Chemical properties and antioxidant activity attributes of *Punica granatum* L. cultivars introduced in Béni Mellal region-Morocco.

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Abstract:

Pomegranate fruit is generally consumed fresh or processed into food products depending mainly on its composition, characteristics, and technological quality. This study aims to assess the variability in terms of physicochemical and biochemical properties of four foreign pomegranate cultivars, newly introduced in Beni Mellal region, to better know their fruit potential in comparison with the local dominant cultivars "*Sefri Ouled Abdellah".* Total polyphenol contents (TPC), total flavonoid contents (TFC), total anthocyanin contents (TAC), soluble sugar contents (SSC), titrable acidity (TA), pH, total soluble solids (Brix°), and juice color (L*, a*, b*, Chroma C*, and hue°) were determined in pomegranate juices. The radical scavenging activity was measured using DPPH to evaluate antioxidant potential. For physicochemical descriptors, the variance was highly significant. The cultivar *Wonderful* has the highest acidity followed by *Smith* (2.449 g/100 mL and 3.11 g/100 mL). *Wonderful* and *Purple Queen* recorded higher values for all biochemical parameters, except for soluble sugars. In fact, cultivars rich in anthocyanins, polyphenols, and flavonoids they presented the highest antioxidant activity. Regarding *Sefri Ouled Abdellah*, this cultivar has the highest values of SSC (154.54 ± 19.08 g/L). This research has highlighted the nutritional and functional potential of the introduced cultivars compared to "*Sefri Ouled Abdellah*" which will help in proposing and orienting their exploitation in Agro-food industries.

Keywords: Antioxidant capacity, Biochemical criteria, physicochemical criteria, *Punica granatum* L.

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Introduction

Pomegranate (*Punica granatum* L.) is a member of the Punicaceae family that grows mostly in semi-arid mild-temperate to subtropical regions. Pomegranate orchards are currently grown commercially in many parts of the world, including the Mediterranean basin, where high quality fruit is produced (Stover and Mercure 2007; Holland et al. 2009). The cropping and production of pomegranate is mainly concentrated in Iran, Afghanistan, India, Mediterranean countries (Morocco, Spain, Turkey, Tunisia, and Egypt) and Middle East countries (Jbir et al. 2008; Melgarejo et al. 2009; Ajal et al., 2015). In Morocco, pomegranate is one of the most important fruit species; with a production that exceeds 113 480 tones from a total area of 12 700 hectares, especially in the region of Béni Mellal, which produce 45 percent of the country's total and is mainly represented by the *Sefri Ouled Abdellah* cultivars (ORMVAT 2002; Walali et al. 2003; Oukabli et al. 2004; MAPMDREF 2018).

The pomegranate fruit is commonly consumed fresh or as juice or used as a source of nutraceutical substances. In fact, organic acids, minerals (such as potassium), vitamins (C, A, and K), and phenolic compounds (which widely exist in the peel, pulp, and seed) are the most important compounds of pomegranate fruits(Gil et al. 2000; Zaouay and Mars 2014; Loukhmas et al. 2020; Chafki et al. 2021; Elfazazi et al. 2022). These phenolic compounds include hydrolysable tannins (punicalagins and punicalins), proanthocyanidins (condensed tannins), anthocyanins, catechins, and phenolic acids (Gallic, ellagic, and chlorogenic, among many others), (Fischer et al. 2011; Qu et al. 2012). Recently, the health benefits of regular consumption of fruits and vegetables have received considerable interest all over the world. This nutritional value consists of many biologically active molecules (fibers, carotenoids, phenolic compounds, vitamins...) (Tomas-Barberan and Gil 2008).

Despite its recognized benefits, the pomegranate remains under exploited in Morocco compared to other producing countries. In fact, pomegranate fruits are locally marketed with low processing and valorization. Moreover, the plant material variability generates heterogeneity of production, and the characteristics of the majority of cultivars, local and introduced, are not known (Haddioui 2012; Loukhmas et al. 2020). The commune of *Ouled Abdellah*, the first zone of pomegranate production in Morocco, is dominated by the *Sefri*

cultivar, which poses marketing problems, especially since the fruit matures at the same time. Varietal diversification is desirable especially by cultivars having different periods of maturity or they have different aptitudes for transformation (varieties of juice).

To the best of our knowledge, although various studies have been performed on pomegranates cultivated in Morocco, no previous studies have been reported on our newly introduced foreign cultivars in Béni Mellal region, which will help the local farmers in selecting cultivars with the best technological quality.

In this context, this study aims to assess physicochemical, biochemical composition, and antioxidant activity of four newly introduced pomegranates cultivars in Béni Mellal region compared to the dominant cultivars *Sefri Ouled Abdellah*. The results of this study will help in evaluating their characteristics and better understanding the quality of the introduced cultivars in order to direct their valorization and commercialization.

1. Materials and Methods

1.1. Plant Materials

Five varieties named *Sefri Ouled Abdellah, Purple Queen, Wonderful, Mollar Roja*, and *Smith.,* were the subject of this study. 50 fruits were manually harvested from 10 pomegranate tree per variety in full maturity during the period between September and November of 2021 from Béni Mellal-Khénifra region of Morocco (table 1). 10 fruits per variety were randomly selected for chemical analysis.

Table 1: Geographic origins of pomegranate fruits of five cultivars cultivated in Morocco.

Cultivars	Country of Origin	Growth area	Latitude north	Longitude west
Purple Queen	Spain	Ouled Yaich , Beni Mellal	32°24'52.0"N	6°26'22.1"W
Wonderful	USA	Sidi Hammadi, Beni Mellal (Deroua)	32°20′43.31″N	6°48′69.52″W
Mollar Roja	Spain	Sidi Hammadi, Beni Mellal (Deroua)	32°20′43.31″N	32°20′43.31″N
Smith	USA	Ouled Yaich , Beni Mellal	32°24'52.0"N	32°24'52.0"N
Sefri Ouled Abdellah	Morocco	Ouled Abdellah, Beni Mellal	32°29′42.0″N	6°26′57.0″W

1.2. Juice extraction

Once the fruits are washed by distilled water and peeled, the seeds are manually separated from the rind, and then the juice is extracted from the arils using a centrifugal (Moulinex JU610D10, France).

1.3. Physicochemical criteria

Titrable acidity (TA), pH, Total soluble solids (TSS), color

The titrable acidity was calculated as the percentage of citric acid by titrating 10 mL pomegranate juice with a solution of NaOH (0.1 N) until pH = 8.1 was reached (IFU 1996). The pH was measured by a pH meter (Hanna HI98161 USA). The total soluble solid was measured as °Brix by a refractometer (Portable Refractometer, VBR50, China), Maturity index (MI) was calculated as the ratio of TSS/TA. (IFU 1991).

Color measurement

Pomegranate juice color Measurements were performed using a Chromameter (CR-410, Konika Minolta Sensing Inc., Osaka (Japan)) which gives the Colorimetric variables L∗, a∗ and b∗. The juice extraction was performed using a juice centrifuge (Easy Fruit Metal Juicer-JU610 Moulinex). After extraction, the juice was homogenized and immediately analyzed.

Colorimetric variables (L*, a*, b*) were measured and color change (ΔE∗ab), chroma (C*) and hue° were calculated from:

$$\Delta^*_{ab=\sqrt{(\Delta L^{*2}+\Delta a^{*2}+\Delta b^{*2})}} \quad (1)$$

$$C^* = \sqrt{(a^{*2}+b^{*2})} \quad (2)$$

$$H^* = tan^{-1}\left(\frac{a}{b}\right) \quad (3)$$

Color measurement was performed on 30 mL samples in Petri dishes against a background of white tiles (Yawadio and Morita, 2007).

1.4. Biochemical criteria

Total phenolic content (TPC)

Total phenols were estimated by the Folin Ciocalteu method described by Singleton et al. (1988). 300µl of diluted juice were added to 1.5 mL of the Folin-Ciocalteu (1/10) mixture. After 5 min of incubation in darkness, 1.2 mL of 7.5% sodium carbonate (Na_2CO_3) solution was added to the previous mixture. The contents of the tubes are mixed using a shaker (Velp scientifica) and incubated for 90 min in darkness at room temperature. The total polyphenol content was reported as milligram equivalents of Gallic acid per gram of dry matter. The reading of the absorbance was made at 765 nm using a UV-Visible spectrophotometer (MAPADA V1100D Visible Spectrophotometer).

Total flavonoid content (TFC)

The total flavonoid content of the juices is determined by spectrophotometry according to the method of Lamaison and Carnat (1990). This method based on the formation of a complex flavonoid-aluminium, having the maximum absorbance at 430 nm. 1 mL of diluted sample was separately mixed with 1 mL of 2% aluminum chloride methanolic solution. After incubation at room temperature for 15 min, the absorbance of the reaction mixture was measured at 430 nm with a UV-Visible spectrophotometer (MAPADA V1100D Visible Spectrophotometer). The flavonoid content was expressed as mg of Rutin equivalent per 100mL of juice.

Total anthocyanins content (TAC)

The TAC was estimated by pH differential method using two buffer systems: potassium chloride buffer pH1.0 (25 mM) and sodium acetate buffer pH4.5 (0.4 M) (Ozgen et al. 2008). Briefly, 0.2 mL of pomegranate juice sample was mixed with 1.8 mL of corresponding buffers and read against water as a blank at 510 and 700 nm.

Absorbance was calculated as:

$A = [(A_{510}-A_{700})\ pH_{1.0} - (A_{510}-A_{700})\ pH_{4.5}]$

$TAC = \left(\frac{A \times MW \times FD \times 100}{MA}\right)$

Where A: absorbance; MW: molecular weight (449.2 g/moL); DF: dilution factor (20); MA: molar absorptivity coefficient of cyanidin-3-glucoside (26.900).

Results were expressed as mg cyanidin-3- glucoside equivalents per 100mL.

Soluble sugars content (SSC)

The determination of total soluble sugars was carried the method described by Dubois et al. (1956). The result was expressed as g Glucose equivalents per 1L.

Radical scavenging activity DPPH

This method is based on the measurement of the capacity of antioxidants to trap the DPPH radical. The effect of each extract on DPPH is measured according to Brand-Williams et al. (1995) 25. 100µl of pomegranate juice sample is added to 2 mL of freshly prepared methanolic solution of DPPH (0.1 mM) incubated in darkness for 30 min. Absorbance reading is performed at 517 nm.

1.5. Statistical analysis

Statistical evaluation of the data was done with the IMB SPSS Statistics V21 software. All data were expressed as means ± SD (n=3). Data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan post hoc test ($P<0.05$). All variables were subjected to bivariate correlation using the Pearson coefficient Results.

1.6. Physicochemical criteria

The results obtained for titrable acidity (TA), pH, and total soluble solid (Brix degree), of different cultivars are presented in Table 2. Significant variability was revealed between pomegranate cultivars for all the parameters studied.

The highest total soluble solid was observed in local cultivar *Sefri Ouled Abdellah* (15.33±0.33), followed by *Smith* (14.16±0.44°) *Mollar Roja* (14.16±0.16), *Wonderful* (14.83±0.16), while *Purple Queen* recorded the lowest value (12.00±0.00 °Brix). On the other hand, *Mollar Roja* and *Purple Queen cultivars*, showed significantly higher pH values (4.51±0.02 and 4.25±0.06 respectively) than those found in *Sefri Ouled Abdellah*, *Smith* and, *Wonderful* (3.890±0.00, 3.11±0.05 and 3.00±0.00 respectively).

Wonderful exhibited the highest TA (2.44±0.12 g /100mL), followed by *Smith* (1.55±0.01 g /100mL), whereas the other cultivars displayed lower juice acidity ranging from 0.15±0.01 g/100mL to 0.19±0.00 g/100mL. Regarding maturity, our cultivars can be classified in two groups according to the classification that has been established by Melgarejo (1993) who reported that sweet varieties have MI = 31-98 while Sweet-sour varieties have MI = 17-24 and Sour varieties have MI = 5-7.

Purple Queen, Mollar Roja, and Sefri Ouled Abdellah can be considered as sweet varieties while, *Wonderful, Smith* are classified as sour varieties.

Table 2: the physicochemical characteristics of the five cultivars studied

Cultivars	pH	TSS	TA	MI
Purple Queen	4.25±0.06^{b}	12.00±0.00^{c}	0.15±0.01^{c}	79.84±8.71^{b}
Wonderful	3.00±0.00^{d}	14.83±0.16^{ab}	2.44±0.12^{a}	6.09±0.65^{c}
Molla Roja	4.51±0.02^{a}	14.16±0.17^{b}	0.16±0.00^{c}	88.54±1.80^{a}
Smith	3.11±0.05^{d}	14.16±0.44^{b}	1.55±0.01^{b}	9.09±0.47c
Sefri Ouled Abdellah	3.89±0.00^{c}	15.33±0.33^{a}	0.19±0.00^{c}	78.12±2.52^{b}

All data were expressed as means ± SD (n=3), letters mean statistically significant differences ($P < 0.05$). Total soluble solids (°Brix); Titrable acidity (TA, g /100mL); MI = (TSS/TA).

Color coordinates of different cultivars are presented in Table 3. The juice color varied from bright red (high values of L*) to dark red (low value of L* and high values of the H) including. *Sefri Ouled Abdellah* had higher values for all parameters (L*, a*, b*, C*), Except for the H* index. While the rest of the cultivars recorded varying values. *Wonderful* displayed

the lowest L*. The lowest a* was recorded by *Smith and Purple Queen* recorded the lowest b*, while *Mollar Roja* recorded the lowest C* and hue°. Based on L*, c* and h indices, which reflect the intensity of the colour, we note that Wonderful variety has the darkest colour of the juice, followed by *Purple Queen* and *Smith* and the other two varieties have a brighter colours.

Table 3 : Color coordinates of the five cultivars studied

Cultivars	L*	a*	b*	C*	hue°
Purple Queen	45.14±3.10^{b}	46.59±0.88^{b}	28.59±1.27^{c}	54.28±1.41^{b}	31.73±0.66^{d}
Wonderful	34.92±0.49^{c}	61.68±1.97^{a}	54.21±2.08^{a}	82.11±2.86^{a}	41.30±0.17^{b}
Molla Roja	66.26±0.27^{a}	6.25±0.16^{d}	11.62±0.11^{d}	19.98±0.17^{c}	35.60±0.81^{c}
Smith	43.45±0.46^{b}	40.12±1.06^{c}	38.05±1.33^{b}	55.29±2.91^{b}	43.50±0.40^{a}
Sefri Ouled Abdellah	68.16±2.91^{a}	65.66±2.74^{a}	58.34±3.71^{a}	87.85±7.80^{a}	41.50±1.15^{b}

L* indicates lightness or darkness, and ranges from black (0) to white (100) a*—color direction from red (a*>0) to green (a*0) to blue (b* <0); Chroma (saturation or vividness) higher the chromaticity more the vividness, lower the chromaticity more the dullness; Hue—an angular measurement in which 0°=red and 90° yellow

1.7. Biochemical criteria

Total Phenolic content (TPC)

Total phenolic content (Table 3) in pomegranate showed very significant variability among all Cultivars. Recorded values were in a range of 120.72–235.77 mg GAE/100mL. *Wonderful* and, *Sefri Ouled Abdellah* had the highest amounts of TPC (235.77±3.26 and, 172.73±4.79 mg GAE/100mL), whereas the lowest values were recorded for *Smith (*120.72±9.38 mg GAE/100mL).

Total Flavonoids content (TFC)

Total Flavonoids content (Table 3) varied from 6.60 to 67.02 mg RE /100mL. *Wonderful* and *Purple Queen* recorded the highest values (67.02 mg RE /100mL and 65.79 mg RE /100mL),

while *Mollar Roja* , and *Sefri Ouled Abdellah* recorded the lowest values (6.60 to 67.02 mg RE /100mL and 6.42 RE mg/100mL).

Total Anthocyanins content (TAC)

There is a great variation in terms of TAC between pomegranate cultivars (7.273-56.00mg/100mL). *Purple Queen* (56.00 ± 0.77 mg/100mL) and *Wonderful* (44.64 ± 4.22 mg/100mL) recorded the highest TA .Whereas the local cultivar *Sefri Ouled Abdellah* had, the lowest value (7.27 ± 0.17 mg/100mL).

Radical scavenging activity DPPH

The DPPH assay (Table 3) ranged from 8.51mg/mL to 4.68 mg/mL. *Wonderful* recorded the highest concentration (8.51±0.58 mg/100mL) followed by *Purple Queen* (7.53±0.39), whereas *Mollar Roja* recorded the lowest concentration (4.68 ± 0.08 mg/100mL).

Soluble sugar content SSC

Total soluble sugars (Table 3) ranged from 61.77 to 154.16 g/L. The highest values were recorded by local cultivar *Sefri Ouled Abdellah (*154.16±19.08 g/L), while the other cultivars displayed lower SSC ranging from 61.77±6.27 to 128.80±30.25g/L.

Table 4 : Biochemical traits of five cultivars:

Cultivars	TPC	TAC	TFC	DPPH	SSC
Purple Queen	132.79±0.48^{cd}	56.00±0.77^{a}	65.79±3.72^{a}	7.53±0.39^{b}	61.77±6.27^{b}
Wonderful	235.77±3.26^{a}	44.64±4.22^{b}	67.02±4.78^{a}	8.51±0.58^{a}	69.021±5.43^{b}
Mollar Roja	134.59±10.03^{c}	8.82±3.11^{c}	6.60±0.49^{b}	4.68±0.08^{d}	88.9±5.43^{b}
Smith	120.72±9.38^{d}	7.92±0.85^{c}	6.76±0.20^{b}	4.90±0.49^{d}	128.80±16.60^{a}
Sefri Ouled Abdellah	172.73±4.79^{b}	7.27±0.17^{c}	6.42±0.24^{b}	5.94±0.43^{c}	154.54±19.08^{a}

All data were expressed as means ± SD (n=3), letters mean statistically significant differences ($P < 0.05$) Total content of soluble sugars (SSC, g GE /L), Total polyphenols content (TPC, mg GAE/100 mL),Total anthocyanins content (TAC, mg /100 mL), Total flavonoids content (TFC, mg RE/100mL), and (DPPH, mg Trolox /100mL) in pomegranate juice.

2. Discussion

2.1. Physicochemical criteria

For total soluble solid our results are in agreement with several other studies reported on pomegranate cultivars (Fadavi et al. 2005; Hmid et al. 2018), while other works (Legua et al. 2012; Russo et al. 2018; Tozzi et al. 2020) recorded values higher than ours. This variability could be related to the fact that TSS of pomegranate juice is controlled by multiple factors such as cultivar, growing area, and maturity (Li et al. 2015), and several studies have also shown a considerable decrease in TSS values under deficit irrigation (Ghosh et al. 2015; Adiba et al. 2021). On the other hand, for TA Fadavi et al. (2005) and Hmid et al. (2018) have also reported similar results with values of 0.4 g/100mL to 2.45 g/100mL and 0.19 to 2.31 g/100 ml respectively. While the values reported by Legua et al. (2012) are relatively higher than ours (0.024 -3.75g /100mL). Concerning maturity, it is generally used to quantify the juice taste and as a maturity index (Rajasekar et al. 2012; Zaouay et al. 2012). Furthermore, the sweet pomegranate is consumed as fresh arils while sour pomegranate is used in the food industry for juice production (Kandylis and Kokkinomagoulos 2020).

According to the results, the juice of *Sefri Ouled Abdellah* has a lighter color and contains more magenta red and yellow components than the other cultivars, while *Wonderful* juice has the darker color. The anthocyanins are responsible for the attractive color of the juice (Ban et al. 2007).

2.2. Biochemical criteria

Pomegranate juice contains a rich concentration of bioactive compounds such as polyphenols, flavonoids, anthocyanins, sugar, and natural antioxidants (Wang et al. 2010; Li et al. 2015).

For TPC our data were lower than those of eight Iranian cultivars (Mousavinejad et al. 2009), while for the *Wonderful* variety, we have almost the same result as Gil et al. (2000) who worked on the same variety. The total flavonoids obtained in the present study were somewhat lower than those reported by Elfalleh et al. (2009) with values from 411 to 575 mg RE /100mL juice. While the values recorded by Hmid et al. (2018) are the closest to ours (14.45-56.99 mg RE /100 mL). However, the differences in TPC and TFC concentration in

pomegranate juice between the present study and those reported by other studies may be related to the growing environment, the degree of maturity and genetic variability(Legua et al. 2012; Li et al. 2015; Attanayake et al. 2018). The results regarding TAC were higher than Alighourchi et al. (2008) and Hmid et al. (2018). Although for the cultivar *Wonderful*, Gil et al. (2000) recorded values close to ours for the same cultivar (30.6 mg/100mL). In addition, the cultivars with higher amounts of polyphenols, total flavonoids and anthocyanins have higher antioxidant activity. These variations can be due to different factors such as genetic variability, growing seasons, agricultural practices, and water stress (Çam et al. 2009; Adiba et al. 2021). Various studies have shown that low temperature stimulates anthocyanin biosynthesis gene expression while high temperature represses it in plants (Lo Piero et al. 2005; Ubi et al. 2006; Schwartz et al. 2009; Wang et al. 2016). The antioxidant activity of pomegranate arils is due to anthocyanin, ascorbic acid, hydrolysable tannins, and phenolic acids, which can be found alone or in combination(Alighourchi et al. 2013; Boussaa et al. 2020).

2.3. Correlation among variables

All variables were subjected to bivariate correlation using the Pearson coefficient to better un derstand the link between the different parameters. Several significant correlation levels at the 0.05 or 0.01 level were found (Tables 5). Antioxidant activities were found to be positively and significantly correlated with TPC, TAC and, TFC, with correlation coefficients of r =0.693**, r =0.861** and r =0.920** respectively. These correlations confirm that TPC, TAC, and TFC are the main constituents contributing to the antioxidant activity of these fruits. In addition, TAC were positively and significantly correlated with TFC (r =0.978**) and, antioxidant activity (r =0.920**), while L* were negatively correlated to TAC (r =-0.642**), and TFC (r =-0.700**). This suggests that darker cultivars contain higher levels of total flavonoids and total antocyanins (Rouholamin et al. 2015).

Table 5 : Matrix of correlation coefficients between colors coordinates and biochemical compound involved in this study

	TPC	TAC	TFC	DPPH	L*	b*	c*	hue°
TPC	1							
TAC	0.340	1						
TFC	0.488	**0.978****	1					
DPPH	**0.693****	**0.861****	**0.920****	1				
L*	-0.333	**-0.642****	**-0.700****	-0.602*	1			
b*	**0.661****	0.048	0.154	0.430	-0.243	1		
c*	**0.658****	0.189	0.277	0.535*	-0.271	**0.985****	1	
hue°	0.341	-0.527*	-0.399	-0.178	-0.138	**0.664****	0.529*	1

Significant and potential correlations were marked in bold

**. Correlation is significant at the 00.01 level (2-tailed).

*. Correlation is significant at the 00.05 level (2-tailed).

3. Conclusion

This study revealed considerable variability among the cultivars *Purple Queen, Wonderful, Mollar Roja, Smith* and *Sefri Ouled Abdellah* cultivated in Béni Mellal region of Morocco. The TPC, TFC, TA, and DPPH were particularly high in the cultivars *Purple Queen* and *Wonderful*, whereas the local cultivar *Sefri Ouled Abdellah* recorded the highest value in SSC (the sweetest cultivar). The results of this study confirmed that the genetic profile of the cultivars has largely contributed to the content of bioactive molecules in the juice. The results of this work represent an important reference to determine the quality of these pomegranate cultivars and to propose innovative and adequate ways for their valorization adapted to their chemical quality. The synthesis of the data allows concluding that the *Sefri Ouled Abdeellah* variety is of very good nutritional quality, especially for fresh consumption. However,

Wonderful and *Purple Queen* varieties can enrich the varietal profile in the Ouled Abdellah area, especially as varieties intended for the production of juice.

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