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Review Article

Recent trends on the quality assessment of Pomegranate: A detailed review

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ABSTRACT

Pomegranate (*Punica granatum*), furthermore to its ancient historical uses, has been well-known to have chemical constituents that improve human health. Pomegranate contains high levels of different phytochemicals including phenolic compounds, fatty acids, sugars, amino acids, aromatic compounds, terpenoids, sterols, alkaloids, indoleamine, etc. Ellagitannins (including punicalagin), ellagic acid, flavonoids, estrogenic flavones, anthocyanidins, punicic acid, and anthocyanins, specifically contributed to marked health beneficial activities of pomegranate. Worldwide, there are more than five hundred pomegranate cultivars with about fifty commercially available cultivars. Furthermore**,** there is a broad diversity in textural, Physico-chemical properties, and the chemical composition of fruits among cultivars grown in many countries. This review covers different studies performed on bio-diverse pomegranate cultivars from different countries. This review highlighted various techniques employed for the quality control of pomegranates including different chemical and physical properties analyses. The study proves that either genetic or environmental conditions can contribute to the various desired traits.

Keywords: *Quality control; Pomegranate; Punica granatum; Bio-diversity; Cultivar.*

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1. Introduction

Pomegranate, *Punica granatum* L., belongs to the Punicaceae family and is considered one of the oldest and most important edible fruits of subtropical and tropical regions, which originated in India and the Middle East. It is cultivated in California, Iran, Chile, Afghanistan, Mediterranean countries (Italy, Turkey, Tunisia, Egypt, Portugal, Syria, Lebanon, Spain, Morocco, and France), and some areas in South Africa, USA, Japan, Russia, and China **[1-4]**. Pomegranate is used in folkloric medicine in the treatment of different diseases like hepatic damage, ulcer, and snakebite, and has recently been considered nature's power fruit. Extracts of whole parts of the fruit have therapeutic benefits and another study proved that the bark, leaves, and roots also have medicinal benefits **[5, 6]**. Fruits of pomegranate are commonly consumed fresh or in commercial products, such as juices, vinegar oil, jam, jelly, and wines while the rind extracts are used as natural nutraceuticals and food preservatives **[7, 8]**.

Pomegranate is a valuable source of phenolic compounds which increased its potential health benefits **[9, 10]**. In murine models and human studies, pomegranate juice and different extracts exerted significant analgesic, anticancer, anti-inflammatory, sexual stimulant, neuroprotective, hypoglycemic, antiatherogenic, antioxidant, antidepressant, antimicrobial, hypolipidaemic, antiviral, antifungal, anti-Alzheimer's, estrogenic, immunomodulatory, skin protective, dental care, cardioprotective, musculoskeletal effects, gastroprotective effects, along with, hepatoprotective, antitrichomonal, anti-obesity, anti-diarrheal and nootropic activity **[11]**. The most characterized phenolic constituents in pomegranate fruit are anthocyanins, flavonoids, phenolic acids, and ellagitannins **[9, 12]**. The most medically beneficial phenolic compounds include ellagitannins (including punicalagin), ellagic acid, flavonoids, punicic acid, anthocyanins, anthocyanidins, and estrogenic flavones. Pomegranate pericarp contains punicalagin, gallic acid, quercetin, catechin, rutin, flavanones, and anthocyanidins. Pomegranate leaves contain tannins (punicafolin and punicalin), glycosides, and flavones. However, Pomegranate flowers contain gallic acid, ursolic acid, and triterpenoids, including Asiatic acid and maslinic. Pomegranate roots and barks contain ellagitannins, such as punicalagin and punicalin, and various piperidine alkaloids **[11, 13]**. Finally, the juice is a crucial source of tannins and phenols, such as punicalagin, punicalin, and ellagic acid, and in red arils genotypes, a high amount of anthocyanins, such as delphinidin, pelargonidin, and cyanidin and their glycosides have been detected. Also, it contains sugars, minerals, and vitamins **[14, 15]**.

Globally, thousands of accessions have been detected with more than 500 cultivars and about 50 commercially available cultivars **[16]**. It has been proved that a broad diversity in several textural, Physico-chemical properties and the composition of fruits occurs between cultivars grown in different countries. Furthermore, it has been proved that the quality of the fruit parameters as color, size, taste, juiciness and seed hardness, and variations in phenolic constituents, sugars, organic acids, and water-soluble vitamins constituents among cultivars are not only influenced by genetics but also could be influenced by the environmental and climate conditions. Due to the huge number of variables that may influence consumer preferences and the manufacturing ability of the fruits, it is significant to consider not only the fruit antioxidant activity and phytochemical composition, but also the physical-chemical and textural properties which are crucial in cultivar characterization, postharvest handling and marketing **[17, 18]**.

This review summarized different studies performed on broad, bio-diverse pomegranate cultivars from various countries. The review explains the changes in the physical properties as color, quality index, total soluble solids, titratable acidity and maturity index, pH, sensorial analysis, organ measurements, and textural properties in different cultivars, as well as the different chromatographic and spectroscopic techniques for characterization of different chemical constituents of pomegranate **(Fig. 1)**. The study proves that both environmental and genetic variables can contribute to different desired traits. Different pomegranate cultivars with their origin are summarized in **Table 1.**

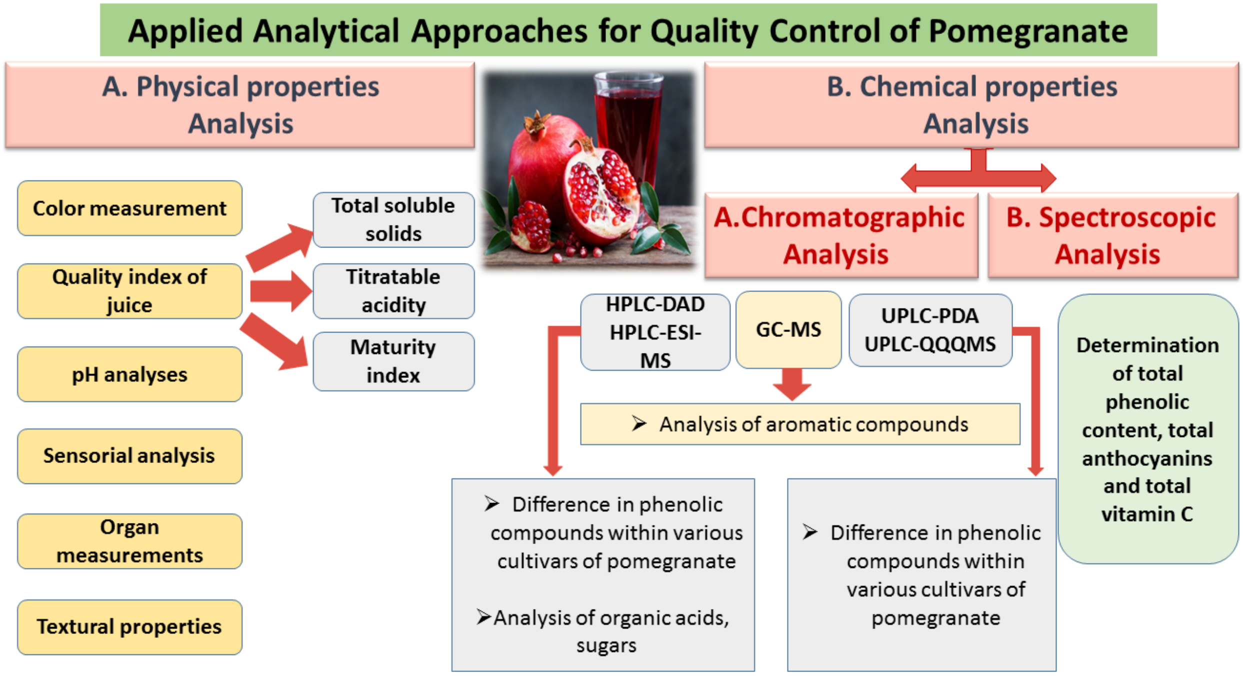

Fig. 1. Applied Analytical Approaches for Quality Control of Pomegranate

Table 1. Different Pomegranate cultivars, code, origin, country of cultivation

Cultivar	Code	Country of Origin	Country of cultivation	Reference
Mollar de Elche 1	ME1	Spain	Sweet Spanish-grown cultivars	**[2]**
Mollar de Elche 2	ME2			
Mollar de Elche 14	ME14			
Casta del Reino de Ojós 2	CRO2			
Piñón Tierno de Ojós 7	PTO7	Spain	Sour-sweet Spanish-grown cultivars	
Piñón Tierno de Ojós 8	PTO8			
Agridulce de Ojós 4	ADO4			
Borde de Albatera 1	BA1	Spain	Sour Spanish-grown cultivars	
Borde de Orihuela 1	BO1			
Mollar de Elche	M.63	Spain		**[19]**
Mollar de Elche	M.55	Spain		
Mollar de Elche	M.49	Spain		
Mollar de Elche	M.29	Spain		
Mollar de Elche	M.Leon.1	Spain		
Mollar de Elche	M.Leon.2	Spain	Spanish- grown cultivars	
Valenciana	V.46i	Spain		
Valenciana	V.111	Spain		

Valenciana	VSN	Spain		
Wonderful	W.3	USA		
Wonderful	W.2	USA		
Wonderful	W.6	USA		
Wonderful	W.5	USA		
Wonderful	W.7	USA		
Wonderful	WSN	USA		
Mollar de Elche 5	ME5	Spain	Spanish- grown cultivars	**[20]**
Mollar de Elche 16	ME16			
Mollar de Elche 17	ME17			
Mollar de Orihuela 6	MO6			
Mollar de Albatera 4	MA4			
Mollar de Albatera 5	MA5			
Borde de Albatera 1	BA1		Sour Spanish-grown cultivars	
Borde de Orihuela 1	BO1			
Borde de Beniel 1	BBE1			
Piñón Tierno de Ojós 5	PTO5		Sour–sweet Spanish-grown cultivars	
Piñón Tierno de Ojós 8	PTO8			**[21]**
Piñón Tierno de Ojós 10	PTO10	Spain		
Mollar de Elche 14	ME14		Sweet Spanish-grown cultivars	
Mollar de Elche 17	ME17			
Valenciana 1	VA1			
Piñón Tierno de Ojós 5	PTO5			
Piñón Tierno de Ojós 8	PTO8			
Mollar de Elche 13	ME13			
Mollar de Elche 14	ME14			
Mollar de Elche 17	ME17			
Mollar de Orihuela 5	MO5			
Mollar de Orihuela 6	MO6			
Mollar de Albatera 3	MA3			
Mollar de Albatera 4	MA4		Spanish- grown cultivars	**[22]**
Valenciana 1	VA1	Spain		
Valenciana 6	VA6			
Valenciana 7	VA7			
Valenciana 11	VA11			
Hizcaznar	HIZ			
Agridulce de Beniel	ADBE1			
Piñón duro de Albatera	PDA1			
Valenciana	VA			

Mollar de Elche	ME		Farmer’s market in Spain	
Wonderful	WOND	USA		
Agha Mandali Save	AMS	Yazd		**[18]**
Alak Shirin Save	ASS	Iran		
Bazmani Pust Nazok	BPN			
Dom Ambaroti	DA			
Khazar Bajestani	KB			
Lili Post Zoloft	LPK			
Malas Pust Sorkh	MPS			
Malas Save	MS			
Malas Yazdi	MY		Iranian- grown cultivars	
Pust Sefeed Dezfo	PSD			
Save Pust Ghermez	SPGh			
Save Pust Sefeed	SPSe			
Shirin Dane Ghermez Ferdows	SDGF			
Shirin Dane Sefeed Ferdows	SDSF			
Shirin Pust Ghermez	SPG			
Shirin Pust Sefeed	SPS			
Shishe Kap	SK			
Torsh Shahvar Ferdows	TSF			
Torsh Shahvar Kashmar	TSK			
Zagh Yazdi	ZY			
Gabsi 1			Sour Tunisian autochthonous cultivars	**[23]**
Mezzi 1				
Mezzi 2				
Mezzi 3				
Garoussi 2				
Gabsi 5	NA	Tunisia	Sweet Tunisian autochthonous cultivars	
Gabsi 9				
Chelfi 1				
Chelfi 3				
Zehri 6				
Garoussi 1				
Tounsi 4				
MG1			Italian old autochthonous varieties	**[14]**
MG2	NA	Italy		
MG3				
Tordimonte A				
Tordimonte B				
Gaeta 1		Italy	Italian grown-cultivars	**[9]**

Gaeta 3				
Gaeta 4	NA			
Tordimonte A				
Itri A				
Formia				
Wonderful		USA		
Sefri				**[15]**
Ounk Hmam		Ouled Abdellah		
Ruby		(Beni Mellal)		
Meski		Morocco		
Grenade Rouge		Al Ouidane		
Grenade Jaune	NA	(Marrakech)	Moroccan- grown	
		Morocco	cultivars	
Mesri		Meknes		
		Morocco		
Kharaji		Bzou		
Hamde		Morocco		
Bouaadime				
Acco			South African-grown cultivars	**[17]**
Arakta				
Bhagwa	NA			
Ganesh				
Herskawitz				
Molla de Elche		Spain		
Ruby				
Wonderful	DPun 81	USA dominant		**[16]**
Commercial Wonderful	PW-1	cultivar		
Sin Pepe	DPUN0082	USA		
Haku-botan	DPUN0007	Japan		
Fleischman	DPUN0028	Unknown		
Salavatski	DPUN0062	Turkmenistan	California-grown cultivars	
Nikitski ranni	DPUN0067	Turkmenistan		
Myagkosemyannyi Rozovyi	DPUN0139	Turkmenistan		
Nusai	DPUN0145	Turkmenistan		
Ovadan	DPUN0150	Turkmenistan		
Kara Gul	DPUN0155	Turkmenistan		
Sweet green-peel	Sweet-GP	Huili, Sichuan	Chinese-grown cultivars	**[24]**
Sweet red-peel	Sweet-RP	China		
Sour red-peel	Sour-RP			
Sour Yunnan Red-peel	Sour-YRP	Mengzi, Yunan		

		China		
Sweet Tai-mountain Red-peel	Sweet-TRP	Taian, Shandong China		
Taishanhong Taishansanbaitian	NA	China	Chinese-grown cultivars	**[25]**

2. QUALITY CONTROL METHODS

2.1. Physical properties

2.1.1. Color measurement

Pomegranate fruit color affects marketability and consumer preference **[17]**. Correspondingly, pomegranate juice color is a critical quality parameter in pomegranate marketing and processing. During fruit ripening, the color increases, and these are doubtlessly correlated to increased production of anthocyanin **[22]**. Previous studies determine the color according to the Color determinations were made in accordance to Values of the CIELAB L^* (brightness or lightness; 100 = white, 0 = black), a^* ($+a^*$ = redness, $-a^*$ = greenness), and b^* ($+b^*$ = yellowness, $-b^*$ = blueness). Color variables were measured by the Minolta chromameter. As well, the a^*/b^* ratio, the hue angle ($H^{\circ *}$), and chroma (C^*) were calculated. The hue angle and chroma had been used as more spontaneously understandable color variables. The color index (CI) was also calculated **[20]**. Mena *et al*. 2011 explored that the Hue angle can be considered as an indicator of the content of anthocyanin since it showed the strongest negative correlation followed by CIE $L*$ and CIE $b*$. Similarly, color parameters among Spanish pomegranate juices (Mollar de Elche, Valenciana) were measured and compared to Wonderful cultivars and significant differences between them were proved. Regarding CIE $b*$: the highest yellowness levels in Valenciana cultivars ranged between 29 (V.111) and 39 (V.46i) followed by Mollar de Elche samples ranging from 21 (M.Leon.1) to 42 (M.29) and then Wonderful ones ranged from 1 (W.2) to 29 (W.5). Valenciana samples showed the highest values of hue angle ranging between 58 and 71, followed by Mollar de Elche and W.5 accession ranging from 35 to 51. While Wonderful showed values lower than 30. However, values of CIE $L*$ of the pomegranate juices proved that Wonderful juices exist in their characteristic darkness unlike Spanish accessions but without observing the differences between them. The lowest brightness was found in W.2 and W.5accessions with values between 0.8 and 17, while the highest lightness was detected in the Mollar de Elche and Valenciana Spanish cultivars with values between 12 (M.Leon.1) and 39 (V.111). As well CIE $a*$ and chroma values did not confirm variation between cultivars groups and varied from 5 (W.2) to 55 (M.29) **[19]**. Calin *et al*. 2011 correlated color parameters with the sweetness or sourness of cultivars. Sweet juices contained the highest values of (a^*) with a mean of 6.56, followed by sour and sour-sweet juices with the mean values of 5.76 and 3.00, respectively. The same was for the C^* value with means of 6.71, 5.80, and 3.05. Moreover, sweet juices contained the highest values of lightness of juices with a mean of 29.24, followed by sour and sour-sweet with the means of 27.89 and 26.90, respectively **[2]**. Legua *et al*. 2012 proved a low correlation value between anthocyanins levels and color index in six Mollar Spanish pomegranate cultivars which rationale that constituents rather than anthocyanins as hydroxycinnamic acids affect aril color in these cultivars **[20]**. Fawole *et al*. 2014 proved that lower total color difference values (TCD) could be considered an index for the maturation stage, especially for cultivars that change the red color of peel rapidly during fruit ripening as the calculated TCD exhibited the difference in the color between skin and aril. Significant differences in TCD were proved between the eight commercially South African-grown pomegranate cultivars, ranging between 20.53 in Ruby and 52.59 in Acco. Therefore there was a significant difference between aril color and fruit skin in the color parameters *a**, *L**, *b**, *C,* and *h*° **[17]**. Beaulieu *et al*. 2015 investigated the color parameter of California-grown pomegranate. Haku-Botan, Fleischman, Myagkofemyannyi Rozovyi, and Sin Pepe showed the highest L* juice color. All these cultivars and Nusai except Haku Botan also showed significantly the greatest C* values. The b* color values showed red and deep red arils are near all cultivars and clustered with citric acid, TA, and total organic acids. Haku-Botan showed extreme values between all cultivars for juice b*, C*, L* and hue, for peel a*, C* and hue in the grouping and it clustered all alone. The two Wonderful cultivars showed similar L* peel color values but were not similar in all remaining color parameters. It could be attributed to soil and climactic changes. Also, a strong positive correlation between juice colors C* and a* was proved **[16]**. Legua *et al*. 2016 evaluated nineteen different pomegranate cultivars (Sixteen cultivars belonged to European pomegranate gene banks in Spain and the remaining cultivars were collected from farmer's markets) in color coordinates and confirmed a significant difference between them. The highest a* values were detected in WOND and HIZ cultivars with means of 6.52 and 6.50, respectively. The garnet color of the juice is found in HIZ, WOND, ME14, and MA4 cultivars as it is characterized by high C* and a* values and low H* and b* values **[22]**.

A color determination according to values of the CIELAB is more objective than subjective visual comparisons so it is considered an accurate and precise method. Finally, the Hue angle can be considered as an indicator for anthocyanin contents, whereas a low correlation was proved between CI and anthocyanin contents **[19, 20]**. As well, lower TCD can be considered an index of maturation status **[17]**. A strong positive correlation between juice colors a* with C* was proved and these color parameters can be correlated with the sweetness or sourness of cultivars **[2]**.

2.1.2. Quality index of juice

2.1.2.1. Total soluble solids (TSS)

Total soluble solids (TSS) determination is crucial for juice quality assessment and the proper selection of cultivars for pomegranate winemaking **[15]**. Climatic changes affect TSS, as fruit cultivated in dry locations contain often higher TSS than those in humid or irrigated farms **[26]**. Varieties and maturity stage of the pomegranate can affect TSS content **[27]**. During maturation, anthocyanin and TSS content increased continuously while the acidity declined **[28]**. TSS content of pomegranate juice was measured by the temperature compensated refractometer and data were given as °Brix. In the studied twenty cultivars grown in Iran, all were suitable for the production of pomegranate juice and consumption because the cultivars contained high levels of soluble solids. The highest TSS content was detected in Torsh Shavar Ferdows (15.07 °Brix) while the lowest one in Agha Mandali Save (11.37 °Brix), with significant differences among the other cultivars **[18]**. Five pomegranate accessions located on the farm of the University of Tuscia showed a significant difference in TSS, where the content ranged from 17.49° to 12.90° Brix in MG1 and Tordimonte A, respectively **[14]**. In contrast, Spanish pomegranates (Mollar de Elche, Valenciana) and Wonderful cultivars showed no significant differences among these cultivars except the Valenciana cultivar, which showed values below 15°Brix. TSS values varied from 13 (V.111) to

18°Brix (WSN) **[19]**. Similarly, there were no significant differences between the juices from different nine Spanish cultivars grouped into sweet (CRO2, ME1, ME2, and ME14), sour-sweet (ADO4, PTO7, and PTO8), and sour (BO1 and BA1) cultivars [2]. In contrast to previously reported Spanish cultivars, Legua *et al*. 2012 proved significant differences between six Mollar Spanish pomegranate cultivars in TSS content. ME17 juice exhibited the lowest value (14.79 °Brix), whereas MO6 showed the largest one (15.81 °Brix) **[20]**. The TSS range in the ten major Moroccan cultivars was comparable to those reported in other cultivars grown in various regions around the world. As well, TSS content significantly differed and ranged from 15.2 (Grenade Jaune) to 17.6° Brix (Bouaâdime) **[15]**. TSS content differed significantly in the eight commercially South African-grown pomegranate cultivars and ranged between 14.04 and 16.32 °Brix, where the highest content was detected in the Wonderful cultivar. These data were following the ranges reported previously by Mena *et al.*, 2011 **[17]**. Among the studied California-grown pomegranate cultivars, DPun 81 cultivar was used as a control and showed the highest TSS content of 17.7 °Brix, followed by the PW-1 cultivar with 17.4°Brix. All remaining cultivars had statistically significantly lower TSS than the control, ranging between 15.70 and 16.40 °Brix, except the Nusai cultivar contained 17.33°Brix. Cultivars that contained the highest TSS content (both Nusai and Wonderful) were considered as sweet-tart, but the lowest content detected in Ovadan (15.7°Brix) was considered sour. These reported values were comparable to those previously reported in different pomegranate accessions from different sources around the world **[16]**. TSS of six Italian pomegranate and Wonderful cultivars varied from 16.12 (Wonderful) to 13.92 °Brix (Gaeta 3). The TSS value of Formia was close to that was detected in Wonderful. There were no significant differences between the TSS value of the other varieties and their value was close to Gaeta 3 **[9]**.

In conclusion, TSS values varied significantly within the previously studied Iranian, Italian, Moroccan, Spanish, and South African cultivars. Furthermore, in other previous studies, there was no significant difference in the Italian and Spanish cultivars.

2.1.2.2. Titratable acidity (TA)

Titratable acidity (TA) was the main factor affecting the taste and classification of pomegranate cultivars **[2]**. Since the acidity content affects the consumer perception of fruit quality. The climatic and growing conditions affect TA content and pomegranate taste **[15]**. Moreover, the genetic make-up of the cultivars affects the acidity level and consumer perceptions **[17]**. In twenty pomegranate cultivars grown in Iran, significant differences were observed for TA, where the content ranged between 0.33 and 2.44 (g/100 g) **[18]**. In five pomegranate accessions located on the farm of the University of Tuscia, Tordimonte A contained the highest value of TA (2.37%), while in MG3 and MG1, TA was 0.48 and 0.45 %, respectively **[14]**. Spanish pomegranates (Mollar de Elche, Valenciana) and Wonderful cultivars showed important statistical differences between cultivar groups, with values differing between 1.9 (M.55) and 29.7 g/kg (W.7). Spanish groups Valenciana and Mollar de Elche obtained the lowest values and were below 2.9 g/kg. In contrast, values in Wonderful cultivars exceeded 5.2 g/kg. These values were comparable to the reported previously in Spanish, Israeli, Turkish, and Iranian fruits **[19]**. The TA mean values of nine Spanish cultivars were 0.26, 0.79, and 1.83% in sweet, sour-sweet, and sour fruits, respectively **[2]**. Legua *et al*. 2012 highlighted that there is a strong correlation between TA and pH, and also between TA and the content of malic acid in six Spanish pomegranate cultivars related to the Mollar cultivar. The values of TA ranged between 0.18 and 0.26% **[20]**. TA values of ten major Moroccan cultivars ranged between 2.4 and 4.8 g/L. Only Hamde showed a TA value of 37.5 g/L of citric acid, close to the Italian Tordimonte A and Iranian TSK reported by Cristofori *et al.* and Tehranifar *et al.*, respectively **[15]**. Fawole *et al.* 2014 noticed that the TA of the eight commercially South African-grown pomegranate cultivars was six-fold more acidic in the Wonderful cultivar (1.16 %) than in the Molla de Elche cultivar (0.19 %) **[17]**. In the studied California-grown pomegranate, DPun 81 juice contained 1.10 %, which was less than the PW-1 cultivar which contained 1.32%. Whereas, the cultivars highest in astringent, sour, and bitter taste such as Kara Gul, Nikiski Ranni, and Haku-Botan were shown to contain higher TA content than DPun 81. Haku-Botan contained the highest value of 2.05% and it was considered sour, sweet-tart, and clear to pale yellow arils. In contrast, cultivars such as Nusai, Sin Pepe, and Fleischman had very low TA, which was lower than DPun 81. Fleischman and Nusai were classified as the sweetest and the fruitiest flavor, followed by Sin Pepe. These reported values were comparable to previously reported in different pomegranate accessions around the world **[16]**. Russo *et al.* 2018 showed that Wonderful and Tordimonte A contained the highest value of TA compared to all six old Italian pomegranate cultivars **[9]**.

In conclusion, TA values varied significantly within the previously studied Iranian and Spanish cultivars, and it was highly correlated with pH and malic acid content.

2.1.2.3. Maturity index (TSS: TA ratio)

The TSS: TA ratio is considered a major parameter for the determination of pomegranate ripeness and quality. In addition, it is crucial for proper selection of cultivars for utilization in the industry as the lowest TSS: TA ratio cultivars are more suitable for utilization in beverage products and food **[17]**. The maturity index (MI) affected the flavor and taste of pomegranate, and it could be used for categorizing the pomegranate cultivars. Martinez et al. (2006) established a classification for Spanish cultivars, with MI from 5 to 7 for sour, MI from 17 to 24 for sour-sweet, and MI from 31 to 98 for sweet cultivars **[18]**. Based on Martinez et al. (2006) classification, twenty pomegranate cultivars cultivated in Iran were classified as follows: TSK, MY, MS, MPS, and TSF as sour, SPGh, SPSe, ZY, DA, BPN, LPK, KB, SK, SDSF, PSD and AMS as sour-sweet and ASS, SPG, SDGF and SPS as sweet, where significant differences were found between cultivars. The values varied between 5.04 and 46.31, where Alak Shirin Save contained the highest value **[18]**. Similarly, MI significantly varied between five pomegranate accessions located on the farm of the University of Tuscia where the values differed from 37.78 in MG3 to 5.43 in Tordimonte A. The MG1, MG2, and MG3 were classified as sweet, while Tordimonte B and Tordimonte A as sour varieties based on Martinez et al classification. Meanwhile, based on Chace et al. suggested that when the acidity of juices is less than 1.8% and MI from 7 to 12, thus pomegranate will be suitable for the fresh market. Therefore, Tordimonte B would be the most appropriate for the fresh market although the Italian market prefers sweet pomegranate varieties **[14]**. MI of Spanish pomegranates (Mollar de Elche, Valenciana) and Wonderful cultivars varied from 5 (W.7) to 89 (M.63). Significant variations were found between cultivars where Spanish accession's lowest value was 52.15, while Wonderful accession's highest one was 29.08 **[19]**. Differences in TA values of nine Spanish cultivars caused a significant variance in MI with the means of 7.94, 19.2, and 60.2 for sour, sour-sweet, and sweet fruits, respectively **[2]**. In another study, six Spanish pomegranate cultivars were classified as sweet since their MI ranged between 59.14 (ME5) and 87.95 (MO6) **[20]**. Similarly, ten Moroccan cultivars were classified as sweet except sour Hamde. The MI significantly varied between cultivars with values of 64.29 in Sefri and 4.48 in Hamde **[15]**. MI values of eight commercially South African-grown pomegranate cultivars were comparable to those reported for Wonderful and Molla de Elche accessions by Mena *et al.*, 2011. The values ranged between 14.28 and 75.77 with significant differences between cultivars. Cultivars can be grouped into three categories: Wonderful as a sour cultivar (14.28), Arakta, Acco, Bhagwa, Ganesh, Herskawitz, and Ruby as sweet-sour (37.48-55.48), and Molla de Elche as a sweet cultivar (75.77) **[17]**. Beaulieu *et al*. 2015 highlighted that there is a strong positive correlation between MI and ascorbic acid, pH, and oxalic acid. While, a strong negative correlation was noticed between MI and total organic acids, citric acid, and TA using Pearson's correlation coefficient. The range of MI among California-grown pomegranate cultivars was comparable to the previously reported in different pomegranate accessions around the world. PW-1 (13.24) and Wonderful control (16.05) ratios were in between other cultivars. Nevertheless, Nusai, Fleischman, and Sin Pepe cultivars contained significantly higher values (66.93-74.29). These values were higher than those reported in the literature (54.74- 71.78) by Fawole & Opara, 2013, and followed higher ratios (64.15-89.28) reported by Mena *et al*. 2011 **[16]**. The MI of six old Italian pomegranates and for the international cultivar Wonderful significantly segregate the cultivars into two main categories, with the lowest ratio were in Tordimonte A (5.88) and Wonderful (7.53) **[9]**.

In conclusion, MI values varied significantly within the previously studied Iranian, Italian, Spanish, Moroccan, and South African cultivars, and it was positively correlated with ascorbic acid, oxalic acid, and pH, while negatively correlated with TA, citric acid, and total organic acids.

2.1.3. pH analysis

The value of pH of pomegranate juice has been analyzed in different studies. Tehranifar *et al*. measured twenty pomegranate cultivars grown in Iran for their pH content. The values ranged between 3.16 and 4.09, with a significant statistical difference among cultivars **[18]**. These values fall following those reported by Mena *et al.*, on Spanish pomegranates (Mollar de Elche, Valenciana) and Wonderful cultivars. The values varied between 3.0 and 4.0. Where, the highest value above 3.6 was exhibited in Valenciana and Mollar de Elche, while Wonderful showed the lowest value below 3.5 **[19]**. Moreover, Calin *et al.* classified nine Spanish cultivars into sweet, sour-sweet, and sour with pH mean values of 4.13, 3.49, and 3.07, respectively **[2]**. Cristofori *et al*. 2011 compared values of pH in Tordimonte A and B which exhibited lower values of 3.1, following values of 3.7 noticed in MG1, MG2, and MG3 **[14]**. Legua *et al*. 2012 measured pH values of six Spanish pomegranate cultivars. The lowest pH value of 3.94 was noticed in MO6, while the highest value of 4.07 was detected in ME5. These values were comparable to previously reported for Spanish cultivars by Mena *et al.* **[20]**. Fawole *et al*. 2014 measured pH values of eight commercially South African-grown pomegranate cultivars. The values varied from 2.96 (Wonderful) to 4.26 (Molla de Elche) **[17]**. Beaulieu *et al*. 2015 highlighted that there is a strong negative correlation between pH with total organic acids, citric, and TA in California-grown pomegranate. Both Wonderful cultivars exhibited the same pH values of 3.05 and 3.06. In Sin Pepe, Fleischman, Salavatski, Ovadan, and Nusai the pH was about 9% greater than Wonderful, as these cultivars contained lower TA than wonderful. Whereas, in cultivars Haku-Botan and Kara Gul, the values were about 7%

lower than Wonderful as these cultivars had the highest TA **[16]**. The pH of six old Italian pomegranates and the international Wonderful noticed significantly lowest value in Tordimonte A and Wonderful compared to other varieties **[9]**.

2.1.4. Sensorial analysis

Mena *et al*. 2011 analyzed Spanish pomegranates (Valenciana, Mollar de Elche) and Wonderful juices for sensory parameters, with significant differences in all parameters except for aroma; therefore aroma is not a characteristic sign of juice. Among studied cultivars, M.55 could be considered an attractive red fruit as it possesses an appealing intermediate color and intensely pleasant taste in the hedonic test. V.46i accession exhibited a very close acidity: sweetness ratio to M.Leon.1 and showed a very light color. Dissimilarities were noticed between Wonderful accessions. Where W.3 had an intense dark color and a moderate ratio, WSN was categorized as highly dark-colored with an acidic and astringent taste. By applying chemometric study, Principle component analysis (PCA) provides information on accessions suitable for juice elaboration in the industry via displaying the main features of each group of accessions. Some accessions of Wonderful juices displayed too much acidity but were rich in bioactive compounds. Whereas, other accessions exhibited interesting antioxidant capacity without high acidity. It is noticed that consumption of Mollar de Elche juices increases as it showed superior organoleptic properties to the other juices, also it contained high polyphenols that are different from anthocyanins **[19]**. Calin *et al*. 2011 concluded that ME2 was the best cultivar suitable for juice processing among the studied nine Spanish cultivars based on physicochemical and sensory results. As ME2 juice was described by high scores of satisfaction degree for fresh pomegranate sweetness (6.2), flavor (3.7) and odor (2.3), medium color scores (5.3), and low sourness scores (2); the total consumer passion for ME2 was 7 out of 10 on the scale. As well, instrumental data aligned with the sensory data as ME2 juices showed the highest value of maturity index (64.2) and total concentration of volatile constituents (10.9 g kg^{-1}, compared with the mean of 2.37 g/kg for the remaining cultivars). By applying the PLS regression biplot, the first two dimensions (PLS1 and PLS2) showed 69% of the difference in the consumer preferences (Y) was clarified by 79% of the variation in the instrumental analysis (X). Acceptance was related to odor, flavor, and juices sweetness. ME2 scored the highest consumer satisfaction especially due to its great content of volatile constituents, mainly terpenes, which made it very aromatic and increased the satisfaction degree for fresh pomegranate flavor. The sweetness perception of the samples was related to MI but not to TSS. In contrast, astringency and sourness were on the contrary side of the plot, and consumers disfavor this characteristic (BA1and BO1). However, astringency has been associated with beneficial health effects for humans due to high polyphenol content and antioxidant activity. Therefore, we could conclude that health and consumer acceptance are not directly correlated in pomegranate juices **[2]**.

2.1.5. Organ measurements

Fruit weight influences consumer preference so it is considered one of the main factors in fruit manufacturing and marketing **[17]**. It is useful to measure the fruit weight, diameter, and length, also calyx diameter and length of the fruits for the design of suitable packaging for fruit storage and handling. The difference in ecological conditions and cultivars causes variation in fruit weight **[18]**. The edible part of the fruits includes the juice and the kernel (woody portion) which forms the aril. Aril yield is an essential property for consumer preferences also for industrial juice processing **[17]**. The wood amount in the edible part of the fruit is described by the woody portion index (WPI) **[23]**. The juice content of the aril is considered one of the critical factors from the industrial point of view **[18]**. Tehranifar *et al*. 2010 measured different parameters in twenty Iranian pomegranate cultivars and proved significant differences in all parameters except the length: diameter ratio of the fruit. Concerning fruit weight, it varied from 196.89 (Shirin Pust Ghermez) to 315.28 g (Shirin Pust Sefeed). Similarly, fruit volume was lowest in Shirin Pust Ghermez (204.24 cm 3) and the highest in Shirin Pust Sefeed (341.35 cm 3). The remaining parameters fruit length, values ranged from 69.49 to 81.56 mm, fruit diameter from 64.98 to 86.88 mm, calyx length from 13.45 to 24 mm, and calyx diameter from 12.52 to 23.96 mm. Tehranifar *et al*. selected Shirin Pust Ghermez as the most promising cultivar as it contained highly preferable features in beverage manufacturing and food processing. Shirin Pust Ghermez showed the minimum skin percentage (32.28%), the maximum aril percentage (65%), and the maximum juice percentage (46.55%). Shirin Dane Ghermez was the second promising cultivar for its bigger fruits. Both cultivars are the cultivars of choice mainly in developing better agronomic potential cultivars. In addition, a variation in fruit skin thickness between studied pomegranates was verified and ranged from 3.13 to 5.36 mm. In addition, there were wide variations in the percentage of aril (37.59–65%), skin (32.28–59.82%), seed (9.44–20.55%), and juice (26.95-46.55%) **[18]**. Cristofori *et al*. 2011 showed significant differences among the five accessions located on the farm of the University of Tuscia in the fruit longitudinal length and equatorial diameter. The fruit weight ranged from 106.8 of Tordimonte A to 297.7 g of MG3. Also, MG3 showed the biggest size. Regarding the shape of the fruit, Tordimonte B was almost rounder than the remaining accessions. MG2 contained the most arils percentage (53.5%), while the lowest was in Tordimonte A (44.5%). Regarding the dry weight of the arils, it ranged from 10.1 (MG1) to 17.9% (Tordimonte B). Fresh weight per aril showed the lowest value in Tordimonte A (0.28 g), and MG1 exhibited the mean of 0.36 g per aril. The seed and aril ratio did not vary in accessions and values ranged between 79.1 and 81.2% with the lowest ratio in Tordimonte A as it contains the highest seed weight (0.070 g) and low weight of aril [14]. Hasnaoui *et al*. 2011 showed that WPI had a significant difference between five sour and seven sweet Tunisian pomegranate cultivars with a mean value in sour twice greater than in the sweet one. The average WPI value ranged from 2.16 (Chelfi 3) to 7.33% (Mezzi 1) with a mean value of 4.43%. It is concluded that sour cultivars with a high woody portion index are considered a useful source for the production of nutraceutical substances as an increase in WPI, increases the unpalatability of sour cultivars. On the other hand, sweet pomegranate cultivars are a good source for fresh juice production with remarkable health benefits, as low WPI makes it valuable for food manufacturing of ready-to-eat arils. Therefore, the cultivar Chelfi 3 is considered the genotype of choice for breeders to produce cultivars with high agronomic potential as it contained the least WPI value and the most aril weight (AW) value. Also, the seed weight (SW), WPI, and AW showed the highest variability between cultivars with coefficients of variation of 42, 29, and 25%, respectively. The WPI is correlated significantly to all seed parameters, except the seed length (SL) **[23]**. The fruit weight of all six Spanish pomegranate cultivars was not affected by cultivar and the ME16 trees produced the largest fruit weight with a value of 351.48 g **[20]**. Fawole *et al.* 2014 showed significant differences in fruit length, weight, sphericity, diameter, volume, surface area, and geometric mean diameter of eight commercially South African-grown pomegranate cultivars. These parameters were highest in Wonderful while lowest in Acco. Fruit weight varied between 274.04 to 509.82 g. The volume varied between 222.52 and 509.82cm3. Regarding fruit size, Wonderful and Ganesh cultivars would be the most promising bigger fruits. The Means of aril content per fruit varied between 123.88 (Molla de Elche) and 305.33 g (Ganesh). Ruby cultivar contained the highest aril yield (68.05 %), while the Wonderful cultivar exhibited the lowest one (47.49 %). Kernel index (KI) which measures the woody portion of the edible part ranged between 6.08-14.81 % **[17]**. In addition, Russo *et al*. 2018 showed that the highest fruit weight of varieties of Italian pomegranates was in Wonderful and Italian cultivars Tordimonte A and Gaeta 1. The remaining cultivars ranged between 228.5 in Formia and 291.6 g in Gaeta 3. Additionally, the fruits of Wonderful were the largest, with the shape slightly rounder than the other varieties. Significant differences were observed between varieties for the equatorial diameter and longitudinal length. The highest arils percentage was detected in Gaeta 1 and Wonderful with values of 63.2 and 62.3%, respectively. The lowest one was detected in Tordimonte A (46.7%) **[9]**.

Shirin Pust Ghermez and Shirin Dane Ghermez in the previously studied Iranian cultivars, Chelfi 3 in the previously studied Tunisian cultivars, and Ganesh and wonderful in the previously studied South African cultivars are preferable in developing cultivars with high agronomic potential. There is a correlation between fruit volume and fruit weight. The WPI is significantly related to SW, AW, seed thicknesses, and seed shape except for the SL.

2.1.6. Textural properties

Differences of cultivars in peel characteristics such as moisture content and thickness may affect the resistance of the fruit to external puncture. Fawole *et al*. proved a significant difference in total fruit puncture force between the eight commercially South African grown pomegranate cultivars. The values ranged from 68.89 (Herskawitz) to 130.98 N (Bhagwa). In addition, there were significant variations in the textural properties such as hardness and toughness of kernels and arils. “Wonderful” cultivar contained the hardest aril and kernel, respectively. Whereas, “Ruby” aril and kernel have the least both **[17]**.

2.2. Chemical Characterization

The chemical structures of the major biologically active secondary metabolites in pomegranate are shown in **Fig. 2**.

2.2.1. Chromatographic techniques

2.2.1.1. HPLC (Phenolics)

Pomegranate is a valuable source of polyphenolic compounds which are present in different fruit parts. In many studies for separation and quantification of polyphenols in the juices and fruit different parts extracts, the main method of choice is high-performance liquid chromatography (HPLC) coupled with spectroscopic and electrospray ionization mass spectrometric (ESI-MS) detection techniques **[9]**. Fischer *et al.* 2011 identified 48 compounds in the mesocarp, peel, arils, and juices prepared from the arils and entire fruit of Peruvian pomegranate of indefinite cultivars obtained from the local market by HPLC-DAD–ESI/MS^n. Among these compounds: 22 ellagitannins, 9 anthocyanins, 7 hydroxycinnamic acids, 4 hydroxybenzoic acids, 2 gallery esters, 2 gallotannins, and 1 dihydroflavonol were detected.

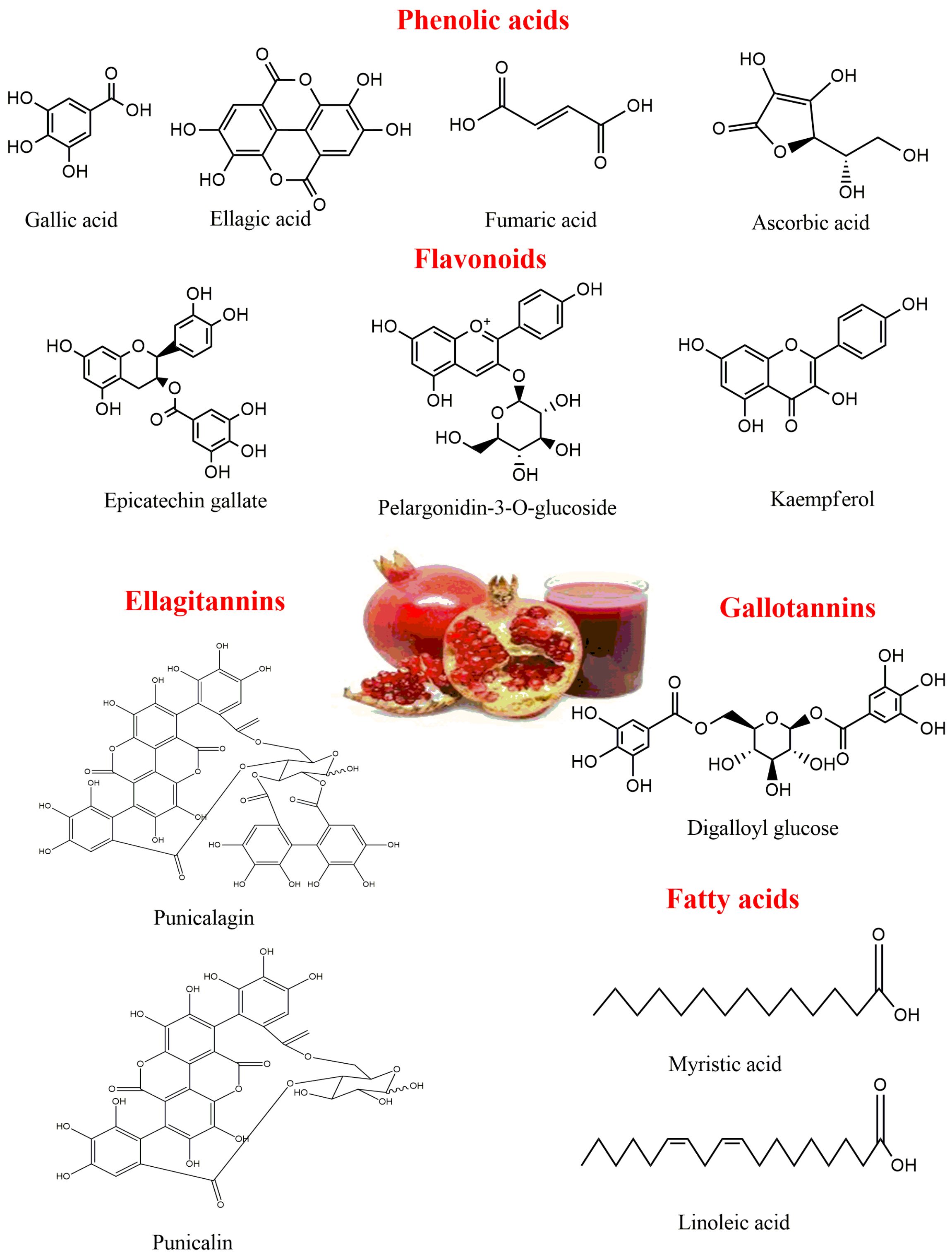

Fig. 2. Chemical structures of the major biologically active secondary metabolites in pomegranate

The ellagitannins were the major in all samples; especially punicalagin and punicalagin were the major quantified ones. Punicalagin ranged between 11 and 20 g/kg mesocarp and peel dry matter in addition to a value of 4 to 565 mg/L in the juices **[29]**. In this study, cyanidin–pentoxide–hexoside, vanillic acid 4-glucoside, brevifolin carboxylic acid, valoneic acid lactone, and dihydrokaempferol-hexoside were firstly reported in pomegranate fruits **[29]**. Another study quantified ellagic acid (EA) derivatives, punicalagin, and anthocyanin contents in juices of Spanish pomegranates (Mollar de Elche, Valenciana) and Wonderful cultivar using HPLC coupled with a photodiode array UV-visible detector. EA derivatives significantly varied between 3 and 160 mg/L. Overall, Wonderful accessions contained higher EA in both free and bound forms than in Valenciana and Mollar de Elche. Regarding punicalagin content, it ranged between 1 and 45 mg/L, with Valenciana cultivars having the highest punicalagin content except for values of more than 40 mg L^{-1} detected in W.2. As for anthocyanin contents, the values varied between 30 and 1080 mg/L with significant differences among accessions. Wonderful accessions showed the highest values, while the Valenciana cultivar showed the lowest ones **[19]**. Legua *et al.* 2012 quantified the total anthocyanin content of juices obtained from six Mollar Spanish pomegranate cultivars by HPLC-DAD, the values ranged between 72.55 (MA4) and 200.21 mg/L (ME5). Cyanidin 3-glucoside was the main anthocyanin for all cultivars except for MA5 with concentrations ranging between 21.47 and 76.42 mg/L. Cyanidin 3,5-glucoside was the second dominant anthocyanin with concentrations ranging from 18.19 to 48.83 mg/L **[20]**. Qu *et al*. 2012 developed a rapid HPLC–UV method that improved sample throughput and facilitated the quantitative assays of four main polyphenols (punicalagin A and B, gallic acid, and ellagic acid) in juices in a single run.

Furthermore, the applicability of this method was evaluated on different products of pomegranate, including handmade juices, marc extracts, and commercial drinks. The method showed good daily reproducibility, good linearity, high recovery rate but low limits of detection (LOD) and quantification (LOQ). Regarding within-day %CV, the values of punicalagin A, punicalagin B, gallic acid and ellagic acid ranged from 2.9 - 5.8%, 2.6 - 6.6%, 2.3 - 6.1% and 1.9 - 6.2%, respectively. But, values of inter-day %CV were 10.2, 11.4, 5.3, and 6.8% for punicalagin A, punicalagin B, gallic acid, and ellagic acid, respectively. Percentages of spike recovery for punicalagin A, punicalagin B, gallic acid, and ellagic acid were 92.4, 95.5, 98.5, and 96.5%, respectively. All pomegranate drinks showed higher concentrations of punicalagin A, punicalagin B, gallic acid, and ellagic acid when compared to handmade juice **[30]**. Similarly, DING *et al*. 2012 developed an accurate, simple, and reproducible HPLC-UV method for the determination of ellagic acid in the extract of pomegranate peel. The method showed a linear range of ellagic acid of 5.36-171.40 μg/mL. The average recovery was 97.82%, and relative standard deviation (RSD) was 1.41% (n = 9) **[31]**. Liu *et al*. 2013 implemented an accurate and reproducible HPLC-UV method for the determination of punicalin, gallic acid, punicalagin, and ellagic acid in the juice. This method showed a linear range of 0.038-0.608 μg for punicalin, 0.020-0.320 μg for gallic acid, 0.074-1.184 μg for punicalagin, and 0.039-0.624 μg for ellagic acid. While the average recoveries values were 95.35, 93.22, 98.00, and 99.84% for punicalin, gallic acid, punicalagin, and ellagic acid, respectively **[32]**.

Most of the reported methods determined phenolic compounds in aqueous and hydroalcoholic extracts (extractable polyphenols), but ellagitannins (ETs) can remain unextracted. Therefore, Garcia *et al*. optimized a method for the determination of pomegranate ellagitannins (extractable and non-extractable) by quantification of the ellagic acid and other products produced after acid hydrolysis using HPLC-DAD-ESI-MS/MS. The most appropriate pretreatment for the highest recovery of the main hydrolyzed products of ETs was hydrolysis by 4 M HCl at 90 ºC for 24 h in water and successive extraction of the pellet with 50:50 v/v DMSO: MeOH. In non-hydrolyzed samples, the LOD and LOQ were valued at 0.06 and 0.20 mg/g for vescalagin, 0.07 and 0.24 mg/g for punicalagin, and 0.01 and 0.03 mg/g for both ellagic acid and gallic acid. But, in hydrolyzed samples the LOD and LOQ, were valued at 0.03 and 0.10 mg/g for ellagic acid and gallic acid. This method showed a high precision with RSD of peak areas less than 5.6, and 7.5% for intra and inter-day precision, respectively. Also, the reproducibility of the method was evaluated by interlaboratory trials that showed a high reproducibility with relative standard deviations of less than 15% through 6 laboratories. The applicability of this method was validated in 11 pomegranate extracts, which showed great variability in both ellagitannin content (from 150 to 750 mg of hydrolysis products/g) and type (galvanic: ellagic acid ratios from 4 to 0.15) of the studied extracts. Also, the applicability was evaluated on different fruit parts: peels, husk, mesocarp, arils, and commercial juices. Husk contained the maximum concentration of ellagic acid and other hydrolyzed products, thereafter the peels and the arils contained only traces amount **[33]**.

To assess the quality of the polyphenols extracted from the peel an efficient and simple HPLC fingerprint method combining similarity alignment and the quantitative assay was developed by testing pomegranate collected from various farms in Shaanxi Lintong of China. The similarities of the 15 characteristic peaks of the pomegranate peel samples were higher than 0.968, which indicated the consistency of the samples from several locations of Lintong. In quantitative analysis, the eight polyphenols contents (including catechin, epicatechin, punicalagin, caffeic acid, chlorogenic acid, rutin, gallic acid, and ellagic acid) comprise 76.0% of the total polyphenols in the peel. Punicalagin represented 76.7% of the eight polyphenols, followed by catechin, ellagic acid, and gallic acid which represented 14.9, 3.3, and 3.1%, respectively. The remaining 4 polyphenols (rutin, epicatechin, caffeic acid, and chlorogenic acid) represented only 2.0%. The method showed good reproducibility as the values of RSD of retention time and area of the peak were below 0.41 and 2.95%, respectively. Moreover, it showed sufficient sensitivity, with LOD for the eight components below 0.047 mg/L. The average recovery rates were 78.6-103.2% and their RSD values were less than 2.96% [34]. For the determination of punicic acid in pomegranate seed oil, a reproducible and accurate method was established using HPLC. The punicic acid was derivatized with ω-bromoacetophenone and triethanolamine was used as a catalyst. The linearity of standard curves of punicic acid was in the range of 0. 0266-0.133 0 g/L and coefficient was 0.999. The average recovery of punicic acid was 98.7%, and RSD was 1.8% **[35]**. Legua *et al*. 2016 identify and quantify 10 phenolic compounds (mainly ellagic acid derivatives and hydrolyzable tannins) and 6 anthocyanins (especially cyanidin 3,5-*O*-diglucoside and cyanidin 3-*O*-diglucoside) in the juices of 19 pomegranate cultivars by HPLC-DAD-ESI-MS/MS. The cultivars VA6, PDA1, PTO8, and WOND showed the highest content of individual phenolic compounds with values of 99.1, 98.2, 87.9, and 80.9µM ellagic acid, respectively. The concentration of the remaining 15 cultivars ranged from 19.3 to 62.0µM ellagic acid.

Cultivars HIZ and WOND showed the highest concentrations of anthocyanins especially cyanidin 3-*O*-diglucoside with values of 259 and 217 µM, respectively, **[22]**. In addition, gallic acid, ellagic acid, rutin, and vitamin C were separated and quantitatively determined in pomegranate freeze-dried juice samples by HPLC. The content of gallic acid, ellagic acid, rutin, and vitamin C was found to be 0.088, 0.046, 0.0072, and 0.02% w/w, respectively. The LOQ and the LOD for all analytes were below 0.095 and 0.07 µg/mL, respectively, which indicated sufficient sensitivity. The method was considered reproducible, accurate, economic, and suitable for the quality control of different commercial products which contain pomegranate as an ingredient. The method was validated according to ICH guidelines for accuracy (97.2-102.5%), precision (0.12-1.87% RSD), and robustness (0.26-2.30% RSD) **[36]**.

Referring to Liu *et al*., Li *et al*. 2016 established a sensitive and validated HPLC–DAD method for quantitative assays of four polyphenols not only in juices but also in different parts of the fruit including flesh, peels, leaves, and seeds from five Chinese cultivars. The peel of Sour-YRP contained a higher concentration of punicalagin A&B (125.23 mg/g) than the remaining polyphenols in the five cultivars. Regarding gallic acid, ellagic acid and punicalin A&B were found only in trace amounts in different pomegranate parts with contents ranging between 0.02 in seeds and 0.41 in Sweet-RP peel, 0.02 in juices and 4.08 in Sweet-RP peel, and 0.01 in juices and 3.91 mg/g in Sweet-RP peel, respectively. Deficiency of punicalagin A&B, punicalin A&B and traces content of ellagic aid, gallic acid in the seeds limited its nutritional value. Four polyphenols (ellagic acid, gallic acid, punicalin A&B, and punicalagin A&B) showed linearity in the range of concentrations of 39–624, 20–320, 38–608, and 74–1184 µg/mL, respectively. The method exhibited good precision for quantitative analysis with intra and inter-day RSD ranging between 0.92 and 2.30 %, good accuracy with the overall recoveries ranging between 94.3 and 100.07 %, good reproducibility with RSD <2.92 %, and good stability as there were no significant changes in all analytes with a storage period of 48 hrs. with RSD <2.98 % **[24]**. Also, punicalagin and six related compounds in the leaf, pericarp, seed, and juice of “Taishanhong” and “Taishansanbaitian” cultivars were determined by an efficient and simple HPLC method. Punicalagin content in the leaves, pericarp, seeds, and juices of both cultivars was greater than the other six compounds. “Taishanhong” pericarp contained the largest punicalagin (138.232 mg/g), shikimic acid (1.528 mg/g), and 3-dehydroshikimic acid (3.125 mg/g) contents. “Taishanhong” leaf contained the highest pentagalloylglucose content (1.694 mg/g) which was 1.7-fold as that present in pericarp, 4.1-fold as in a seed, and 2.3-fold as in juice. This method showed high sensitivity and feasibility as the LOD values ranged between 0.15 and 0.24 mg/g. Also, it showed high precision, repeatability, and good stability. The recovery rates were between 98.9 and 102.5%, and the RSD values were less than 2.2% **[25]**. Similarly, different phenolic compounds in peel, juice, and pulp of 6 different Italian cultivars and one international cultivar were measured by two various separation methods. The first method determined anthocyanin, while the second determined ellagitannins, phenolic acids, and flavonoids. Thirty-five phenolic compounds were detected, and 28 of them were classified into four major phenolic compound categories: flavonoids, phenolic acids, anthocyanins, and hydrolyzable tannins. As well, the quantitative assay was performed using a mixture of 9 phenolic compounds. Peels contained the highest phenolic compounds with means of a total concentration of 121.1 mg/g), while juices are the lowest with means of a total concentration of 0.5 mg/ml. Regarding anthocyanins, were the highest in juices (ranging from 29.9% to 73.2%), followed by pulp (4.9–23.1%) and below the LOD in the peel. Ellagitannins were the major compounds in peel (ranging from 39.7 to 84.2%), but they present at lower percentages in juice and pulp. Total flavonoid content in pulps was rich in syringe hexoside and catechin, whereas, the peel and juices were rich in catechin. Finally, in all analyzed samples, phenolic acid content was below 8% of total phenolic components **[9]**.

2.2.1.2. UPLC (Phenolics)

In addition to HPLC, many phenolic constituents were identified and quantified in pomegranate by ultra-performance liquid chromatography (UPLC). Nuncio-Jáuregui *et al.* identified and quantified major derivatives of ellagic acid (MDEA) of the thinning and ripe fruits from nine Spanish pomegranate cultivars by LC-PDA–QTOF/MS and UPLC-PDA. Thirty-five MDEA were identified and the total content of MDEA was greater in thinning fruits with values ranging from 3521 to 18,236 mg/100 g dry matter (dm) than in ripe fruits with values from 608 to 2905 mg/100 g dm. The three major compounds in thinning fruits were: HHDP-gallery-hexoside, punicalagin isomer, and granatin B which comprised 36.4, 19.9, and 7.3% of MDEA total concentration, respectively. Whereas, ellagitannin was the major compound in ripe fruits and accounted for 42.9% of MDEA total concentration. As well, cultivar variations affected the concentration of MDEA, In thinning fruits, it ranged from 3521 to 18,236 mg/100 g dm, while in ripe ranged from 608 to 2905 mg/100 g dm **[21]**. In another study, Beaulieu *et al.* 2015 implemented a quantifiable and rapid method to classify the three main anthocyanin groups in the whole fruit and juices of the studied California-grown pomegranate by UPLC-UV. All cultivars contained the cyanidin group as the predominant class of anthocyanidins and a strong positive correlation was verified between cyanidin and total anthocyanidins using Pearson's correlation coefficient. The maximum cyanidin and total anthocyanidins concentrations were in Salavatski juices and PW-1 cultivars. Whereas, DPun 81 control concentration was in the middle. The highest levels of delphinidin were in Ovadan, Salavatski, PW-1, and DPun 81 control juices. Pelargonidin was not detected only in Haku-Botan and was always lower in all cultivars **[16]**. Wang *et al.* 2016 developed and validated a sensitive, simple, fast, and accurate UPLC-QQQMS method to simultaneously determine seven compounds (chebumeinin A, chebumeinin B, chebulagic acid, chebulic acid, pentagalloyl glucose, corilagin, and gallic acid) in dried whole pomegranate, dried pomegranate peels, and fresh pomegranate peels and seeds. The correlation coefficients were higher than 0.99 with linear ranges of 0.003 4–13.875 0, 0.013 5–13.875 0, 0.003 4–13.875 0, 0.216 8–55.500 0, 0.867 2–55.500 0, 0.216 8–55.500 0, and 0.003 4–13.875 0 μg/mL in chebumeinin A, chebumeinin B, chebulic acid, pentagalloyl glucose, corilagin, chebulagic acid and gallic acid, respectively. The average recovery rate ranged from 99.44 to 105.54% and the intra and inter-day RSDs were 0.11–0.95% and 0.14–1.21%, respectively **[37]**.

The major advantages of UPLC over conventional HPLC are higher sensitivity, improved resolving power, shorter run-time, and less solvent consumption.

2.2.1.3. HPLC (Organic acids)

The organic acid content in pomegranate is greatly affected by cultivar (genotype) and agro-climatic conditions **[15, 23]**. The organic acid profile plays a vital role in the sensory qualities of pomegranate juice, improves health benefits, and controls the juice quality and shelf life via controlling microorganism incidence in fruit and byproducts **[15]**. Moreover, organic acids improve antioxidant activity by acting as a synergistic antioxidants **[22]**. Various organic acids were separated and detected in pomegranate juice. L -malic and citric acid are the major organic acids detected in juices obtained from Spanish pomegranates (Mollar de Elche, Valenciana) and Wonderful cultivars. L -malic acid was slightly greater than citric acid in both Valenciana and Mollar de Elche cultivars. In the wonderful cultivar, malic acid was not different than in the Spanish cultivars, but citric acid concentration was higher than 3.8 g/L with low–medium content in W2 and W3 (around 4 g/L). Oxalic acid was also detected but only in three cultivars: W3, W5, and W6. The concentrations were below 0.3 g/L. In contrast to authors Zhang *et al*. **[38]** who stated that tartaric acid was not found in pomegranate, tartaric acid was identified and quantified in all the juices with concentrations ranging from 0.17 to 0.41 g/L **[19]**. Another study quantified the organic acid contents of seeds and the juices of five sour and seven sweet Tunisian pomegranate cultivars by HPLC. Both fumaric acid and acetic acid couldn't be detected contrasting previously reported studies that quantified both acids in Spanish and Iranian juices. Both tartaric and ascorbic acids were detected in a few amounts. Succinic acid and oxalic acid contents are much greater in sweet than in sour cultivars, with means of 0.219, 0.513 g/100 g for oxalic and succinic acids, respectively. Finally, the major organic acids in the fruit are citric and malic. Citric acid was the main organic acid in sour cultivars and it is 15 times more than sweet ones, whereas, malic acid was the major in sweet cultivars. The correlation between citric acid content and the sourness of the fruits was proved. Mezzi 2 cultivar showed the highest citric acid content, the strongest acidic taste, and also the highest total organic acid content **[23]**.

Legua *et al*. 2012 confirmed by HPLC that malic was the main organic acid in the juices of ten major Moroccan cultivars (ranged between 0.31 and 1.56 g/100 g) followed by quinic acid (ranged between 0.063 and 1.19 g/100 g). With the presence of a lower concentration of oxalic, citric, and succinic acids with values of 0.011-0.11, 0.018-3.22, and 0.032- 0.36 g/100 g, respectively. Fumaric acid was detected in trace amounts **[15]**. Whereas, Legua *et al*. 2012 highlighted that there are strong correlations between citric acid and total acids. Citric acid was the main organic acid detected in Mollar Spanish cultivars and ranged between 0.15 and 0.22 g 100^{-1} juice, followed by malic acid ranging between 0.052 and 0.065 g 100^{-1} juice in ME5, ME16, and ME17 cultivars, and oxalic acid ranged from 0.047 to 0.051 g 100^{-1} juice in MA4, MA5, and MO6 cultivars. Also, tartaric acid was detected in Mollar pomegranate juice and ranged between 0.021 and 0.028 g 100^{-1} juice. Traces of ascorbic, succinic, quinic, lactic, acetic, and fumaric acids were also found in the aril juice **[20]**. Besides, Beaulieu *et al*. 2015 investigated that citric acid was the main organic acid in the studied California-grown pomegranate juices. Sour or tart was described for cultivars with high citric acid content. Fumaric and ascorbic acids were not found in Ovadan and Haku-botan. Sweet Sin Pepe and Fleischman cultivars and sweet-tart Nusai had significantly lower organic acids, especially citric acid but were characterized by the highest level of oxalic and malic acid. Meanwhile, Myagkosemyan Rozovyi contained about twofold higher total organic acid content than the other sweet cultivars, except Salavatski, also Ovadan, Salavatski, Kara Gul, and Nikitski Ranni contained high levels of total organic acids, especially citric acid. Furthermore, the correlation between parameters was proved by Pearson's correlation coefficient. The highest correlation was between citric acid and total organic acids. Oxalic acid showed correlations with ascorbic acid and pH. Finally, oxalic acid showed a negative correlation with TA **[16]**. Legua *et al*. 2016 investigated a significant difference in organic acid contents of the juices of nineteen pomegranate cultivars (Sixteen cultivars belonged to European pomegranate gene banks in Spain and the remaining cultivars were collected from farmer's market). The results showed that the malic and quinic acids were the major acids mainly in sweet cultivars, but citric was the main in the sour-sweet and sour cultivars. In general, sour cultivars contained the highest levels of total acids, followed by the sour-sweet and sweet cultivars with values of 2.64, 2.06, and 1.30 g/100 ml, respectively. Cultivars HIZ and WOND contained the highest concentrations of organic acids **[22]**.

Liquid chromatography is a technique widely used to determine organic acids in many fruits and vegetables due to its sensitivity, selectivity, and reproducibility. Also, there is no prior derivatization needed as occurred in GC techniques for short-chain organic acids.

2.2.1.4. HPLC (Sugars)

The balance between organic acid and sugar contents determines the taste of the fruit. Sugar contents in pomegranate are greatly affected by cultivar (genotype) and agro-climatic conditions **[15, 23]**. Organic acid and sugar composition indicate the fruit quality parameters. In addition, it is crucial to evaluate the fruit maturity, ripeness, and storage conditions **[20]**. Tehranifar *et al*. 2010 showed significant differences between twenty Iranian pomegranate cultivars in total sugars. The concentrations of total sugars ranged from 13.23 (Agha Mandali Save) to 21.72 g/100 g (Save Pust Sefeed) **[18]**. Mena *et al*. 2011 noticed that fructose and glucose, which are considered the chief sources of sweetness and energy, were the major sugars in Spanish pomegranates (Mollar de Elche, Valenciana) and Wonderful cultivars. The concentration of fructose ranged between 60 and 90 g/L in V111 and W6, respectively. Meanwhile, values of glucose ranged from 70 to 100 g/L in V111 and WSN, respectively. All juices contained fructose higher than glucose, with a glucose to fructose (G/F) ratio ranging from 0.88 to 0.96. The results found are in agreement with the G/F ratio of 0.8–1.0 as stated by the Association of the Industry of Juices and Nectars of the European Union [19]. These results were comparable to that reported by Hasnaoui *et al*., where fructose and glucose constitute the majority of sugars and fructose was predominant with fewer quantities of arabinose in five sour and seven sweet Tunisian pomegranate cultivars. Sugar content varied from 17.77 to 19.98 g/100 g in sour cultivars and from 13.13 to 16.55 g/100 g in sweet ones. Furthermore, a relation between sourness and sugar contents of arils of pomegranate could be concluded as fructose and glucose contents were greater in sour cultivars than in sweet ones. For sour cultivars, fructose values ranged from 9.46 to 10.61 g/100 g, and for sweet ones from 7.21 to 9.02 g/100 g. Only sweet cultivars Gabsi 9 and Garoussi 1 have an advantage in juice and fruit traits as they contained higher fructose than glucose as fructose is 1.3 times glucose. Finally, sucrose was less dominant as it was quantified only in Gabsi 1 (208 mg/100 g) and Chelfi 3 (32 mg/100 g). Whereas, maltose, galactose, and sorbitol could not be detected **[23]**. Similarly, Legua *et al*. 2012 confirmed in ten major Moroccan cultivars that fructose and glucose were the major sugars with values ranging from 7.8 to 10.4 and 6.9 to 8.6 g/100 g, respectively. Sugar contents varied from 16.1 to 19.3 g/100 g. Sorbitol and sucrose are found in trace or undetectable amounts **[15]**. In contrast to previously reported, Legua *et al*. 2012 verified that cultivars did not affect the sugar content in six Mollar Spanish cultivars. Moreover, the mean levels of the G/F ratio in this study ranged from 1.65 to 1.92. Glucose was the highest concentration and comprises 60 to 64% of the total sugars, followed by fructose, maltose, and sucrose. Glucose showed very strong correlations with total sugars. Maltose and sucrose were detected in all studied cultivars **[20]**. Legua *et al*. 2016 confirmed that Glucose and fructose were the main sugars in juices of nineteen pomegranate cultivars (Sixteen cultivars belonged to European pomegranate gene banks in Spain and the remaining cultivars were collected from farmer's market). Significant differences between cultivars in the sugar contents were noticed. It ranged between 9.41 (WOND) and 15.3 g/100 mL (ME13) and the highest values detected in Mollar de Elche cultivars **[22]**.

2.2.1.5. Gas Chromatography (Volatile component)

The aroma of pomegranate juices is determined by a mixture of chemical constituents (e.g. aldehydes, alcohols, terpenes, esters, ketones) whose concentration is usually low and differences in these compounds depend on the cultivar, maturity stage, climatic conditions, industrial factors such as harvest and postharvest treatments, storage and processing conditions. It is valuable to determine different chemical groups as it influences the consumer preference for juices and affects sensory quality. Monoterpenes in juices (such as limonene, α-pinene β-pinene, β-myrcene, and γ-terpinene) were correlated with good consumer preferences. Whereas, aldehydes in juices (such as hexanal, hexanol, and *cis*-3-hexanol) were associated with poor consumer preferences **[2]**. Calin *et al*. 2011 identified 18 volatile compounds, including alcohols, aldehydes, monoterpenes, monoterpenoids, and linear hydrocarbons, in nine Spanish cultivars of fresh juices using hydro-distillation for isolation of compounds then analyzed using gas chromatography-mass spectrometry. The major compounds were *trans*-2-hexenal, 3-carene, α-terpineol, and α-terpinene, and the volatiles total concentration ranged between 1.7 and 10.9 g kg^{-1}. Cultivar ME2 was considered the most suited for juice processing as it contained a high concentration of 3-carene and α-terpineol **[2]**. Fawole *et al*. 2014 identified fewer compounds compared to Calin *et al*., 15 volatile constituents were identified and classified into six categories like alcohol, aldehydes, terpenes, carboxylic acid, ketones, and esters in the juices of eight commercially South African-grown pomegranate cultivars. The alcohol group was the predominant one and ranged from 32.5 to 54.9 %. Therefore, it could be one of the most important and useful classes of compounds for cultivar classification **[17]**. The volatile profile of the California-grown germplasm was composed of 29 compounds containing 9 alcohols, 8 terpenes, 5 esters, 4 aldehydes, 2 ketones, and 1 aromatic phenol. Compounds of six carbon atoms such as hexanal, 1-hexanol, (E)-2- hexenal, (Z)-3-hexenal, and (Z)-& (E)-3-hexanol was the major compound in the volatile profiles of all cultivars. By applying chemometric analysis, PCA showed that terpenes and aldehydes are useful for the characterization of cultivars [16].

Gas chromatography, combined with hyphenated techniques, was a highly recommended tool for the isolation and identification of various flavor components in pomegranate juice as aldehydes, alcohols, terpenes, and esters. It is a typical most suited technique for volatile oil components analysis.

2.2.2. Spectroscopic techniques

2.2.2.1. Total phenolic content

Pomegranate juice contains the highest phenolic content as compared to other juices such as sour cherry, turnip, and red grape juice **[18]**. Moreover, phenolic compound production was affected by several external factors including environmental and climatic changes. Several studies verified that genetic variability can also lead to changes in the biosynthesis of phenolic metabolites **[14]**. Thus the analysis of phenolic content is important to determine varieties with high antioxidant activity **[22]**. The total phenolic content of the juice was measured by Folin-Ciocalteu's technique using gallic acid as a standard. A study on twenty Iranian pomegranate cultivars showed significant variation in total phenolic concentration (TPC) and the values ranged between 295.79 (Torsh Shahvar Kashmar) and 985.32 mg gallic acid equivalents (GAE)/100 g (Malas Pust Sefeed) [18]. Another study verified significant variation between juices of different Spanish pomegranates (Mollar de Elche, Valenciana) and Wonderful cultivars. TPC ranged from 1500 to 4500 mg GAE/L. However, the values in Spanish origin were very consistent but compared to Wonderful accessions, large differences were found as W.3 has twofold higher than Mollar de Elche and Valenciana pomegranates **[19]**. In addition, Cristofori *et al.* 2011 proved a significant difference between five pomegranate accessions located on the farm of the university of Tuscia. The values ranged from 651.4 (MG1) to 1103.1 mg (GAE)/L per FW (MG2) **[14]**. Similarly, significant variation detected between ten major Moroccan cultivars and TPC ranged from 41.01 to 83.43 mg/100 g. The highest concentrations were noticed in Hamde sour cultivar while the lowest was in Mesri. Therefore, it is recommended that Hamde sour cultivar will be suitable for juice production and for promoting health benefits **[15]**. TPC in eight commercially South African grown pomegranate cultivars differs considerably from those reported for Moroccan pomegranate cultivars. The amount of TPC significantly varied among the cultivars and fell in the range between 140.08 and 530.55 mg/100 ml. Herskawitz cultivar showed the highest content which is fourfold the lowest content in the Ganesh cultivar **[17]**. In addition, there were significant differences between nineteen different pomegranate cultivars (Sixteen cultivars belonged to European pomegranate gene banks in Spain and the remaining cultivars were collected from farmer's markets). The values ranged between 90 and 145 mg GAE/100 ml. The cultivars HIZ, ME13, and VA1 contained higher TPC content with values of 145, 132, and 125 mg GAE/100 mL, respectively **[22]**. Li ***et al*.** 2016 established a validated method for quantitative analysis of TPC in different parts of the fruit including leaves, flesh, peels, seeds, and juices from five Chinese cultivars. TPC and 4 polyphenols values varied significantly in different parts of the fruit. Flesh and peel contained higher TPC content than leaves, seeds, and juices. The peel and flesh represented higher than 83 % of the total TPC content of an entire pomegranate. Among the cultivars, Sour-YRP contained the maximum TPC content (688.61 mg/g) followed by Sweet-TRP (602.98 mg/g). Whereas, Sweet-GP, Sweet-RP, and Sour-RP cultivars contained slightly low TPC content with values of 581.69, 522.41, and 557.98 mg/g, respectively. Regarding the maturity stage of pomegranate, low-maturity contained the highest values of TPC (878.22 mg/g). While the lowest one was in high maturity (581.89 mg/g) **[24]**. TPC content of the juices of six old Italian pomegranate cultivars and for Wonderful ranged from 0.87 to 1.93 mg of GAE/ml. Gaeta 1 variety showed the highest value and Gaeta 4 showed the lowest one. Regarding peel and pulp results, peel samples TPC content was 20–45 times higher than the pulp samples. The pulp samples ranged from 3.19 to 8.89 mg of GAE/g of fresh weight, while peel samples ranged from 90.0 to 137.3 mg GAE/g of fresh weight **[9]**.

2.2.2.2. Total anthocyanins

The red, purple, or blue color of the fruits and the color of pomegranate juice are mainly due to anthocyanins content. As well, anthocyanins contribute to the antioxidant activity of fruits **[18]**. Significant differences in the content of total anthocyanins of the twenty Iranian pomegranate cultivars were verified. The total anthocyanins were measured by the pH differential method by using two buffer systems and the absorbance was detected for samples diluted in the two different buffers at 510 and 700 nm after 15 min. of incubation. Malase Yazdi contained the highest total anthocyanins content (30.11 mg cy-3-glu/100 g) **[18]**. There were also significant differences between five pomegranate accessions collected and located on the farm of the University of Tuscia. Anthocyanins analysis in the juice was performed based on the Mancinelli *et al*. and the extracts were filtered, and their absorbance was detected at 530 nm. The maximum value was in MG3 and the minimum one in MG1. While similar values were detected between the other accessions **[14]**.

2.2.2.3. Total vitamin C

Compared to other fruits such as citrus, pomegranate is not characterized by its high content of vitamin C. However, the detection of this vitamin is crucial from an industrial point of view, since nonenzymatic browning leads to the degradation of vitamin C to hydroxymethyl furfural. This browning may affect the quality of the slightly colored juices, whereas brown compounds affect human acceptance. In addition, vitamin C accelerates anthocyanins loss and decreases its stability. Therefore, the determination of vitamin C content is very crucial to evaluate the final juice quality and detect any damage caused by different processing steps **[19]**. A study on twenty Iranian pomegranate cultivars showed statistically significant variation in ascorbic acid content (9.91–20.92 mg/100 g) **[18]**. In contrast, another study showed no differences between different Spanish pomegranates (Mollar de Elche, Valenciana) and the Wonderful cultivar however, they were detected among cultivars. Vitamin C content varied between 80 and 200 mg/L. Higher values were detected in W6, W7, and M.Leon2. Therefore, these juices would be the most liable to unaccepted browning **[19]**.

Declarations

Ethics approval and consent of participation

Not applicable

Consent of publication

Not applicable

Data and materials availability

All data produced or analyzed throughout this study are included in the current manuscript.

Competing interests

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