

## RESEARCH ARTICLE

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**Physico-chemical characteristics and antioxidant activity of pomegranate (*Punicagranatum L.*) fruit cultivated in Albania**ARIOLA MORINA<sup>1\*</sup>, RENATA KONGOLI<sup>1</sup>, LUZIANA HOXHA<sup>1</sup>, TATJANA KOKAJ<sup>2</sup>, MAMICA SALAJ<sup>1</sup>, IBRAHIM HOXHA<sup>3</sup><sup>1</sup>Faculty of Biotechnology and Food, Agricultural University of Tirana/PajsiVodina, Kodër Kamëz, Tiranë, Albania<sup>2</sup>Institute of Plant Genetic Resource, Agricultural University of Tirana/PajsiVodina, Kodër Kamëz, Tiranë, Albania<sup>3</sup>University "Haxhi Zeka", Pejë / Rruga"UÇK-së" 30000 Pejë, Kosovo

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**Abstract**

The pomegranate (*Punicagranatum L.*) fruit deserves special attention by consumers interested at consuming nutritional food with excellent taste. Pomegranate is a highly valued crop and is widely cultivated in Mediterranean countries. Based on the maturity index (sugar/acid ratio) pomegranate fruit cultivars can be classified into three groups: sweet, sour-sweet and sour. Pomegranate juice is well known for its health beneficial compounds which can be attributed to its total polyphenolic compounds and high level of antioxidant activity. In this research paper the effects of maturity on the chemical composition (pH, total acidity and total soluble solids), total polyphenolic contents, total anthocyanins contents, vitamin C content and antioxidant capacities of pomegranate juices cultivated in different areas in Albania were assessed. The most widespread pomegranate variety in Albania is named *Devedishe* that grows mostly in hilly areas of Albania. Fruit weigh ranged from 116.3 g to 463.5 g. Arils were separated manually from the fruits after they were washed and peeled off, then the physico-chemical properties of fresh juice prepared from the arils were evaluated. The pH of juice ranged from 2.98 to 3.85, titratable acidity (TA) from 0.55 to 2.9 g/100 ml juice (citric acid), total soluble solids ranged from 13.5 to 18.6 (<sup>0</sup>Brix), maturity index (MI) from 4.7 to 33.03. Total polyphenolic content shows considerable amounts that ranged from 459.4 mg/L GAE to 1448.1 mg/L GAE, total anthocyanins are between 115.22 mg/L and 554.4 mg/L cyanidine-3-glucoside, total flavonoides ranged from 86.94 mg/L to 423.89 mg/L catechin. Total antioxidant activity measured by ABTS method and DPPH ranged from 2123.46 mg/L to 7172.84 mg/L and 45.13% to 88.13% respectively.

**Keywords:** Pomegranate; chemical properties; antioxidant activity.**1. Introduction**

The pomegranate (*Punicagranatum L.*) is one of the oldest fruits that have not changed much throughout the history [1]. The pomegranate cultivation and usages are deeply embedded in human history and its utilization has been found in many ancient cultures as food as well as a medical remedy [2]. The fruit is classified as a berry [3] consisting many closely packed red grains (arils), and irregular segments separated by non-edible white piths and thin membranes. Each aril contains a seed surrounded by edible juice pulp. Optimal conditions for the pomegranate exist in Mediterranean type, arid climates with high exposure to sunlight, annual total precipitation of 170-560 mm, mild winters with minimal temperature not lower than -12°C and hot dry summers without precipitation during the last stages of the fruit development. Under such conditions, the fruit can reach its optimal size, color and sugar concentration, without the danger of peel splitting [4]. Due to very favorable climatic conditions in Albania the pomegranate grows spontaneously and it has produced a huge genetic variability. Almost every region in the country has its own sort of cultivated pomegranates. Apart from the cultivated type, the wild pomegranate is grown as well, and it has found here its natural habitat. From a great massif covered with wild pomegranates on the slopes around Shkodra and Lezha in the north of Albania to its organic cultivation in many parts of the coastal lowland area down south to Saranda bordering Greece, pomegranate growing has

enormous potential in the country. The plant is very resistant to insects and disease infections and grows well in almost all soil types and it requires neither irrigation nor pruning. There is not so much innovation required and on the other side, much care should be devoted towards the preservation of the best fruit characteristics and creation of new varieties with improved qualities.

Consumers across the world are becoming more interested in foods with health promoting functions as they gain more awareness of the links between food and health [5]. Epidemiological studies have revealed that consumption of fruits and vegetables with high phenolic content correlates with reduced cardio- and cerebrovascular diseases and cancer mortality [6,7]. Phenolic compounds produce their beneficial effects by scavenging free radicals. Recently, there has been an increasing interest in determining dietary sources of antioxidant phenolics, and red fruits and their juices such as grape and different berry juices have received attention due to their high antioxidant activity [8]. Pomegranate juice has become more popular because of the attribution of important biological actions [9]. Numerous studies over the past decade have shown that pomegranate juice contains higher levels of antioxidants compared to other fruit juices and beverages [8, 10]. Some authors evaluated the commercial polyphenol-rich beverages available in the U.S. market for their total phenolic compounds, antioxidant activity and antioxidant function on cholesterol (ability to inhibit low-density lipoprotein (LDL) oxidation) [11]. The ranking of the beverages on the basis of the average amounts of total phenolic compounds was: Pomegranate juice > red wine > Concord grape juice > blueberry juice > black cherry and cranberry juice > orange juice, iced tea beverages and apple juice. Both antioxidant activity and antioxidant functionality of the beverages were highly correlated with their total phenolic content levels. The major class of pomegranate phytochemicals is the polyphenols (phenolic rings bearing multiple hydroxyl groups) that predominate in the fruit [12]. The predominant polyphenols in pomegranate juices are flavonoids, condensed tannins and hydrolysable tannins. Flavonoids are mainly found in the peel and arils and include flavonols, flavanols and anthocyanins; hydrolysable tannins are found in the peel, membrane and pith include gallotannins and ellagitannins, while condensed tannins are found in the peel and juice [12]. Organic acids such as gallic acid, chlorogenic acid and citric acid are mainly found in the juice. This paper wants to overcome the existing knowledge gap, at least partly, by elucidating the physicochemical and phytochemical characteristics of the pomegranate variety 'Devedishe' which is commonly grown in Albania.

## 2. Material and Methods

For this study were collected fresh pomegranate fruits of Devedishe variety from Shkodër (D/Sh), Valias (D/V), Lezhë (D/L), Berat (D/B), Institute of Plant Genetic Resources (D/IPGR), Tivarash variety cultivated at IPGR (T/IPGR), Ekotip variety cultivated at IPGR (E/IPGR), Kokërrmadh cultivated in Valias (K/V) and Wild variety cultivated in Dajt (W/D). All samples were harvest in 2017 at their maturity stages based on subjective evaluation of the texture of fruit and skin color and all fruits had a firm texture and reddish color. The fruits were first flushed by tap water before the peel, pulp and seed fractions were carefully separated. Peel and pulp were separated manually after measurement of fruit fresh mass. Fruits were weighted on a balance of accuracy of 0.001 g. Aril, juice and seed mass were measured as above. Then the arils and juices were analyzed for major chemical compositions and antioxidant activity.

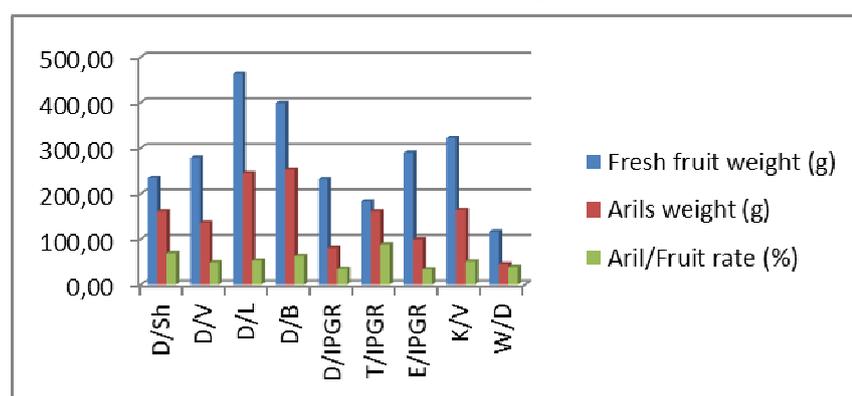
### 2.1. Chemical analysis

Total soluble solids (TSS) measurement expressed as the refractive index of fresh pomegranate juice samples was determined according to AOAC method using a Bellingham+Stanley Ltd. Abbe 60/DR refractometer and the results are reported as degree Brix (<sup>0</sup>Brix). Total titratable acidity (TTA) was estimated by titration of fresh pomegranate juice with 0.1 N NaOH to the end point of pH 8.2 and expressed as citric acid per 100 g of juice. The pH measurements were performed using a digital pH meter calibrated at 21°C, using standard buffer solution at pH 4 and 7. Moreover, maturity index (MI) was also calculated as the ratio of TSS to TTA, according to Nuncio-Jáuregui et al. 2015 [13]. Total phenolic (TP) content of pomegranate juices was determined colorimetrically using Folin-Ciocalteu's reagent as described by Singleton & Rossi, 1965 [14]. Samples (50 µL) were mixed with Folin-Ciocalteu reagent and 7.5% sodium carbonate. The mixture was allowed to stand for 30 min at room temperature in the dark before the absorbance was measured spectrophotometrically at 760 nm using

UV/Vis spectrophotometer Libra S22 (Bichrom, UK). The total phenolic content is expressed as mg/L Gallic acid equivalents (mg/L GAE) using a gallic acid (50 – 500 mg/L) standard curve. Total flavonoid content was measured using aluminum chloride colorimetric assay [15]. An aliquot (1mL) of sample was added to 10 mL volumetric flask containing distilled water. Then 0.3 mL NaNO<sub>2</sub> was added to the flask and after 5 min, 0.3 mL AlCl<sub>3</sub>. At 6th min, 2 mL NaOH (1 M) was added and the total volume was made up to 10 mL with distilled water. The solution was mixed completely and the absorbance level was measured versus prepared reagent blank at 510 nm. Total flavonoid content is expressed as mg/L catechin equivalents (mg/L CE). Anthocyanin's content was measured according to the pH differential method [16]. Absorbance of an aliquot of 1ml was diluted 1:10 with solution pH 1.0 and pH 4.5. The absorbance of the mixtures was then measured at 535 and 700nm against distilled water. Calculation of the anthocyanins concentration was based on a cyanidin-3-O-glucoside and results are expressed as mg/L cyanidin-3-glucoside equivalents (mg/L C3G). *ABTS assay*. Antioxidant activity was determined using ABTS radical scavenging assay [17]. ABTS and potassium persulfate mixture was kept in the dark at room temperature for 16 h before use. For the analysis, the stock solution was diluted in aqueous methanol 80% (v/v) until the absorption at 734nm was 0.7± 0.02. 10µl of sample was mixed with 990µl of ABTS reagent. The percentage inhibition of absorbance at 734nm after 6min at room temperature was calculated and plotted as a function of antioxidant concentration. The result is expressed as mg/L ascorbic acid equivalents (mg/L AAE). *DPPH assay*. The antioxidant capacity of the juice extracts was studied through the evaluation of the free radical-scavenging effect on the 1,1-diphenyl-2-picrylhydrazine (DPPH) radical. The determination was based on the method proposed by Brand-Williams et al. [18]. Pomegranate juice was diluted with methanol:water (6:4) and the absorption was measured after mixed with 0.1mM DPPH while 2ml of 80% aqueous methanol was used as a blank solution. The mixtures were incubated in the dark for 30 min and the absorbance was measured at 517nm. The percentage of inhibition was calculated as:  $\text{Inhibition \%} = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}$  and the results are expressed as the percentage of inhibition of the DPPH.

### 3. Results and Discussion

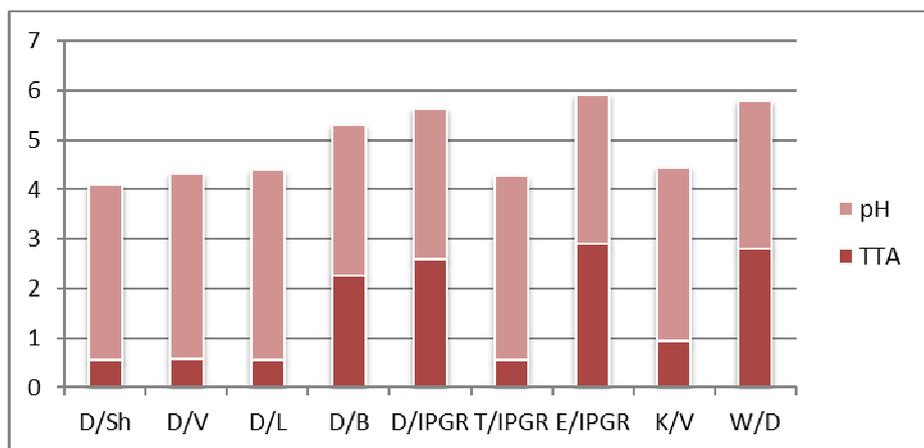
Physical characteristics of pomegranate variety Devedishe, Tivarash, Ecotip, Kokërrmadh and Wild variety are presented at Figure 1. The results show that maximum fresh pomegranate weight range from a minimum 116.27g to 463.48g represented from wild pomegranate and Devedishe variety respectively. “Devedishe” pomegranate cultivar grown in different areas of Albania show a maximum fresh fruit weight reached in Lezha area and the minimum fruit weigh is found in pomegranate samples taken from Institute of Plant Genetic Resources with a value of 231.44g (Figure 1). In several previous studies, a wide variation was found on fruit mass of pomegranate cultivars that varied between 150 and 568 g [19].



**Figure 1.** Mean values of main physical characteristics of pomegranate juices from Devedishe, Tivarash, Ecotip, Kokërrmadh and Wild variety grown in Albania.

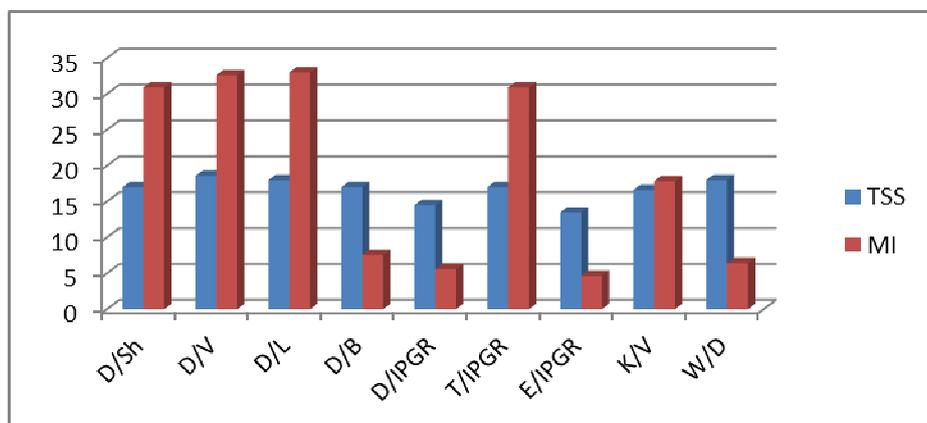
Arils weight is one of the most important parameters from an industrial point [20] because it affects the yield of juice and the results showed a range from 45.11g to 252.47g presented from wild variety and Devedishe cultivated in Berat, but when we present aril weight/fruit weight rate we see that the highest value 88% is

presented from variety “Tivarash” cultivated at IPGR and the lowest value 34% and 35% presented from “Ecotip” and “Devedishe” cultivated at IPGR.



**Figure 2.** Mean values of pH and total titratable acidity (TTA) expressed as g citric acid/100g juice of pomegranate juices from Devedishe, Tivarash, Ekotip, Kokërrmadh and Wild variety grown in Albania.

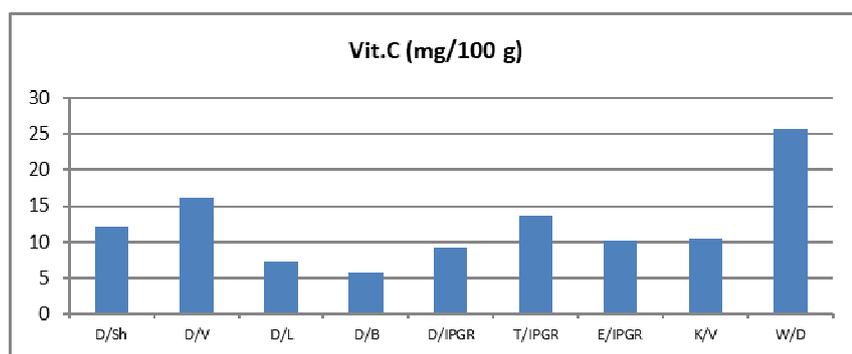
The highest value of pH is 3.85 (Devedishe/Lezhe) and 3.74 (Devedishe/Valias), while the lowest value is 2.98 (wild pomegranate) and 3.02 (Ekotip/IPGR) (Figure 2). This parameter defines the acidic taste of pomegranate juice [21]. Citric acid is the predominant acid in pomegranate [22]. Kulkarni & Aradhia stated that acidity decreases at the time of maturation and is associated with increasing in the sugar content [23]. The titratable acidity showed values from 0.55g/100g of juice (Devedishe/Shkodër; Tivarash/IPGR) to 2.8g and 2.9g/100g of juice (Wild/Dajt and Ekotip/IPGR respectively) (Figure 2). The results for total titratable acidity (TTA) and pH showed a clear relation.



**Figure 3.** Mean values of total soluble solids (TSS) ( $^{\circ}$ Brix) and maturity index (MI) (expressed as TSS/TTA) of pomegranate juices from Devedishe, Tivarash, Ekotip, Kokërrmadh and Wild variety grown in Albania.

Türkmen and Ekşi reported that according to AIJN proposal, the minimum  $^{\circ}$ Brix of pomegranate juice should be 14.0  $^{\circ}$ Brix indicates the percentage of water-soluble solids in fruit juice and can be affected by many factors: cultivar, growth region, year of production and maturity level of the fruit [24]. With regard to TSS, all juice samples presented values that ranged as low as 13.5 $^{\circ}$ Brix (Ekotip/ IPGR) to the highest value of 18.6  $^{\circ}$ Brix (Devedishe/Valias) (Figure 3). The MI is related to the taste and flavor of pomegranate, and some authors have used it to classify pomegranate cultivars [20]. Other researchers have noted wide ranges for this parameter, for example, Sharman & Bist reported that MI value was 95.16 [25]. Following classification was proposed by Martinez et al. [26] for the values of maturity index in Spanish cultivars: maturity index (MI) = 5 - 7 for sour, MI = 17 - 24 for sour - sweet and MI = 31 - 98 for sweet cultivars [26]. Cam et al. and Martinez et al. stated the maturity index (MI = TSS/TA) is responsible for the taste and flavor of pomegranate and quality of the cultivars depends in this factor [26,27]. Based on these results, Ekotip, wild variety and Devedishe/Berat are classified as

sour (4.7, 6.4, 7.6, respectively), Kokërrmadh/Valias as sour-sweet, whereas Devedishe variety cultivated in Shkodër, Lezhë and Valias are classified as sweet.



**Figure 4.** Mean values of vitamin C expressed as mg/100g juice of pomegranates from Devedishe, Tivarash, Ekotip, Kokërrmadh and Wild variety grown in Albania.

The highest content of vitamin C is found in wild pomegranate (25.8 mg/100g), followed by Tivarash/IPGR (13.6 mg/100g) and the lowest in Devedishe/Lezhe (7.23 mg/100g) and Devedishe/Berat (5.64 mg/100g) (Figure 4). According to results, the cultivars and climacteric condition of cultivation areas have a very important role in the amount of total soluble solids, pH, titratable acidity and vitamin C [20]. Phenolic compounds in fruits and vegetables have important contributions to sensory attributes, as well as potential health benefits [28]. Notably, loss of astringency is a desirable change that occurs during maturation and ripening of pomegranate fruits that is primarily due to decreases in the level of phenolic compounds during maturation [23,29].

**Table 1.** Mean values and standard deviation of total phenolic content (TP as mg/L Gallic acid equivalent), total flavonoids (TF as mg/L catechin equivalent), total anthocyanins (TA as mg/L cyaniding-3-glucoside equivalent) and antioxidant activity using ABTS method (mg/L ascorbic acid equivalents AAE) and DPPH method (%).

Nr.	Code	TP (mg/L GAE)	TF (mg/L CE)	TA (mg/L C3G)	ABTS (mg/L AAE)	DPPH (%)
1	D/Sh	925.35±0.58	223.89±0.08	554.40±0.24	3358.02±0.10	71.95±0.026
2	D/V	1029.31±0.76	351.67±0.13	115.22±0.30	4320.99±0.49	71.03±0.04
3	D/L	868.65±1.00	162.78±0.13	327.30±0.12	4827.16±0.23	73.31±0.01
4	D/B	459.41±0.79	151.67±0.12	143.61±0.31	3000±0.16	55.74±0.03
5	D/IPGR	831.29±0.85	196.11±0.09	133.59±0.46	3629.63±0.31	59.77±0.01
6	T/IPGR	1251.09±0.91	423.89±0.04	283.05±0.25	4506.17±0.16	74.61±0.02
7	E/IPGR	691.68±0.13	207.22±0.40	154.46±0.08	3197.53±0.30	56.60±0.02
8	K/V	316.44±0.21	86.94±0.09	102.09±0.22	2123.46±0.18	45.13±0.01
9	W/D	1448.12±0.42	396.11±0.18	267.18±0.08	7172.84±0.20	88.13±0.01

The results presented at Table 1 show that the highest quantity of total phenolic content for samples of pomegranate juices is 1448.12±0.42 mg/L GAE (Wild variety) and 1029.31±0.76 mg/L GAE (Devedishe/Valias) while the lowest is 459.41±0.79 mg/L GAE (Devedishe/Berat) and 316.44±0.21 mg/L GAE (Kokërrmadh/Valias). Samples of pomegranate juices from Tivarash variety cultivated in IPGR showed the highest content of total flavonoids (423.89 ±0.04 mg/L CE) followed by Wild variety with 396.11±0.18 mg/L CE, while the lowest content 86.94±0.09 mg/L CE is found in Kokërrmadh/Valias. One of the most important quality characteristics of pomegranate is the red pigmentation of seeds and juice, which is affected by the anthocyanin concentration and the chemical structures of the individual anthocyanins [30, 31]. Anthocyanins content on pomegranate juice ranged from the highest quantity of 554.4±0.24 mg/L C3G (Devedishe/Shkoder)

followed by 327.3±0.12 mg/L C3G (Devedishe/Lezhe) to the lowest quantity of 115.22±0.3 mg/L C3G (Devedishe/Valias) and 102.09±0.22 mg/L C3G Kokërrmadh/Valias. The highest and the lowest antioxidant activity using ABTS method were detected in wild variety of pomegranate (7172.84±0.20 mg/L AA) and Kokërrmadh/Valias (2123460±0.18 mg/L AA) respectively (Table 1). Antioxidant activity measured by the bleaching of the radical DPPH showed that the highest activity is presented from Wild variety (88.13±0.01%) followed by Devedishe/Lezhe (73.31±0.01%) and the lowest activity from Kokërrmadh/Valias (45.13±0.01%). Our results are in comparable with reported levels of antioxidant activity in other researches were 10.37–67.46 % for seven cultivars of pomegranate juices in Turkey and 18.6–42.8 % for eight pomegranate juices from Iran [10, 33]. The differences in the genetic variability led to the variation in the biosynthesis of phenolic compounds among cultivars. These results show a close correlation between total phenolic content and antioxidant activity of pomegranate juices grown in Albania.

#### 4. Conclusions

This study showed differences in some physical and chemical properties of four pomegranate cultivars grown in Albania that can be affected by genetic variability, area of growth and climacteric condition. Based on these results Ekotip, Wild variety and Devedishe/Berat are classified as sour, Kokërrmadh/Valias as sour-sweet, whereas Devedishe variety cultivated in Shkodër, Lezhë and Valias are classified as sweet. The four pomegranate varieties showed that the chemical characteristics like vitamine C, phenolic contents, anthocyanins contents and flavonoids contents highlighting that the pomegranate fruit varieties grown in Albania could be a very good source of antioxidants. The wild variety exhibits high antioxidant capacity and vitamine C, which make it a high promising variety as a genetic resource for creating potential cultivars used for consumer consumption and food industry. However, more studies of physical and chemical properties of pomegranate are required.

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