Research Article

Kara Mohammed*, Hamza Saghrouchni, Youness El Abdali, Assouguem Amine, Nouha Haoudi, Mohamed El fadili, Aya Amar, Tareq Hamijo, Isil Var, Riaz Ullah, Zerhouni Abdou Rachid, Bahhou Jamila

Phytochemical and physicochemical studies of different apple varieties grown in Morocco

https://doi.org/10.1515/chem-2023-0205
received October 23, 2023; accepted February 6, 2024

Abstract: The apple is an important part of the human diet and is one of the most popular fruits in Morocco, with varieties that are now suitable for human consumption. This study aims to clarify several aspects of apples, such as their physical and chemical compositions and nutrient profiles. Total polyphenols, flavonoids, tannins, and anthocyanins were determined using spectrophotometer and organic acid compounds were identified by high-performance liquid chromatography-ultraviolet. The results show a significant difference between the several varieties under study in terms of their physico-chemical and phyto-chemical characteristics. In general, the studied apples are juiciness, with a percentage ranging from 60 to 71%. The pH values demonstrate how acidic the apples are. The Brix parameter denotes values greater than 13°Brix. Total polyphenols and condensed tannins (1154.65 ± 13.54 µg EAG/g and 514.09 ± 32.40 µg EAT/g, respectively) are more present in the Ahjjani variety than they are in other varieties. This demonstrates their good nutritional quality while not being consumable. However, the Story variety has a predominance of flavonoids (75.074 ± 2.309 µg QE/g) and flavones (45.074 ± 2.09 µg QE/g). The detection of organic acids has shown that the acid succinic is the most abundant in all the varieties of studied apple fruits. These results allow us to infer that non-consumable varieties are also important dietary sources of bioactive molecules, notably for polyphenols. The fact that these kinds can be used to produce other byproducts is therefore highly intriguing.

Keywords: polyphenols, flavonoids, HPLC, phytochemical, apple

*** Corresponding author: Kara Mohammed,** Laboratory of Biotechnology, Conservation and Valorisation of Natural Resources (LBCVNR), Faculty of Sciences Dhar El Mahraz, Sidi Mohamed Ben Abdallah University, BP 1796 Atlas, Fez 30000, Morocco, e-mail: mohammed.kara@usmba.ac.ma
Hamza Saghrouchni: Department of Biotechnology, Institute of Natural and Applied Sciences, Çukurova University, Balcalı/Sarıçam, 01330 Adana, Turkey
Youness El Abdali: Laboratory of Biotechnology, Environment, Agri-food and Health (LBEAS), Faculty of Sciences Dhar El Mahraz, Sidi Mohamed Ben Abdellah University, Fez 30050, Morocco
Assouguem Amine: Laboratory of Functional Ecology and Environment, Faculty of Sciences and Technology, Sidi Mohamed Ben Abdellah University, PO. box 2202 Imouzzer Street, Fez 30000, Morocco
Nouha Haoudi: Laboratory of Biotechnology, Conservation and Valorisation of Natural Resources (LBCVNR), Faculty of Sciences Dhar El Mahraz, Sidi Mohamed Ben Abdallah University, BP 1796 Atlas, Fez 30000, Morocco; National Institute for Agricultural Research (INRA), BO 578, Meknes, Morocco
Mohamed El fadili: LIMAS Laboratory, Faculty of Sciences Dhar Mahraz, Sidi Mohamed Ben Abdellah University, Fez 30000, Morocco
Aya Amar: Department of Plant and Crops, Faculty of Bioscience Engineering, Ghent University, Ghent, Adana, Belgium
Tareq Hamijo: Faculty of Agriculture, Department of Horticulture, Çukurova University, 01330 Balcalı, Adana, Turkey
Isil Var: Department of Food Engineering, Faculty of Engineering, Çukurova University, 01250, Sarıçam, Adana, Turkey
Riaz Ullah: Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia
Zerhouni Abdou Rachid, Bahhou Jamila: Laboratory of Biotechnology, Conservation and Valorisation of Natural Resources (LBCVNR), Faculty of Sciences Dhar El Mahraz, Sidi Mohamed Ben Abdallah University, BP 1796 Atlas, Fez 30000, Morocco

1 Introduction

The apple (*Malus domestica*) is one of the most widely grown and consumed fruits. A wide variety of apple varieties thrive in different climatic regions. Morocco's apple varietal profile is a combination of multiple imported types. The Golden Delicious dominates the spread of these kinds, with Starking Delicious and Starkimson serving as its pollinators. Red Chief, Golden Smoothie, and Gloster are also cultivated there [1]. In areas with mild winters, the phenological and physiological variety of 27 apple cultivars was demonstrated in earlier research by Oukabli et al. While some of them can tolerate the climatic and educational conditions of these places, others are not well suited to them [2]. Certain cultivars can be grown in mountainous places where temperatures are moderate and elevations range from 500 to 800 m: Black Stayman, Einschiemer, Vistabella, Ozark Gold, Gala Royal, Anna, and Dorset Golden [1,2].

Various pomological traits were investigated in the work of [3] to investigate the pomological diversity of apple germplasm growth in Morocco using the descriptors of the International Board for Plant Genetic Resources (IBPGR, 1992) and the International Union for the Protection of New Varieties of Plants [4]. This work has shown that the varietal profile of apple in Morocco is diversified. In addition to the varieties introduced from outside of Morocco, there are local cultivars with specific properties of adaptation to each region of Morocco, such as Oumlile, Ahmri, Mticha, Labiad, Amlale, Oumlile beldi, Azougar, Beldi, Lahlou, Talhlout, Lahmar, and Zarbana [3]. Some of these varieties are marketable, and the apple varieties are labeled in Morocco as local products. Three varieties are mentioned, the Midelt Apple, Imilchel Apple, and Haouz Apple. The Midelt apple is a combination of Golden Delicious, Starkimson, and Starking Delicious varieties [5]. The "Imilchil apple" encompasses the whole circle of Imilchil (Imilchil, Bouznou, Outarbate, Amouguer, and Aït Ihya). The "Haouz apple" variety, derived from Golden Delicious, Starking Delicious, and Royal Gala varieties, is grown exclusively in the geographical area of the rural commune of Asni in the province of Al Haouz [6]. The number of new varieties continues to evolve while keeping their traceability by the National Office of Sanitary Safety of Food Products.

The physico-chemical study of different apple cultivars grown in Morocco is essential to understand the inherent properties and chemical compositions of these fruits. Physical attributes, such as size, shape, color, and texture, directly influence the marketability and acceptance of apples in the fresh produce sector. These parameters are vital in selecting apples that meet the demands of consumers while optimizing storage and transportation logistics. Additionally, texture attributes, such as crispiness and juiciness, are crucial in defining the sensory experience of consuming apples and are highly regarded in the food industry for apple-based product development [7].

The physicochemical properties of apples play a pivotal role in shaping consumer preferences and determining the suitability of specific cultivars for various applications. From a chemical standpoint, apples are a rich source of various bioactive compounds that contribute to their nutritional value and potential health benefits. Organic acids, sugars, and soluble solids content determine the taste and sweetness of apples, whereas antioxidants, vitamins, and minerals confer health-promoting properties [8]. Apples are particularly rich in several types of polyphenols, including resveratrol, flavonoids, and phenolic acids such as quercetin, catechins, epicatechins, anthocyanins, procyanidin, hydroxycinnamates, dihydrochalcones, and chlorogenic acids [9–11]. These compounds are highly correlated with the antioxidant activity. According to several studies, despite their low market value, the old apple varieties are considered to be a principal source of bioactive compounds compared to the commercial varieties [11,12].

Understanding the chemical composition of different apple varieties is integral to assessing their overall nutritional quality and their contributions to a balanced diet [7,13]. The chemical properties of apples vary according to several factors such as variety, stage of ripening, and geological origin [7]. In Morocco, to our knowledge, there are a few studies that were carried out on the chemical composition of some apple varieties.

This research aims to determine the physicochemical properties of different varieties of apples in Morocco and highlights the many facets of apples, including their physical characteristics, chemical constituents, and nutritional profiles.

The physico-chemical study of the different varieties of apples grown in Morocco will provide a better understanding of the diversity of these emblematic fruits. Through the meticulous analysis of their physical and chemical attributes, we strive to unlock the full potential of apples as a valuable and versatile agricultural commodity, contributing to sustainable food systems and promoting human health and well-being.

2 Materials and methods

2.1 Plant material

Fresh, mature, and ripe cashew apples were picked from a local plantation in Imouzzer Kandar, Morocco (at 33°44′ North, 5°01′ West) during the maturity season. The fruits were transported straight from the site of collection to the Laboratory.

Nine varieties were selected for this study. Two varieties, known as the Lbeldi (P1) and Ahjjani (P2) apples, are unmarketable fruits, due to their small size and wild character, while the seven varieties (P3–P9) are among the dessert apples most widely grown and marketable in Morocco. The description of the different varieties is shown in Table 1 (Supplementary S1).

2.2 Physicochemical characterization of apples

To characterize the physicochemical quality of apples, the following parameters are studied: juiciness, pH, soluble solid content, and dry matter (DM). These parameters are measured using apple juice.

Determining DM involves heating a quantity of the apple sample at 105°C until a constant mass is obtained. It is expressed as a percentage. Determining the juiciness of apples is based on compressing the fruit to extract juice from a defined quantity of apple. The results are calculated as the fraction of the volume of juice extracted (in mL) over the quantity of apple (g) put under pressure. The values obtained are expressed as a percentage (%, v/p). The pH is measured using a 210 pH microprocessor electro-meter. The total soluble solids (TSS) content of the juice is measured by Brix degree using a pocket refractometer (0–40% Brix) (Model ATAGO Pocket Refractometer).

2.3 Phytochemical characterization

To characterize the phytochemical quality of apples, the following parameters are studied: total polyphenols, total flavonoids, flavonols, flavones, anthocyanins, and condensed tannins (CT). The levels of these various components are determined using apple extracts.

2.3.1 Preparation of the extracts

Apple extracts were prepared using the method described by [14]. Fresh apples were washed with tap water and air-dried. Using a slicer, the apples were cut lengthways into 12 pieces, with the seeds removed. The pieces are crushed at a relatively low temperature using a blender for 10 s to obtain a grind. A volume of 50 mL of 80% aqueous methanol was added to 10 g of the material obtained, and the whole was reground at maximum speed for 1 min and then filtered through filter paper. For optimum extraction, the crushed material was washed twice with 30 mL of methanol. The whole mixture was evaporated under vacuum at a temperature of 40°C. The product obtained is the apple extract, which will be stored at 4°C for later use. The extraction yield (*R*) is determined using the formula proposed in the literature.

2.3.2 Total polyphenolic content

The total polyphenol content of the various samples was determined using the method described by Kara et al. [15]. This involves mixing 0.1 mL of Folin–Ciocalteu reagent (25%) with 0.1 mL of apple extract diluted in methanol (10 mg extract/mL MetOH). The mixture was then vortexed vigorously and 2 mL of sodium carbonate solution (2%) was added. After 30 min incubation at room temperature, the optical density was measured spectrophotometrically at 760 nm.

2.3.3 Total flavonoids content

Yang et al. [16] described the procedure for flavonoid determination. One milliliter of each methanolic extract (10 mg/mL) is added to 1 mL of 2% aluminum chloride methanolic solution. After 15 min incubation at room temperature, the absorbance of the samples was measured in an ultraviolet (UV)–visible spectrophotometer at 430 nm [16].

2.3.4 Flavones et flavonols

Flavones and flavonols were determined by mixing 0.5 mL of the methanolic extract in a test tube with 1.5 mL of ethanol, 0.1 mL of the methanolic solution of $AlCl_3$ (10%; v/v), 0.1 mL of CH_3COONa, and 2.8 mL of distilled water. The solution was incubated at room temperature for 30 min. A UV–visible spectrophotometer was used to measure absorbance at 415 nm [15].

Table 1: Studied apple varieties

Varieties	ID	Description	Date of harvest
Ahjjani	P1	Small size, red skin, flesh not very melting, acidic, astringent and sour.	September
Lbeldi	P2	Size of cherry, melting flesh, acidic, astringent and sour.	September
Starking Delicious	P3	Shiny dark red skin, elongated shape, delicately sweet, juicy.	September
Red Delicious	P4	Red skin, elongated shape, melting and floury flesh, delicately sweet, juicy.	September
Story	P5	Intense and very attractive red skin, very dense and firm, very sweet with very low acidity.	October
Gala	P6	Hard but smooth skin, with pink-orange stripes on a yellow background. Sweet, slightly acidic with a hint of bitterness, firm, juicy, very crisp and tonic.	September
Golden Limosin	P7	Yellow, sweet, slightly acidic, firm and juicy, very crisp and invigorating.	October
Golden Delicious	P8	Yellow, delicately sweet, juicy, sweet taste, crunchy texture, attractive, uniform shape.	October
Granny Smith	P9	Tart, very firm, crunchy, juicy, cookable or chewable, fairly thick-skinned.	September

2.3.5 Total anthocyanins

Total anthocyanins are determined using the pH difference method described by Lee et al. [17], which is used to calculate total anthocyanin content. The samples were dissolved in buffers of pH 1.0 and 4.5. The absorbance of the solutions was measured at 510 and 700 nm.

The following equation is used to calculate the final absorbance and total anthocyanin content:

$$\text{Total anthocyanins } (\mu g/g) = \frac{A \times \text{MV} \times \text{FD} \times 10^3}{\varepsilon \times l}$$

where $A = (A510\text{–}A700)_{\text{pH } 1,0} - (A510\text{–}A700)_{\text{pH } 4,5}$, MW: tmolecular weight of 3-glucose cyanidin (449.2 g/mol), DF: dilution factor, ε: molecular extinction coefficient, L mol^{-1} cm^{-1}, and l: pathlength (1 cm).

2.3.6 CT

The calorimetric test, as described by [18], is used to determine the tannin content of the samples. Briefly, 100 µL of the apple extract sample (10 mg/mL) is mixed with 500 µL of Folin–Ciocalteu and 1 mL of sodium carbonate (7.5%). Absorbance was measured at 760 nm after 30 min incubation. The values for CTs in vinegar are expressed in equivalent micrograms of tannic acid per gram of extract (µg EAT/g).

2.3.7 Identification of organic acids using high-performance liquid chromatography (HPLC)-UV

Organic acids were carried out using HPLC according to the method described by Bozan et al. [19].

To extract the organic acids, 50 mg of the sample and 1 mL of ultra-distilled water were mixed. The mixture was placed in an ultrasonic water bath at 80°C for 15 min and then sonicated and centrifuged at 5,500 rpm for 15 min. The mixture was then filtered (Whatman nylon syringe filters, 0.45 µm, 13 mm/diameter) prior to analysis by HPLC (Shimadzu LC 20 A VP) equipped with a UV detector (Shimadzu SPD 20 A VP), and an 87 H column (5 µm, 300 mm × 7.8 mm [I.D.], Transgenomic). The injection volume is 20 µL, and the detection wavelength is 210 and 242 nm.

The identification of organic acids and the determination of peaks are based on the retention time of the peaks and the comparison of spectral data according to five standards used, malic, citric, succinic, fumaric, and L-ascorbic acids. The acids identified were evaluated according to the calibration curves of the corresponding standards. Results are expressed in mg per 100 g.

2.4 Statistical analysis

The graphs used in this study were created using GraphPad Prism 8.0.1. A one-factor analysis of variance (ANOVA) and Tukey's post hoc test were used for statistical analysis. Differences were considered significant at $p < 0.05$. Principal components analysis (PCA) was performed using Minitab 19.1 statistical software.

3 Results and discussion

3.1 Physico-chemical characterization of fruit

The pH values recorded in our study appear to be similar for most of the varieties studied. They ranged from 3.40 ± 0.148 to 4.66 ± 0.30 (Table 2). According to the statistical ANOVA, the pH values for Ahjjani (P1) and Lbeldi (P2) and Golden Delicious (P8) are lower than those recorded for the other varieties. These values indicate the acidic nature of apples. The high acidity of the two unmarketable varieties Ahjjani (P1) and Lbeldi (P2) can be explained by their richness in organic acids, as shown in Table 3. Generally, our results are in line with several studies which also note that the variation in pH values is between 3 and 5 [7].

The °Brix values vary according to the studied varieties, ranging from 13.00 ± 0.00 to 21.050 ± 0.212 °Brix (Table 2). The average is 17.77 °Brix and the standard deviation is 2.85 °Brix. This index is proportional to the level of soluble sugars. These values also show that these samples are a better indicator of taste quality. These results are higher than those found by Coseteng and Lee for apples at the end of ripening before storage [20].

Total DM represents the soluble and insoluble DM contained in the apple varieties studied. The results of this analysis show that DM varies according to the variety studied. The Red Delicious (P4) and Gala (P6) varieties contained very high quantities compared with the other varieties, respectively, 35.030 ± 0.424 and 35.24 ± 0.424% (Table 2). The Ahjjani variety (P1) represents an intermediate value (26.55%), while the Lbeldi (P2), Story (P5), Golden Limosin (P7), Red Delicious (P8), and Granny Smith (P9) varieties have almost similar quantities, not exceeding 22.4%. In comparison with the results of [21], our samples show high DM values, particularly for Golden Delicious and Granny Smith. These authors found quantities of less than 16.92% in these two varieties, of which 67.60 and 56.61% represent the percentage of soluble DM in the two

Table 2: Physicochemical parameters of different varieties studied

Varieties	ID	pH	Brix °B	Dry matter (%)	Juiciness (%, p/v)
Ahjjani	P1	3.50 ± 0.14[b]	21.10 ± 0.14[a]	26.55 ± 1.34[b]	61.06 ± 1.50[b]
Lbeldi	P2	3.50 ± 0.00[b]	21.05 ± 0.21[a]	20.79 ± 0.28[c]	60.65 ± 1.20[b]
Starking Delicious	P3	4.50 ± 0.14[a]	16.09 ± 0.16[d]	21.17 ± 0.11[c]	69.22 ± 0.73[a]
Red Delicious	P4	4.30 ± 0.28[a]	17.00 ± 0.42 [cd]	35.03 ± 0.42[a]	68.66 ± 0.13[a]
Story	P5	4.66 ± 0.28[a]	19.00 ± 0.57[b]	21.55 ± 0.30[c]	69.58 ± 0.97[a]
Gala	P6	4.46 ± 0.08[a]	18.50 ± 0.71[bc]	35.24 ± 0.42[a]	68.70 ± 0.57[a]
Golden Limosin	P7	4.00 ± 0.14[a]	20.00 ± 0.57[ab]	22.39 ± 0.26[c]	68.25 ± 0.49[a]
Golden Delicius	P8	3.40 ± 0.15[b]	14.50 ± 0.00[e]	15.37 ± 0.52[d]	68.74 ± 0.23[a]
Grany Smith	P9	4.00 ± 0.00[a]	13.00 ± 0.00[e]	14.97 ± 0.17[d]	71.00 ± 0.42[a]
Total		36.32 ± 1.2	160.24 ± 2.78	213.06 ± 3.82	605.86 ± 6.24
Max		4.66 ± 0.28	21.1 ± 0.71	35.24 ± 1.34	71 ± 1.5
Min		3.4 ± 0.14	13 ± 0.00	14.97 ± 0.11	60.65 ± 0.13
Average		4.04 ± 0.13	17.80 ± 0.31	23.67 ± 0.42	67.32 ± 0.70
SD		0.48 ± 0.10	2.87 ± 0.26	7.39 ± 0.37	3.75 ± 0.45

The letters (a–e) mean the significant difference between the means in the same column.

varieties, respectively. In fact, apples are characterized by their high content of polysaccharides, polyphenols, and water-soluble vitamins such as vitamin C and B vitamins B1–B12 [7,21]. Our study shows that DM content depends on the variety, and the difference in soluble DM content between the different varieties studied can be explained by the texture and biochemical composition of the fruit.

The results obtained for the juiciness parameter are shown in Table 2. The values recorded range from 60.65 ± 1.202 to 71.00 ± 0.42%. The Ahjjani (P1), Lbeldi (P2), and Red Delicious (P4) varieties had significantly lower values than the other varieties. These results can be explained by the ripeness, texture, and/or firmness of the fruit. As shown by Mehinagicen et al., juiciness is more strongly expressed in fresh, crunchy fruits (Starking Delicious, Story, Gala, Golden Limosin, Golden Delicious, Fuji, and Granny Smith) than in the generally less appreciated mealy fruits (Red delicious, etc.). Moreover, this property is strongly influenced by the choice of variety and the length of storage. Juiciness decreases significantly between the harvest date and after 5 months' storage, whatever the stage of ripeness at harvest [22].

3.2 Phytochemical characterization

3.2.1 Extraction yield

The results shown in Figure 1 show the rate of extraction of metabolites from apple fruit. It can be clearly seen that the rate varies according to variety. Statistical analysis shows that the difference is significant at $p < 0.05$.

However, extraction yields were very high for the Ahjjani (P1) and Golden Delicious (P8) varieties, with yields 18.7 ± 0.141 and 17.1 ± 0.99%, respectively. These varieties contain high content of polar and soluble solid compounds,

Table 3: Organic acids quantified in different apple varieties (mg/100 g)

Varieties	ID	L-Ascorbic acid	Citric acid	Succinic acid	Fumaric acid
Ahjjani	P1	107.96	ND	1807.84	ND
Lbeldi	P2	462.86	104 267.2	61911.46	ND
Starking delicious	P3	ND	ND	11345.28	43.98
Red delicious	P4	ND	ND	3683.54	5.96
Story	P5	ND	ND	7247.88	32.14
Gala	P6	ND	ND	17525.5	134.36
Golden limosin	P7	ND	ND	2749.86	4.42
Golden delicious	P8	ND	214.62	235.88	5.62
Granny smith	P9	ND	ND	ND	ND

ND: not detected.

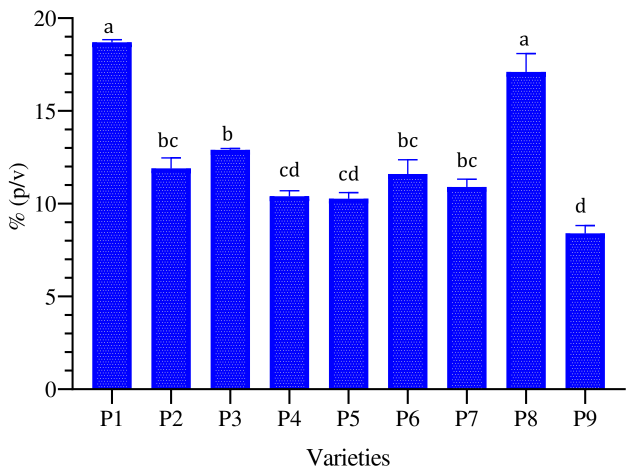

Figure 1: Metabolite extraction yield from apple fruit.

such as polysaccharide, organic acids, and polyphenols. The other varieties had yields of no more than 12.9 ± 0.07%. Granny Smith (P9) had the lowest yield (8.4 ± 0.424%).

3.2.2 Total polyphenolic content

The results obtained are shown in Figure 2 and show a significant difference between the different varieties studied. The maximum content was observed in Ahjjani apples (P1) with a concentration of 1154.65 ± 13.54 µg EAG/g of extract. The minimum value was recorded in the Golden Limosin variety (P7) with a concentration of 82.63 ± 16.52 µg EAG/g of extract. Our results remain significantly lower than those found by [23]. These authors showed that the average total polyphenol content varied from 66.2 to 211.9 mg/100 g of fresh fruit.

From these results, we can see that the quantity of polyphenols depends on the geographical origin, genotype, and

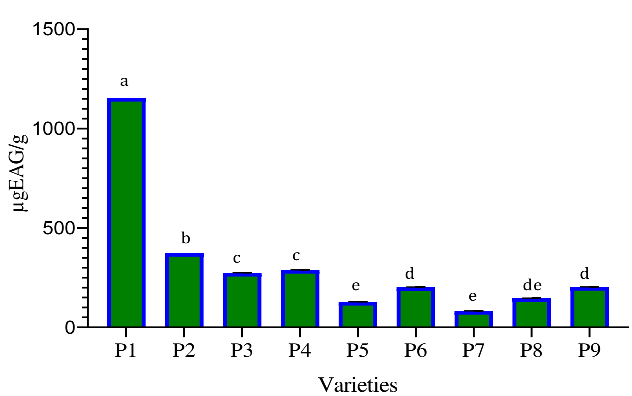

Figure 2: Total polyphenol content in the different varieties studied (µg EAG/g extract).

varietal profile. The two unmarketable varieties Ahjjani (P1) and Lbeldi (P2) are very rich in polyphenols compared with the marketable varieties tested in this study. These results can be explained by the fact that, in the region studied, these varieties are not treated or maintained by farmers. As a result, and to resist the various biological and abiotic attacks, they favor the synthesis of polyphenols. Thus, determining the polyphenol content of unmarketable apple varieties was therefore a crucial element of this research. It showed that the compounds contained in the apples tested could constitute a healthy alternative and, consequently, easily compete with commercial varieties used to make by-products such as vinegar, jam, and other products.

Similarly, these results are in line with those of Kahle et al. [23] and Soler et al. who noted that total polyphenol content varies from 154 to 178 mg/L in marketable apples. For older varieties of cider apples, however, levels vary between 261 and 970 mg/L [24].

Hydroxycinnamic acid (HCA) derivatives, flavan-3-ols (monomeric and oligomeric), flavonols and their conjugates, dihydrochalcones, and procyanidins are among the phenolics found in different apple cultivars [25]. In addition, studies have shown that several factors can influence the polyphenol content in fruit, namely the extraction method used, the varietal profile, the genotype, the growth stage, the storage method, and the storage time [20,26–29]. Among the polyphenols contained in apple varieties, chlorogenic acid and (−)-epicatechin are the most dominant components, while the (+)-catechin is the weakest component.

Indeed, Kahle et al. have shown that the main HCA present in apple fruit is chlorogenic acid, accounting for 87% of total polyphenols. Furthermore, with regard to dihydrochalcones, which are mainly found in apple fruit, phlorizidine (phloretin 2′-β-D-glucoside) and phloretin 2′-β-D-xylosyl-(1→6)-β-D-glucoside are the main components [23]. Other polyphenol molecules are identified by [13], such as hyperoside, isoquercitrin, avicularin, rutin, and quercitrin. Hyperoside is the main quercetin glycoside present in apple fruit.

3.2.3 Total flavonoid content

The results for flavonoids and flavones are highly variable and depend on the apple variety (Figures 3 and 4). By performing an ANOVA on the results obtained, we can clearly see a significant difference between the different varieties. The maximum levels of flavonoids (75.074 ± 2.309 µg EQ/g) and flavones (45.074 ± 2.309 µg EQ/g) were obtained for the Story variety (P5).

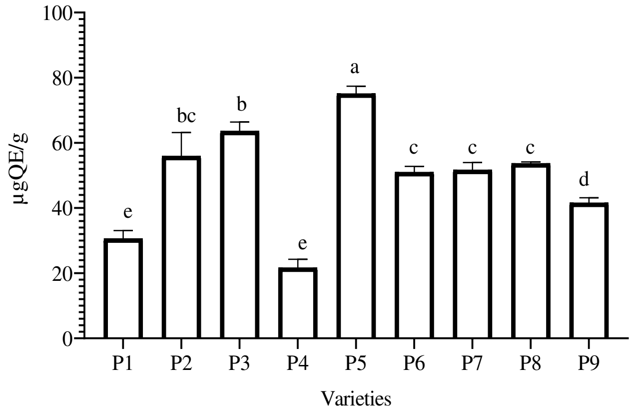

Figure 3: Total flavonoid content in the different varieties studied (μg EQ/g extract).

In fact, flavonoids are synthesized in particular sites of the plant and are responsible for the color and aroma of the fruit [30], which is the case for the red varieties studied in our study, such as Lbeldi (P2), Starking Delicious (P3), Red Delicious (P4), Story (P5). and Gala (P6). In addition, flavonoids play a role in frost resistance and drought resistance and may play a functional role in plant heat acclimatization and frost tolerance [31]. The high flavonoid content of these varieties makes them an important source of antioxidants, anti-cancer, and anti-microbial agents.

Compared with other phenolic compounds, flavanols are often abundant in fruit. In apples, two sub-classes of flavanols have been identified: catechin and oligomeric procyanidins are the main class of apple polyphenols, accounting for more than 80%, followed by HCA (1–31%), flavonols (2–10%), dihydrochalcones (0.5–5%), and, in red apples, anthocyanins (1%) [11].

Apart from the varietal profile, other factors can influence total flavonoid levels. Several studies have shown that ripening and storage period can modify flavonoid levels. According to the same authors, cold storage can lead to a drop in flavonoid content. This is due to the cessation of synthesis and conversion of ortho-diphenols (ODPs) into other compounds. In the literature, it is also noted that flavonols increase except for catechins, which decrease during storage.

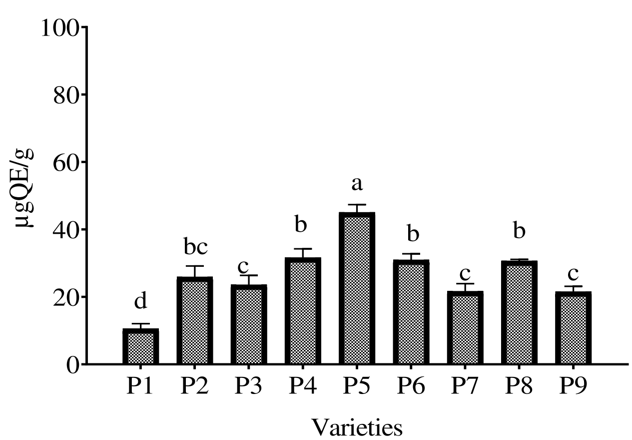

Figure 4: Flavone/Flavonol content in the different varieties studied (μg EQ/g extract).

3.2.4 CTs

CTs are among the phenolic compounds that represent significant quantities in apple fruit. In our study, we found that the CT content was very high in the Ahejjani variety (P1) compared with the other varieties, with a content of 514.09 ± 32.40 μg EAT/g (Figure 5). However, the other varieties (P2 to P9) had similar values, ranging from 17.128 ± 0.239 to 62.015 ± 8.054 μg EAT/g of extract.

The maximum content obtained for P1is explained by the fact that this variety has a bitter and astringent taste. Indeed, flavan-3-ols and tannins have long been considered to be responsible for this taste aspect in fruit [32–34]. In addition, According to the study of Bravo et al., CT and soluble polyphenols cross-link with proteins and inhibit digestive enzymes, thereby impacting protein digestibility and possibly increasing endogenous nitrogen excretion [35]. Therefore, the use of the Ahjjani variety (P1) must take into account its high CT content, which must either be eliminated or extracted and valorized in the industrial sector.

3.2.5 Anthocyanins

Anthocyanins belong to the group of pigments responsible for coloring, particularly fruit. In contrast to the other parameters, the anthocyanin levels obtained in the

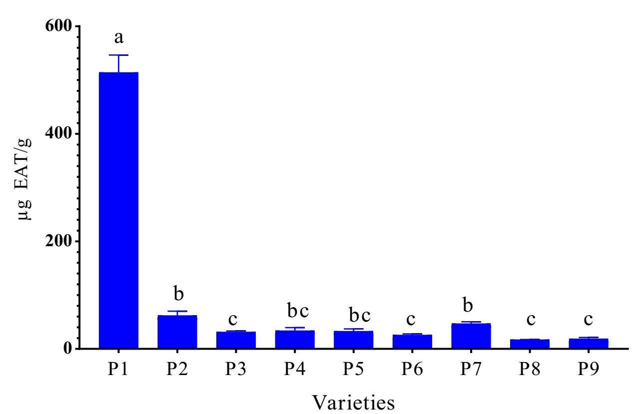

Figure 5: CT content in the different varieties studied (μg EAT/g extract).

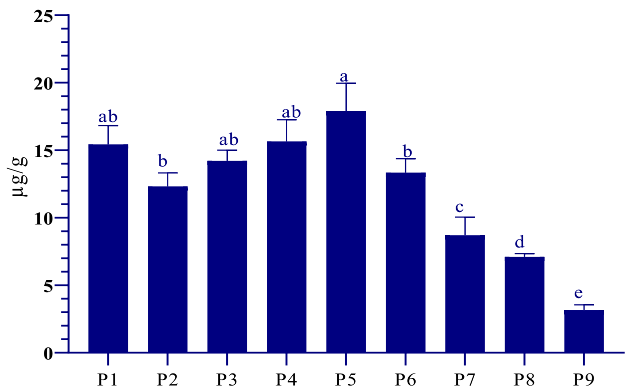

Figure 6: Anthocyanin content in the different varieties studied (μg/g).

different varieties studied were slightly variable. Values ranged from 3.160 ± 0.386 μg EAT/g for the variety (P9) to 17.900 ± 2.057 μg EAT/g for the variety (P5). The high content of anthocyanins is observed in the red varieties (P1 to P6) in comparison with the yellow and green ones (P7, P8, and P9) (Figure 6). This result can be explained by the nature of the pigments contained in apple fruit, and especially in the peel of red-colored apples [10]. As reported by Harborne, the main classes are red to violet monomeric anthocyanins [36]. In plants, these molecules are also involved in adaptation to the climate change, UV light exposure, mechanical damage, pathogen attacks, flooding, low-oxygen conditions, and other types of stress [31,37,38]. The presence of anthocyanins in the fruit can contribute to human health as anti-inflammatory, antioxidant, anti-obesity, and anti-diabetic agents [39].

3.2.6 Identification of organic acids by HPLC

The organic acids in our samples were determined by HPLC-UV using four reference molecules (Table 3 and Supplementary S2).

The results obtained are expressed in mg/100 g of extract. L-Ascorbic acid was observed in the Ahejjani (P1) and Lbeldi (P2) varieties with concentrations of 107.96 and 462.86 mg/100 g of extract, respectively. Citric acid was only found in the Lbeldi (P2) variety with a maximum concentration of 104,267.2 mg/100 g and Golden Delicious (P8) with a content of 214.62 mg/100 g, respectively.

Except for the Granny Smith variety (P9), succinic acid was identified in almost all the varieties studied. As for

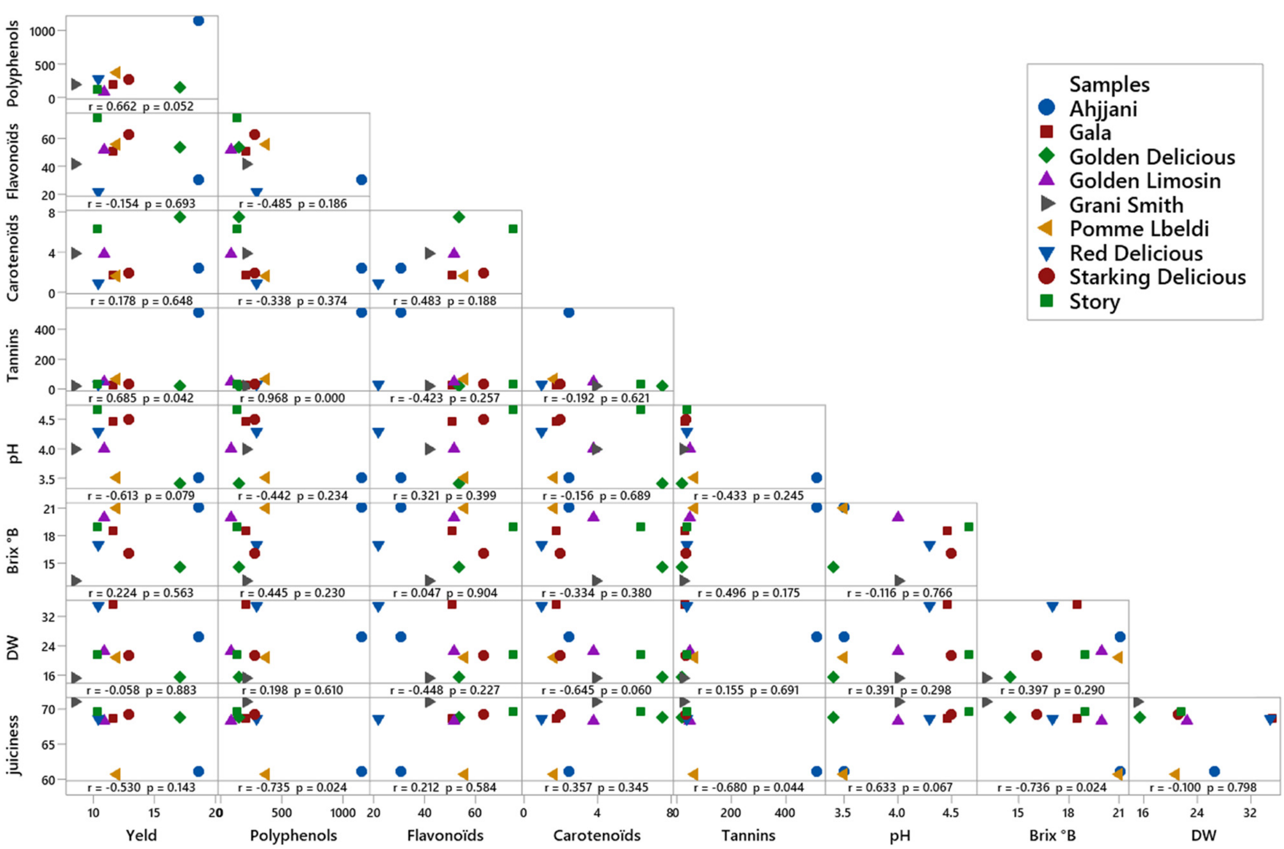

Figure 7: Pearson correlation between different studied parameters.

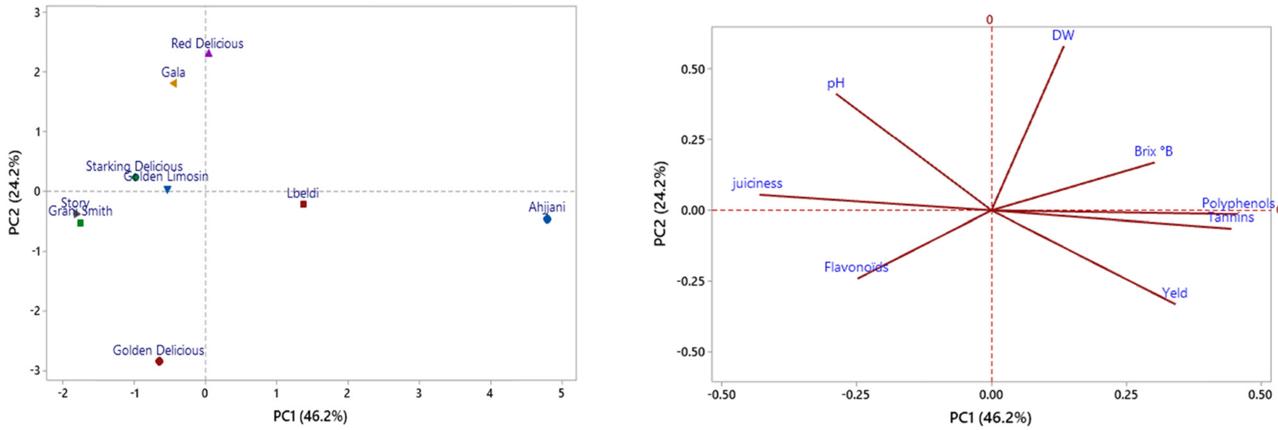

Figure 8: Principal component analysis of different studied parameters.

fumaric acid, its concentration was found to vary from 4.42 mg/100 g in the Gala variety (P6) to 134.36 mg/100 g in the Golden Limosin variety (P7), while it was not identified in the Ahjjani (P1), Pomme Lbeldi (P2), and Granny Smith (P9) varieties. In general, apples are known to contain little ascorbic acid [40].

Other organic acids identified by Kunicka-Styczyńska and Pogorzelski in apples include (*S*)-(−)-malic acid, which accounts for over 90% of the acids, pyruvic acid, tartaric acid, citric acid, lactic acid, and succinic acid [41]. Indeed, according to the study carried out by Ma et al. [42], apples of the genus Malus accumulate mainly malic acid and citric acid, while wild (unmarketable) fruit has significantly higher levels of organic acid content than marketable fruit. The organic acid content of fruits and juices influences their flavor, stability, nutrition, acceptability, and keeping quality. In addition, they are important in the processing of gelling products, as they affect the gelling property of pectin. The sugar/acid ratio determines the taste and flavor of apples [7,43]. Therefore, the processing of these varieties must consider the content of these organic acids.

3.3 Principal component analysis and correlation between different parameters

In the dataset shown in Figure 7, polyphenols and tannins exhibit a positive correlation with Yeld (r = 0.662 and r = 0685, respectively) indicating that hydro-methanolic extraction was optimized. Tannins were correlated positively with polyphenols (r = 0.968), which is logical since tannins are classified among the polyphenols. Polyphenols, tannins, and brix have a negative correlation with juiciness (r = −0.680, r = −0.735, and r = −0.736, respectively). In addition, Juiciness reveals a strong negative correlation with Tannins (r = −0.680), polyphenols (r = −0.735), and Brix (r = −0.736). The large quantity of juice in the fruit reflects a high-water content, which has a negative influence on the quantity of soluble solids and therefore on the Brix value. These results also show that during the extraction process, a large quantity of insoluble polyphenols and tannins remain attached to the apple residue (pomace). These correlations offer insights into the relationships among the various variables in the dataset.

Figure 8 shows the score plot (A) and the loading plot (B) of different parameters used in this study. The first principal component (PC1) explains the highest amount of variance in the data (46.2%). PC1 is mainly influenced positively by Polyphenols, Yield, Tannins, and Brix °B, while negatively influenced by Flavonoids, pH, and Juiciness. This axis is mainly associated with the two unmarketable apple varieties, particularly Ahjjani Variety. This explains by the fact that these varieties are rich in polyphenols, tannins, and soluble solids.

The second principal component (PC2) explains the second-highest amount of variance (24.2%). PC2 is positively influenced by pH, Brix °B, and DW and negatively influenced by Flavonoïds. The third principal component (PC3) explains 13.3% of the total variance. PC3 is positively influenced by Flavonoïds and Brix °B and negatively influenced by Polyphenols and Juiciness. These results clearly show that unmarketable varieties are very important thanks to their richness in bioactive compounds and can be used in processing.

4 Conclusion

The aim objective of this study was to determine the phytochemical properties of different varieties of apples in

Morocco. Physicochemical and phytochemical including polyphenols, flavonoids, CTs, anthocyanins, and organic acid compounds were determined. The apples studied were generally juicy with an acid pH. The Brix parameter represents values above 13°Brix. Total polyphenols and CTs were higher in the Ahjjani variety than in the other varieties. This shows their good nutritional quality even though they are not marketable. Flavonoids, on the other hand, are dominant in the Story variety. The identification of organic acids showed that succinic acid was the most abundant in all the apple varieties studied. These results lead us to conclude that the unmarketable varieties are also considered an important source of bioactive compounds with high nutritional value, particularly polyphenols. As a result, the production of these cultivars should increase and be used to produce dietary supplements, vinegar, and other products. It is therefore worth pointing out that these varieties can be used to produce other by-products such as compote, jam, and vinegar. A more detailed understanding of the variability in phytochemical characteristics and fruit quality of these apple varieties will be useful for the future selection of a new apple genotypes with improved dietary quality and processing characteristics suitable for the manufacture of apple-based products.

Acknowledgments: Authors wish to thank Researchers Supporting Project Number (RSP2024R110) at King Saud University Riyadh Saudi Arabia for financial support.

Authors contributions: M.K., H.S., and N.H. – conceptualization; A.A., M.F., I.V., and R.U. – formal analysis; M.K., Y.A., T.H., and I.V. – investigation; M.K., N.H., A.Amar., and J.B. – writing – original draft; M.K., A.Z., and N.H. – software; M.K., H.S., M.E., and N.H. – writing – review and editing; A.Z. and J.B. – supervision. All authors have read and agreed to the published version of the manuscript.

Conflict of interest: The authors declare no conflict of interest.

Ethical approval: The conducted research is not related to either human or animal use.

Data availability statement: All data generated or analyzed during this study are included in this published article.

References

[1] Oukabli A. Le pommier, une culture de terroir en zone d'altitude, bulletin mensuel d'information et de liaison du pntta N°115. INRA. 2004 [cited 2022 Mar 2]. https://inrameknes.info/? p = 1686.
[2] Oukabli A, Laghezali M, Chahbar A. Phenology and fruiting of apple in a zone with mild winters (Morocco). Fruits. 1998 [cited 2022 Mar 8];2(53):105–17. https://www.infona.pl//resource/bwmeta1.element.elsevier-e536b841-3785-3d0a-a1f3-820f9c5a2e62.
[3] Khachtib Y, Bouda S, Ait Bella Y, Haddioui A. Pomological Evaluation of Apple (Malus x domestica Borkh) Germplasm in Morocco. Phytomorphol Int J Plant Morphol. 2020 jun;70:1–9.
[4] UPOV. International Union for the Protection of New Varieties of Plants (UPOV). 2005 [cited 2022 Mar 8]. https://www.upov.int/portal/index.html.en.
[5] MAPM, bulltin officiel N° 6218. bulletin officiel, texte particulier, N° 6218-29 Safar 1425 (2-1-2014). 2014.
[6] MAPM, bulltin officiel, No 6854. bulltin officiel, No 6854 – 11 joumada II 1441 (6-2-2020), Arrêté du ministre de l'agriculture, de la pêche maritime, du développement rural et des eaux et forêts n° 4014-19 du 21 rabii II 1441 (18 décembre 2019) portant reconnaissance de l'indication géographique «Pomme du Haouz» et homologation du cahier des charges y afférent. [Internet]. 2020. http://www.onssa.gov.ma/images/reglementation/reglementation_connexe/SDOQ/BO_6854_Fr_IG_Pomme_du_Haouz.pdf.
[7] Wu J, Gao H, Zhao L, Liao X, Chen F, Wang Z, et al. Chemical compositional characterization of some apple cultivars. Food Chem. 2007 Jan [cited 2022 Sep 29];103(1):88–93. https://www.sciencedirect.com/science/article/pii/S0308814606006157.
[8] Tsao R, Yang R, Young JC, Zhu H. Polyphenolic profiles in eight apple cultivars using high-performance liquid chromatography (HPLC). J Agric Food Chem. 2003 Oct [cited 2022 Jul 23];51(21):6347–53. doi: 10.1021/jf0346298.
[9] Jakobek L, Boc M, Barron AR. Optimization of ultrasonic-assisted extraction of phenolic compounds from apples. Food Anal Methods. 2015 Nov [cited 2021 Jun 30];8(10):2612–25. http://link.springer.com/article/10.1007/s12161-015-0161-3.
[10] Jakobek L, García-Villalba R, Tomás-Barberán FA. Polyphenolic characterisation of old local apple varieties from Southeastern European region. J Food Compos Anal. 2013 Sep [cited 2023 Dec 23];31(2):199–211. https://www.sciencedirect.com/science/article/pii/S0889157513000781.
[11] Wojdyło A, Oszmiański J, Laskowski P. Polyphenolic compounds and antioxidant activity of new and old apple varieties. J Agric Food Chem. 2008 Aug;56(15):6520–30.
[12] Djina B, Vučeta J, Biljana L. Old apple varieties in central montenegro. Agric For. 2013 May;59:217–23.
[13] Liaudanskas M, Viškelis P, Kviklys D, Raudonis R, Janulis V. A comparative study of phenolic content in apple fruits. Int J Food Prop. 2015 May [cited 2022 Sep 29];18(5):945–53. doi: 10.1080/10942912.2014.911311.
[14] Pearson DA, Tan CH, German JB, Davis PA, Gershwin ME. Apple juice inhibits human low density lipoprotein oxidation. Life Sci. 1999 [cited 2021 Dec 19];21(64):1913–20. https://www.infona.pl//resource/bwmeta1.element.elsevier-55eaa43f-3bb6-30cf-8d15-4fb42ed4304d.
[15] Kara M, Assouguem A, Fadili ME, Benmessaoud S, Alshawwa SZ, Kamaly OA, et al. Contribution to the evaluation of physicochemical properties, total phenolic content, antioxidant potential, and antimicrobial activity of vinegar commercialized in Morocco. Molecules. 2022 Jan [cited 2022 Mar 13];27(3):770. https://www.mdpi.com/1420-3049/27/3/770.
[16] Yang JF, Yang CH, Liang MT, Gao ZJ, Wu YW, Chuang LY. Chemical composition, antioxidant, and antibacterial activity of wood vinegar

from Litchi chinensis. Molecules. 2016 Aug [cited 2021 Feb 6];21(9):1150. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6274201/.
[17] Lee J, Durst RW, Wrolstad RE, Collaborators, Eisele T, Giusti MM, et al. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the PH differential method: collaborative study. J AOAC Int. 2005 Sep [cited 2023 May 8];88(5):1269–78.https://academic.oup.com/jaoac/article/88/5/1269-1278/5657437.
[18] Smit CJB, Joslyn MA, Lukton A. Determination of tannins and related polyphenols in foods. Anal Chem. 1955 Jul [cited 2021 Apr 25];27(7):1159–62. doi: 10.1021/ac60103a035.
[19] Bozan B, Başer KHC, Kara S. Quantitative determination of naphthaquinones of Arnebia densiflora (Nordm.) Ledeb. by an improved high-performance liquid chromatographic method. J Chromatogr A. 1997 Oct [cited 2022 Jun 22];782(1):133–6. https://www.sciencedirect.com/science/article/pii/S0021967397004603.
[20] Coseteng MY, Lee CY. Changes in apple polyphenoloxidase and polyphenol concentrations in relation to degree of browning. J Food Sci. 1987 [cited 2022 Oct 1];52(4):985–9. https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2621.1987.tb14257.x.
[21] Karakasova L, Stefanoski A, Rafajlovska V, Klopceska J. Technological characteristics of some apple cultivars. Acta Hortic. 2009;825(825):559–64
[22] Mehinagic E, Madieta E, Cedex A. Comment mesurer objectivement la jutosité des pommes? Revue suisse Vitic Arboric Hortic. 2009;41(2):127–31.
[23] Kahle K, Huemmer W, Kempf M, Scheppach W, Erk T, Richling E. Polyphenols are intensively metabolized in the human gastrointestinal tract after apple juice consumption. J Agric Food Chem. 2007 Dec;55(26):10605–14.
[24] Soler C, Soriano JM, Mañes J. Apple-products phytochemicals and processing: A review. Nat Prod Commun. 2009 May [cited 2021 Dec 19];4(5):1934578X0900400504. doi: 10.1177/1934578X0900400504.
[25] Naczk M, Shahidi F. Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. J Pharm Biomed Anal. 2006 Aug [cited 2022 Jul 19];41(5):1523–42. https://www.sciencedirect.com/science/article/pii/S0731708506003062.
[26] Matthes A, Schmitz-Eiberger M. Polyphenol content and antioxidant capacity of apple fruit: effect of cultivar and storage conditions. 2009;86:6.
[27] Macheix JJ, Fleuriet A, Jay Allemand C. Les composés phénoliques des végétaux. Un exemple de métabolites secondaires d'importance économique. PPUR presses polytechniques. 2005. 212 p.
[28] Kahle K, Kraus M, Richling E. Polyphenol profiles of apple juices. Mol Nutr Food Res. 2005 Aug;49(8):797–806.
[29] Kahle K, Kempf M, Schreier P, Scheppach W, Schrenk D, Kautenburger T, et al. Intestinal transit and systemic metabolism of apple polyphenols. Eur J Nutr. 2011 Oct;50(7):507–22.
[30] Janick J. Plant breeding reviews. Vol. 25. United States of America: John Wiley & Sons; 2008. p. 493.
[31] Samanta A, Das G, Das S. Roles of flavonoids in Plants. Int J Pharm Sci Technol. 2011 Jan;6:12–35.
[32] Lea AGH, Arnold GM. The phenolics of ciders: Bitterness and astringency. J Sci Food Agric. 1978 [cited 2022 Jul 29];29(5):478–83. https://onlinelibrary.wiley.com/doi/abs/10.1002/jsfa.2740290512.
[33] Robichaud JL, Noble AC. Astringency and bitterness of selected phenolics in wine. J Sci Food Agric. 1990 [cited 2022 Jul 29];53(3):343–53. doi: 10.1002/jsfa.2740530307.
[34] Thorngate JH, Noble AC. Sensory evaluation of bitterness and astringency of 3R(−)-epicatechin and 3S(+)-catechin. J Sci Food Agric. 1995 [cited 2022 Jul 29];67(4):531–5. doi: 10.1002/jsfa.2740670416.
[35] Bravo L, Saura-Calixto F, Goni I. Effects of dietary fibre and tannins from apple pulp on the composition of faeces in rats. Br J Nutr. 1992 May;67(3):463–73.
[36] Harborne JB. The Flavonoids: Advances in Research since 1980. New York, NY: Springer; 2013. 630 p.
[37] Bakshi A, Gilroy S. Chapter 17 – Analysis of plant flooding response. In: Jez J, editor. Methods in enzymology. Academic Press; 2023 [cited 2023 Dec 25]. p. 461–91. (Biochemical pathways and environmental responses in plants: Part B; vol. 680). https://www.sciencedirect.com/science/article/pii/S0076687922003688.
[38] Kent K, Hölzel N, Swarts N. Chapter 10 – Polyphenolic compounds in sweet cherries: A focus on anthocyanins. In: Watson RR, Preedy VR, Zibadi S, editors. Polyphenols: Mechanisms of action in human health and disease. 2nd edn. Academic Press; 2018 [cited 2023 Dec 25]. pp. 103–18. https://www.sciencedirect.com/science/article/pii/B9780128130063000106.
[39] Kang I, Lee Y, Lee M. Chapter 28 – Anthocyanins: What they are and how they relate to obesity prevention. In: Watson RR, editor. Nutrition in the prevention and treatment of abdominal obesity. 2nd edn. Academic Press; 2019 [cited 2023 Dec 25]. p. 409–30. https://www.sciencedirect.com/science/article/pii/B9780128160930000288.
[40] Lemmens E, Alós E, Rymenants M, De Storme N, Keulemans WJ. Dynamics of ascorbic acid content in apple (Malus x domestica) during fruit development and storage. Plant Physiol Biochem. 2020 Jun;151:47–59.
[41] Kunicka-Styczyńska A, Pogorzelski E. L-malic acid effect on organic acid profiles and fermentation by-products in apple wines. Czech J Food Sci. 2009;27(Special Issue 1):S228–31.
[42] Ma B, Yuan Y, Gao M, Li C, Ogutu C, Li M, et al. Determination of predominant organic acid components in malus species: Correlation with Apple domestication. Metabolites. 2018 Oct [cited 2022 Oct 4];8(4):74. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6316603/.
[43] Petkovsek MM, Stampar F, Veberic R. Parameters of inner quality of the apple scab resistant and susceptible apple cultivars (Malus domestica Borkh.). Sci Hortic. 2007 Sep [cited 2023 Dec 25];114(1):37–44. https://www.sciencedirect.com/science/article/pii/S0304423807001963.