC in FW. When measured in dry weight (DW), both 'Gala' and 'Vadiello_03′ showed an increase in flesh TPC with altitude. For 'Gala', TPC increased from 104.36 at CITA to 322.59 mg GAE/100 g DW at Albarracín, while for 'Vadiello_03' flesh TPC increased from 223.76 at CITA to 357.66 mg GAE/100 g DW at Bescós de Garcipollera. No significant effect was observed for flesh TPC in DW in the other four accessions.

Concerning individual sugars, fructose, the predominant sugar in apple fruit, was strongly influenced by growing altitude. For all the studied accessions, fructose concentration increased with altitude, except for the 'Esperiega de Ademuz' accession, where fructose decreased from 68.52 at low altitude to 48.57 g/Kg FW at medium altitude. A similar trend was observed for sorbitol content. Sorbitol concentrations increased with altitude for all apple accessions, except for 'Allueva', where it decreased from 22.09 at CITA (202 m) to 11.69 g/Kg FW at Albarracín (1182 m). No significant altitude effect was observed on any individual sugar for 'Torres Albarra_01′. On the other hand, the effect of altitude varied depending on the accession. Glucose decreased with altitude in 'Esperiega de Ademuz' (from 23.30 at CITA, 202 m, to 14.01 g/Kg at Ligüerre, 483 m), while it increased in 'Allueva' (from 8.12 at lower altitude vs. 16.18 g/Kg at higher altitude). For sucrose, the trend was also dependent on the accession. In 'Vadiello_03′, lower sucrose values were obtained at higher altitude (63.43 at Cita, 202 m, to 36.40 g/Kg at 'Bescós de Garcipollera', 929 m), while an increase in sucrose content with altitude was found in both 'Gala' (46.44 at CITA, 202 m, to 62.12 g/Kg at Albarracín, 1182) and 'Pomera del Pais' (41.98 at Ligüerre, 483 m, to 47.75 g/Kg at Bescós de Garcipollera, 929 m).

A significant effect of altitude was also observed on the total sugars content in the fruit for both 'Gala' and 'Pomera del Pais'. In both samples, the higher the altitude at which the fruits were grown, the higher the total sugars concentration. Specifically, total sugar content increased from 124.64 to 164.05 g/Kg in 'Gala', and from 132.98 to 152.89 g/Kg in 'Pomera del Pais'.

4. Discussion

The present study had two main objectives. The first was to characterize the organoleptic quality of 36 apple genotypes from the CITA germplasm bank. The second was to examine the effect of altitude on five apple accessions and one commercial cultivar ('Gala') grown at two different altitudes.

The values of biochemical traits observed was within the ranges described for *Malus* x *Domestica* Borkh cultivars regarding fruit quality traits (Aprea et al., 2017; Mignard et al., 2021a), as well as for physicochemical attributes such as peel and flesh phenolics (Cvetković et al., 2024; Mignard et al., 2021a) and individual sugars (Aprea et al., 2017; Castel et al., 2020; Cvetković et al., 2024; Mignard et al., 2022). As expected, the 36 apple accessions exhibited considerable variability across the studied parameters, consistent with previous reports (Cice et al., 2023; Drogoudi et al., 2008; Mignard et al., 2023b; Millán-Laleona et al., 2023). This genetic variability, with accessions identified as unique genotypes by Pina et al. (2014) and Pereira-Lorenzo et al. (2017), contributed to the **variation in both physical and biochemical fruit traits, highlighting the important contribution of autochthonous apple phytogenetic resources to genetic diversity**.

The significant positive correlations between total sugars and individual sugars (sucrose, glucose, fructose, and sorbitol) were consistent with known metabolic pathways in apples (Aprea et al., 2017). The positive correlation between glucose and fructose, along with the negative correlation between glucose and sucrose, reflects the interconnectedness of these sugars in both primary and secondary fruit metabolism (Durán-Soria et al., 2020). **Changes in their concentrations can indicate different stages of ripening and responses against biotic and abiotic stresses**. Moreover, fructose, which had the highest correlation with total sugars, is the major contributor to the overall sweetness of apples and plays a key role in consumer satisfaction (Davies et al., 2022).

The negative correlation between ripening index and peel TPC is also noteworthy. During ripening, starch is converted into sugars, enhancing apple fruit sweetness and texture (Aprea et al., 2017). This process likely explains why sweeter fruits with higher sugar content tend to have lower phenolics concentrations, reflecting a trade-off between primary and secondary metabolites, as reported in previous studies (Aprea et al., 2017; Caretto et al., 2015). Additionally, TA was positively correlated with peel TPC, suggesting that more acidic apples may have higher antioxidant capacities, as phenolics contribute to both acidity and antioxidant properties (Cvetković et al., 2024).

Although the one-way ANOVA revealed significant variation based on accession origin only for TPC in flesh, it is interesting to note that non-Spanish commercial cultivars such as ‘Fuji’, ‘Gala’, ‘Golden’ and relatives tended to cluster together in the PCA, indicating similar biochemical profiles. Previous studies on a large set of apple genotypes conserved in seven Spanish collections (1453 diploid accessions) allowed the discrimination of an Iberian gene pool of apple cultivars, which were separated from a wide set of foreign cultivars using a common set of microsatellites markers (Pereira-Lorenzo et al., 2017). Additionally, Mignard et al. (2023a) revealed that Spanish native accessions (94, comprising 72 diploid and 22 triploid accessions) from the apple germplasm bank established at the Experimental Station of Aula Dei (EEAD-CSIC) stand out for their distinct organoleptic and nutritional qualities when compared to non-Spanish cultivars (92, comprising 78 diploid and 14 triploid accessions), which tend to group together.

Changes in modern apple phenotypes, including polyphenolics content, soluble solids content, and flesh firmness, have been observed over the centuries, with a reduction in phenolics linked to a decrease in bitterness (Davies et al., 2022). High phenolics content in apples is associated with important health-promoting properties but, it is also linked to increased enzymatic browning (Miranda et al., 2023) and sometimes to astringent or bitter flavors. As a result, some breeding programs have historically focused on improving the visual appeal and flavour of fruit, often unintentionally leading to a reduction in phenolic content. This highlights the need for a more balanced breeding approach that preserves both nutritional and organoleptic quality (Watts et al., 2021). In this study, most of the local mountainous apple accessions contained a higher phenolic content than commercial cultivars and can therefore be considered a better source of bioactive compounds (Drogoudi et al., 2008; Millán-Laleona et al., 2023). In conclusion, **‘New World’ cultivars generally have lower phenolic content than ‘Old World’ cultivars, although there is an increasing focus and interest on improving fruit quality, including phenolic content and antioxidant bioactive compounds** for their human health benefits (Bars-Cortina et al., 2017; Gibney et al., 2019; Ho et al., 2020; Millán-Laleona et al., 2023; Tamura et al., 2020).

Indeed, ‘Reineta’ was the only non-Spanish cultivar that was distinctly separated from the other commercial cultivars in the PCA analysis, and it was also the only non-Spanish triploid cultivar. As noted in previous studies (Greaves et al., 2024; Mignard et al., 2022), triploid accessions typically exhibited different biochemical profiles, with higher levels of bioactive compounds, titratable acidity, and carbohydrate content. Despite their relatively low fertility, triploid accessions could be valuable resources for future breeding programs due to their favorable organoleptic and nutritional traits. Factors such as **ploidy (diploid/triploid) and origin (autochthonous accession/non-Spanish commercial cultivar) underscore the key role of genotype in shaping the physicochemical attributes of apple**.

Apple production at **high altitudes** is currently present in many regions of Spain, especially in Soria, Aragón and Catalonia (Iglesias et al., 2018). A few studies from different countries, such as Italy (Donati et al., 2006; Stainer et al., 2000), Spain (Iglesias et al., 2016; 2018) and New Zealand (Chagné et al., 2016; Iglesias et al., 2016), have highlighted the significant influence of temperature, in particular differences between valley and hilly areas, on key fruit quality parameters such as firmness, crushable acidity, which increase in higher altitude areas, as well as slower ripening rates. Understanding the variation in these traits is essential for improving fruit quality and promoting sustainable and local agricultural practices. In our study, differences in some of these parameters have also been observed. Notably, the ‘Gala’ and ‘Pomera del Pais’ accessions showed a significant increase in fruit firmness at higher altitudes. As reported by other studies, fruit firmness may increase with altitude because of temperature and solar radiation, which can induce changes in cell wall structure associated with calcium absorption and the duration of the cell division period, both of which are positively influenced by altitude (Charles et al., 2018; Navarro-Serrano et al., 2020). Nevertheless, flesh firmness, in hilly regions, can be greater compared to that of the same cultivar grown in valley sites but for a similar ripening stage, measured by the starch-iodine test (Donati et al., 2006: Iglesias et al., 2018). However, the lack of significant effects on the other four accessions suggests that altitude influences some varieties more than others, likely due to genetic background differences in fruit development and ripening.

New traits that may be influenced by altitude have been also assessed in this study such as bioactive compounds (such as polyphenols) and individual sugar profiles of apple autochthonous accessions from both valley and hilly regions. Interestingly, no significant altitude-effect on TPC in fruit peel was observed when expressed on a dry weight basis. However, on a fresh weight basis, TPC varied significantly for ‘Torres Albarra_01′ and ‘Vadiello_03′. For ‘Torres Albarra_01′, TPC increased with altitude, while it decreased for ‘Vadiello_03′. In the flesh, ‘Gala’, ‘Torres Albarra_01′, and ‘Vadiello_03′ showed **significant increases in TPC at higher altitudes, suggesting that cooler temperatures, increased temperature range between day and night, and increased light and UV exposure may enhance phenolic biosynthesis** (Li et al., 2013; Mignard et al., 2021a). Similar findings were reported by Li et al. (2021a) for ‘Gala’ and other accessions in China. This indicates a complex interaction between environmental factors and genetic traits. Total phenolics play a key role in protecting fruits from UV and high light exposure (Li et al., 2013), and their accumulation is likely linked to the photoprotective function of phenolics under intense UV radiation (Li et al., 2013). Previous studies have also shown that agroclimatic conditions can boost phenolic content in apple peel (Karagiannis et al., 2020; Li et al., 2021a) and flesh (Mignard et al., 2021a), suggesting a shared regulatory mechanism for phenolic compound accumulation across different fruit tissues, with increased light and UV exposure. **The response of phenolic compounds to altitude varied in both magnitude and intensity depending on the genetic background of the apple accessions**.

Furthermore, the study emphasized the strong influence of altitude on sugar concentrations, particularly fructose and sorbitol. Li et al. (2021b) reported an increase in sucrose at higher altitudes for ‘Gala’, consistent with our findings. While similar trends were observed for other sugars, further comparisons for ‘Gala’ were not statistically supported. The significant increase in fructose with altitude, except for ‘Esperiega de Ademuz’, suggests that **higher altitudes may promote sugar accumulation, likely due to slower fruit development and ripening, allowing for greater carbohydrate synthesis** (Iglesias et al., 2018; Tijero et al., 2021). However, the variation in sugar content among accessions, especially the contrasting effects on glucose and sucrose, highlights the complexity of fruit metabolism influenced by both altitude and genetic factors. Notably, the increased total sugar content in 'Gala' and 'Pomera del Pais' with altitude reinforces the idea that **altitude can significantly enhance fruit sweetness, a key trait for consumer acceptability**. This emphasizes the potential for growers to select high-altitude sites to improve the sweetness and overall quality of apple crops. Indeed, the Pyrenees and the mountainous Iberian System, characterized by a diverse climate and rich ecological diversity, are home to many endemic species (Pironon et al., 2022). **This biodiversity, combined with the region's climatic conditions, makes the Pyrenees and the mountainous Iberian System from in northeastern Spain a key area for agricultural adaptation, particularly in the context of climate change.**

5. Conclusions

This study highlights the high biodiversity present in apples from the northeastern region in Spain, represented by the CITA apple collection, particularly in terms of organoleptic and nutritional qualities. Spanish accessions exhibited higher phenolic content, while their sugar profiles varied widely depending on the accession's origin and genetic background. These findings underscore the importance of preserving autochthonous phytogenetic resources. Despite the study's limitations, with data collected from only one year, the notable differences observed in the same accessions grown in distinct regions emphasize the crucial influence of geographic location and more specifically of the altitude in shaping organoleptic profiles. In particular, the higher total phenolic content (TPC) observed in accessions grown at higher altitudes may be associated with an enhanced antioxidant potential, suggesting possible positive implications for human health. The variation in bioactive compounds, such as polyphenols and carbohydrates, in relation to altitude underscores the need for further research into how different growing conditions can influence the human health benefits of apples, especially considering the combined effect of phenolic compounds and sugar composition on nutritional quality and consumer intake. Moreover, in addition to biochemical and organoleptic traits, agronomic performance parameters, such as yield, fruit size, fruit color, and tolerance to major diseases, are critical. All these traits strongly depend on the adaptability and resilience of the cultivars, which are essential for both autochthonous accessions and commercial cultivars to support sustainable production, benefiting both environmental conservation and growers' profitability.

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CRediT authorship contribution statement

P. Mignard: Writing – original draft, Methodology, Formal analysis, Data curation. **C.M. Cantín:** Writing – review & editing, Writing – original draft, Supervision, Formal analysis, Conceptualization. **L. Castel:** Resources, Methodology, Formal analysis, Data curation. **A. Pina:** Writing – review & editing, Validation, Resources, Investigation, Formal analysis, Data curation. **J. Gonzalez:** Writing – review & editing, Validation, Methodology, Formal analysis. **P. Errea:** Writing – review & editing, Validation, Resources, Investigation, Formal analysis, Data curation.

Declaration of competing interest

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

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2026.114739.

Data availability

Data will be made available on request.

References

Amblar-Francés, M.P., Ramos-Calzado, P., Sanchis-Lladó, J., Hernanz-Lázaro, A., Peral-García, M.C., Navascués, B., Dominguez-Alonso, M., Pastor-Saavedra, M.A., Rodríguez-Camino, E., 2020. High resolution climate change projections for the Pyrenees region. ASR 17, 191–208. https://doi.org/10.5194/asr-17-191-2020.

Aprea, E., Charles, M., Endrizzi, I., Laura Corollaro, M., Betta, E., Biasioli, F., Gasperi, F., 2017. Sweet taste in apple: the role of sorbitol, individual sugars, organic acids and volatile compounds. Sci. Rep. 7, 44950. https://doi.org/10.1038/srep44950.

Asma, U., Morozova, K., Ferrentino, G., Scampicchio, M., 2023. Apples and apple by-products: antioxidant properties and food applications. Antioxidants 12, 1456. https://doi.org/10.3390/antiox12071456.

Bars-Cortina, D., Macià, A., Iglesias, I., Romero, M.P., Motilva, M.J., 2017. Phytochemical profiles of new red-fleshed apple varieties compared with traditional and new white-fleshed varieties. J. Agric. Food Chem. 65 (8), 1684–1696. https://pubs.acs.org/doi/abs/10.1021/acs.jafc.6b02931.

Boudichevskaia, A., Kumar, G., Sharma, Y., Kapoor, R., Singh, A.K., 2020. Challenges and strategies for developing climate-smart apple varieties through genomic approaches. Genomic Designing of Climate-Smart Fruit Crops. Springer International Publishing, Cham, pp. 23–71. https://doi.org/10.1007/978-3-319-97946-5_2.

Butkeviciute, A., Viskelis, J., Liaudanskas, M., Viskelis, P., Janulis, V., 2022. Impact of storage controlled atmosphere on the apple phenolic acids, flavonoids, and anthocyanins and antioxidant activity in vitro. Plants 11, 201. https://doi.org/10.3390/plants11020201.

Calvo-Castro, L.A., Lobo-Vázquez, M., Gómez-González, J.C., Arnáez-Serrano, E., Zamora-Fallas, G., Sánchez-Zúñiga, K., Centeno-Cerdas, C., 2022. Bioactive potential of tropical highland apple (*Malus* domestica cv. Anna) crude extract: opportunities for food waste revalorization. Futur. J. Pharm. Sci. 8, 57. https://doi.org/10.1186/s43094-022-00447-z.

Cantin, C.M., Gracia, A., 2022. Intrinsic and extrinsic attributes related to the influence of growing altitude on consumer acceptability and sensory perception of fresh apple. J. Sci. Food Agric. 102, 1292–1299. https://doi.org/10.1002/jsfa.11656.

Caretto, S., Linsalata, V., Colella, G., Mita, G., Lattanzio, V., 2015. Carbon fluxes between primary metabolism and phenolic pathway in plant tissues under stress. Int. J. Mol. Sci. 16, 26378–26394. https://doi.org/10.3390/ijms161125967.

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.

Chagné, D., Kirk, C., How, N., Whitworth, C., Fontic, C., Reig, G., Iglesias, I., 2016. A functional genetic marker for apple red skin coloration across different environments. Tree Genet Genome. 12, 1–9. https://doi.org/10.1007/s11295-016-1025-8.

Charles, M., Corollaro, M.L., Manfrini, L., Endrizzi, I., Aprea, E., Zanella, A., Corelli Grappadelli, L., Gasperi, F., 2018. Application of a sensory–instrumental tool to study apple texture characteristics shaped by altitude and time of harvest. J. Sci. Food Agric. 98, 1095–1104. https://doi.org/10.1002/jsfa.8560.

Cice, D., Ferrara, E., Magri, A., Adiletta, G., Capriolo, G., Rega, P., Di Matteo, M., Petriccione, M., 2023. Autochthonous apple cultivars from the campania region (southern italy): bio-agronomic and qualitative traits. Plants 12, 1160. https://doi.org/10.3390/plants12051160.

Cvetković, B., Bajić, A., Belović, M., Pezo, L., Dragojlović, D., Šimurina, O., Djordjević, M., Korntheuer, K., Philipp, C., Eder, R., 2024. Assessing antioxidant properties, phenolic compound profiles, organic acids, and sugars in conventional apple cultivars (*Malus* domestica): a chemometric approach. Foods 13, 2291. https://doi.org/10.3390/foods13142291.

Davies, T., Watts, S., McClure, K., Migicovsky, Z., Myles, S., 2022. Phenotypic divergence between the cultivated apple (*Malus* domestica) and its primary wild progenitor (*Malus* sieversii). PLoS One. 17, e0250751. https://doi.org/10.1371/journal.pone.0250751.

Donati, F., Gaiani, A., Guerra, W., Stainer, R., Berra, L., 2006. Comparazione sensoriale e strumentale di mele provenienti da diversi areali italiani. Rivista Di Frutticoltura E Di Ortofloricoltura. 68 (11), 63–70.

Drogoudi, P.D., Michailidis, Z., Pantelidis, G., 2008. Peel and flesh antioxidant content and harvest quality characteristics of seven apple cultivars. Sci. Hortic. 115, 149–153. https://doi.org/10.1016/j.scienta.2007.08.010.

Durán-Soria, S., Pott, D.M., Osorio, S., Vallarino, J.G., 2020. Sugar signaling during fruit ripening. Front. Plant Sci. 11. https://doi.org/10.3389/fpls.2020.564917.

Fadanelli, L., Comai, M., Dorigoni, A., Mattivi, F., Boschetti, A., 2005. Influence of crop load and production site on quality of 'Golden delicious' apples during storage. Acta Hortic. 749–756. https://doi.org/10.17660/ActaHortic.2005.682.98.

FAOSTAT, Food and Agricultural Organization. http://faostat.fao.org/ (Accessed 04th November of 2024) [WWW document], 2024.

García-Ruiz, J.M., Lasanta, T., Ruiz-Flano, P., Ortigosa, L., White, S., González, C., Martí, C., 1996. Land-use changes and sustainable development in mountain areas: a case study in the Spanish Pyrenees. Landsc. Ecol. 11, 267–277. https://doi.org/10.1007/BF02059854.

Gibney, E.R., Milenkovic, D., Combet, E., Ruskovska, T., Greyling, A., González-Sarrías, A., de Roos, B., Tomás-Barberán, F., Morand, C., Rodriguez-Mateos, A., 2019. Factors influencing the cardiometabolic response to (poly)phenols and phytosterols: a review of the COST action POSITIVe activities. Eur. J. Nutr. 58, 37–47. https://doi.org/10.1007/s00394-019-02066-6.

Gracia, A., Cantín, C.M., 2022. Effects of consumers' sensory attributes perception on their willingness to pay for apple cultivars grown at different altitudes: are they different? Foods 11, 3022. https://doi.org/10.3390/foods11193022.

Greaves, E., Davies, T., Myles, S., Migicovsky, Z., 2024. Comparing diploid and triploid apples from a diverse collection. BioRxiv. https://doi.org/10.1101/2024.07.17.603958.

Ho, K.K., Ferruzzi, M.G., Wightman, J.D., 2020. Potential health benefits of (poly) phenols derived from fruit and 100% fruit juice. Nutr. Rev. 78, 145–174. https://doi.org/10.1093/nutrit/nuz041.

Iglesias, I., Bonany, J., Batlle, I., Cantin, C.M., Troggio, M., Allan, A.C., Friend, A., Espley, R.V., Lin–Wang, K., Chagné, D., Volz, R.K., 2016. The development of redskinned apples adapted to the warm climates of South European countries. Chr. Hort. 56 (1), 9–14.

Iglesias, I., Garanto, X., Cortada, G.E., Farré, X., 2018. La manzana en altitud: resultados y balance de nueve años de actuación. Revista. De Fruticultura. 6–81.

Karagiannis, E., Michailidis, M., Tanou, G., Scossa, F., Sarrou, E., Stamatakis, G., Samiotaki, M., Martens, S., Fernie, A.R., Molassiotis, A., 2020. Decoding altitude-activated regulatory mechanisms occurring during apple peel ripening. Hortic. Res. 7, 120. https://doi.org/10.1038/s41438-020-00340-x.

Lattanzio, V., 2013. Phenolic compounds: introduction. Natural Products. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 1543–1580. https://doi.org/10.1007/978-3-642-22144-6_57.

Li, P., Ma, F., Cheng, L., 2013. Primary and secondary metabolism in the sun-exposed peel and the shaded peel of apple fruit. Physiol. Plant 148, 9–24. https://doi.org/10.1111/j.1399-3054.2012.01692.x.

Li, Y., Sun, H., Li, J., Qin, S., Niu, Z., Qiao, X., Yang, B., 2021a. Influence of genetic background, growth latitude and bagging treatment on phenolic compounds in fruits of commercial cultivars and wild types of apples (*Malus* sp.). Eur. Food Res. Technol. 247, 1149–1165. https://doi.org/10.1007/s00217-021-03695-0.

Li, Y., Sun, H., Li, J., Qin, S., Yang, W., Ma, X., Qiao, X., Yang, B., 2021b Effects of genetic background and altitude on sugars, malic acid and ascorbic acid in fruits of wild and cultivated apples (*Malus* sp.). *Foods* 10, 2950. https://doi.org/10.3390/foods10122950.

Meyer, J.D., 2014. Apple-producing Family Farms in South Tyrol: an Agriculture Innovation Case Study. Publisher: Food and Agriculture Organization of the United Nations (FAO). ISBN (Hardback): 978-92-5-108365-9.

Michalska, A., Łysiak, G., 2015. Bioactive compounds of blueberries: post-harvest factors influencing the nutritional value of products. Int. J. Mol. Sci. 16, 18642–18663. https://doi.org/10.3390/ijms160818642.

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* x *domestica* Borkh). Sci. Hortic. 285, 110142. https://doi.org/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. 109–114. https://doi.org/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. Agronomy 12, 827. https://doi.org/10.3390/agronomy12040827.

Mignard, P., Font i Forcada, C., Giménez, R., Moreno, M.A., 2023a. Population structure and association mapping for agronomical and biochemical traits of a large Spanish apple germplasm. Plants 12, 1249. https://doi.org/10.3390/plants12061249.

Mignard, P., Giménez, R., Moreno, M.A., 2023b. Postharvest performance of 17 apple accessions grown in NE Spain after long-term cold storage. J. Appl. Bot Food Qual. 96, 55–66.

Millán-Laleona, A., Bielsa, F.J., Aranda-Cañada, E., Gómez-Rincón, C., Errea, P., López, V., 2023. Antioxidant, antidiabetic, and anti-obesity properties of apple pulp extracts (*Malus* domestica Bork): a comparative study of 15 local and commercial cultivars from Spain. Biol. (Basel) 12, 891. https://doi.org/10.3390/biology12070891.

Ministry of Agriculture, Fisheries, and food via the network of weather stations of the Agroclimatic Information System for Irrigation (Sistema de Información Agroclimática para el Regadío - SiAR).https://servicio.mapa.gob.es/websiar/SeleccionParametrosMap.aspx?dst=1.

Miranda, C., Irisarri, P., Arellano, J., Bielsa, F.J., Valencia, A., Urrestarazu, J., Pina, A., Santesteban, L.G., Castel, L., Errea, P., 2023. Assessment of flesh browning diversity in apple germplasm collections phenotyped by image analysis. Acta. Hortic. 483–488. https://doi.org/10.17660/ActaHortic.2023.1362.64.

Navarro-Serrano, F., López-Moreno, J.I., Azorin-Molina, C., Alonso-González, E., Aznarez-Balta, M., Buisán, S.T., Revuelto, J., 2020. Elevation effects on air temperature in a topographically complex mountain valley in the Spanish Pyrenees. Atmosphere (Basel) 11, 656. https://doi.org/10.3390/atmos11060656.

Parajuli, R., Thoma, G., Matlock, M.D., 2019. Environmental sustainability of fruit and vegetable production supply chains in the face of climate change: a review. Sci. Total Environ. 650, 2863–2879. https://doi.org/10.1016/j.scitotenv.2018.10.019.

Parry, M.L., 2019. Climate Change and World Agriculture. Routledge. https://doi.org/10.4324/9780429345104.

Pereira-Lorenzo, S., Urrestarazu, J., Ramos-Cabrer, A.M., Miranda, C., Pina, A., Dapena, E., Moreno, M.A., Errea, P., Llamero, N., Díaz-Hernández, M.B., Santesteban, L.G., Laquidain, M.J., Gogorcena, Y., Urbina, V., Dalmases, J., Ascasíbar-Errasti, J., Royo, J.B., 2017. Analysis of the genetic diversity and structure of the Spanish apple genetic resources suggests the existence of an Iberian genepool. Ann. Appl. Biol. 171, 424–440. https://doi.org/10.1111/aab.12385.

Pina, A., Urrestarazu, J., Errea, P., 2014. Analysis of the genetic diversity of local apple cultivars from mountainous areas from Aragon (Northeastern Spain). Sci. Hortic. 174, 1–9. https://doi.org/10.1016/j.scienta.2014.04.037.

Pironon, S., Gómez, D., Font, X., García, M.B., 2022. Living at the limit in the Pyrenees: peripheral and endemic plants are rare but underrepresented in protection lists. Divers Distrib. 28, 930–942. https://doi.org/10.1111/ddi.13487.

Planton, G., 1995. Apple maturity: cTIFL-eurofru starch code and the starchmeter. The apple starch test as a decision help for harvest. *Infos CTIFL* (France). ISSN 0758-5373.

Porritt, S.W., 1964. The effect of temperature on postharvest physiology and storage life of pears. Can J. Plant Sci. 44, 568–579. https://doi.org/10.4141/cjps64-111.

R Core Team, 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org.

Rodríguez, A., Pérez-López, D., Centeno, A., Ruiz-Ramos, M., 2021. Viability of temperate fruit tree varieties in Spain under climate change according to chilling accumulation. Agric. Syst. 186, 102961. https://doi.org/10.1016/j.agsy.2020.102961.

Royo Díaz, J.B., Miranda, C., Santesteban, G., Errea, P., 2017. Método armonizado para la caracterización pomológica del manzano (*Malus* × *domestica* Borkh). Monografías INIA 29.

Sahu, N., Saini, A., Behera, S.K., Sayama, T., Sahu, L., Nguyen, V.T.V., Takara, K., 2020. Why apple orchards are shifting to the higher altitudes of the Himalayas? PLoS One 15, e0235041. https://doi.org/10.1371/journal.pone.0235041.

Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic 16, 144–158. https://doi.org/10.5344/ajev.1965.16.3.144.

Stainer, R., Steffanelli, D., Lanzoni, S., Pellegrino, S., Sansavini, S., 2000. Valutazione sensoriale e strumentale di mele di diversa provenienza. Rivista Di Frutticoltura. 7/8, 53–62.

Stewart, A.L., Ahmed, S., 2020. Effects of climate change on fruit nutrition. Fruit Crops. Elsevier, pp. 77–93. https://doi.org/10.1016/B978-0-12-818732-6.00007-1.

Swarup, S., Cargill, E.J., Crosby, K., Flagel, L., Kniskern, J., Glenn, K.C., 2021. Genetic diversity is indispensable for plant breeding to improve crops. Crop Sci. 61, 839–852. https://doi.org/10.1002/csc2.20377.

Tamura, Y., Tomiya, S., Takegaki, J., Kouzaki, K., Tsutaki, A., Nakazato, K., 2020. Apple polyphenols induce browning of white adipose tissue. J. Nutr. Biochem. 77, 108299. https://doi.org/10.1016/j.jnutbio.2019.108299.

Tijero, V., Girardi, F., Botton, A., 2021. Fruit development and primary metabolism in apple. Agronomy 11, 1160. https://doi.org/10.3390/agronomy11061160.

UPOV (International Union for the Protection of New Varieties of Plants), 1974. *Examination of distinctness, Uniformity and Stability and the Development of Harmonized Descriptions of New Varieties of Plants* (Document TG/1/3). UPOV, Geneva.

Watkins, R., Smith, R.A., 1983. Descriptor List for Apple (*Malus*). In: IBPGR/CEC AGPR/IBPGR/82/71, EUR 8354/1. Rome, Italy. https://www.bioversityinternational.org/e-library/publications/detail/apple-descriptors/. ISBN: 9290431016.

Watts, S., Migicovsky, Z., McClure, K.A., Yu, C.H.J., Amyotte, B., Baker, T., Bowlby, D., Burgher-MacLellan, K., Butler, L., Donald, R., Fan, L., Fillmore, S., Flewelling, J., Gardner, K., Hodges, M., Hughes, T., Jagadeesan, V., Lewis, N., MacDonell, E., MacVicar, L., McElroy, M., Money, D., O'Hara, M., Ong, Q., Campbell Palmer, L., Sawler, J., Vinqvist-Tymchuk, M., Rupasinghe, H.P.V., DeLong, J.M., Forney, C.F., Song, J., Myles, S., 2021. Quantifying apple diversity: a phenomic characterization of Canada's apple biodiversity collection. Plant. People Planet. 3, 747–760. https://doi.org/10.1002/ppp3.10211.