Original article

# Evaluation of physicochemical characteristics of pomegranate (*Punica granatum* L.) fruit during ripening

**Mehdi Zarei[1*], Majid Azizi[1], Zeinolabedin Bashir-Sadr[2]**

[1] Dep. Hortic. Sci., Fac. Agric., Ferdowsi Univ. Mashhad, Mashhad, P.O Box 91757-1163, Iran
m_zarei_63@yahoo.com

[2] Ind. Sci. Res. Inst. Iran, PO Box 37575-111, Tehran, Iran

* Correspondence and reprints

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## Evaluation of physicochemical characteristics of pomegranate (*Punica granatum* L.) fruit during ripening.

**Abstract — Introduction**. Pomegranate fruit is a good source of bioactive compounds. Although data about the importance of pomegranates in human nutrition has increased extensively in the last years, the fruit physical and chemical characteristics of some Iranian pomegranate cultivars during fruit ripening have not been investigated in detail yet. Therefore, the evaluation of physicochemical characteristics of pomegranate fruit cv. 'Rabbab-e-Fars' at three different stages, from fruit set to ripening, was the aim of the present study. **Materials and methods**. Fruit fresh weight and volume, and peel, aril, juice and seed percentage were measured as physical features and total soluble solids, pH, titrable acidity, ascorbic acid, total sugars, anthocyanins, phenolics and tannins, condensed tannins, and antioxidant activity in the juice were evaluated as the chemical properties. **Results**. The highest percentage of aril (57.86%) and juice (48.01%) and lowest percentage of peel (42.13%) and seed (10.49%) were observed in 140-day-old fruits. A significant increase in concentrations of total soluble solids and total sugars were recorded during fruit ripening. The ascorbic acid content decreased significantly, while the amount of total anthocyanins increased significantly with fruit maturity. Ripe fruit, which had a low titratable acidity (1.35 $g \cdot 100\ g^{-1}$ of fruit juice), showed a correspondingly high pH (3.23). The levels of total phenolics, total tannins, condensed tannins and antioxidant activity declined significantly during fruit ripening. **Conclusion**. Our results provide important information on the changes in physical and chemical properties of pomegranate fruit during ripening, which is very useful for determination of the fruit quality.

**Iran Islamic Republic / *Punica granatum* / fruits / proximate composition / physicochemical properties / antioxidants**

## Évaluation des caractéristiques physico-chimiques des fruits de *Punica granatum* L. pendant la maturation.

**Résumé — Introduction**. Le fruit de *Punica granatum* est une bonne source de composés bioactifs. Bien que les données sur l'importance de ce fruit en nutrition humaine se soient bien développées durant ces dernières années, ses caractéristiques physiques et chimiques pendant la maturation n'ont pas encore été étudiées en détail pour certains cultivars iraniens. Par conséquent, nous avons évalué les caractéristiques physico-chimiques de grenades (cv. Rabbab-e-Fars) à trois étapes différentes de leur développement, de la nouaison à la maturation. **Matériel et méthodes**. Le poids à l'état frais et le volume du fruit et le pourcentage de peau, d'arille, de jus et de graines ont été mesurés en tant que caractéristiques physiques et les solides solubles totaux, le pH, l'acidité titrable, l'acide ascorbique, les sucres totaux, les anthocyanines, les composés phénoliques et les tannins, les tannins condensés et l'activité antioxydante dans le jus ont été évalués comme propriétés chimiques. **Résultats**. Le pourcentage le plus élevé d'arille (57.86 %) et de jus (48.01 %) et le plus bas pourcentage de peau (42.13 %) et de graines (10.49 %) ont été observés sur des fruits au stade de 140 jours après la nouaison. Une croissance significative de la concentration des solides solubles totaux et des sucres totaux a été enregistrée pendant la maturation du fruit. La teneur en acide ascorbique a diminué de manière significative tandis que la quantité d'anthocyanines totales a augmenté significativement avec la maturité de fruit. Le fruit mûr, qui a eu une faible acidité titrable (1.35 $g \cdot 100\ g^{-1}$ de jus de fruit), a montré également un pH élevé (3.23). Les teneurs en composés phénoliques totaux, tannins totaux, tannins condensés et l'activité antioxydante ont diminué de manière significative pendant la maturation du fruit. **Conclusion**. Nos résultats fournissent des informations importantes sur les changements des propriétés physiques et chimiques du fruit de *P. granatum* pendant sa maturation ; celles-ci sont très utiles pour la détermination de la qualité du fruit.

**Iran République islamique / *Punica granatum* / fruits / composition globale / propriété physicochimique / antioxydant**

## 1. Introduction

Pomegranate (*Punica granatum* L.) belongs to the Punicaceae family and is one of the oldest delicious edible fruits. It has been cultivated widely in many tropical and subtropical regions and its cultivation has increased in recent years [1]. Iran is one of the main producers and exporters of pomegranate in the world. The total pomegranate production of Iran was 670,000 t in 2005 [2], and its production is rapidly increasing year by year. Fruit is consumed fresh or processed into juice, jams, syrup and sauce. Pomegranate is also used, traditionally, as medicine [3].

The edible part (aril) of the fruit constitutes 52% of the total fruit weight (w/w), comprising 78% juice and 22% seeds [4], though it depends on the cultivar. Pomegranate juice is an important source of anthocyanins, especially the 3-glucosides and 3,5-diglucosides of delphinidin, cyanidin and pelargonidin [5], and some of the phenolic compounds and tannins such as punicalin, pedunculagin, punicalagin and ellagic acid [4]. In addition, the peel of pomegranate contains substantial amounts of polyphenols such as ellagic tannins, ellagic acid and gallic acid [6].

The popularity of pomegranate is mainly due to a protective role in prevention of oxidation of both low- and high-density lipoprotein, blood pressure, inflammation, atherosclerosis, prostate cancer, heart disease and HIV-1 [7–11]. These beneficial effects have been attributed to the high level of antioxidant activity [12]. Phenolic acids, anthocyanin and ascorbic acid, either alone or in combination, are responsible for the antioxidant activity of pomegranate [13].

Recently, the high antioxidant activity of the extracts from different parts of pomegranate fruit such as the peel, juice and seeds have been reported [12–15]. Antioxidant activity and chemical changes in pomegranate arils during fruit development were analyzed by Kulkarni and Aradhya [4]. Various reports have shown significant variations in organic acids, phenolic compounds, sugars, water-soluble vitamins and mineral composition of pomegranates during the years [16, 17]. Al-Maiman and Ahmad have also studied changes in physical and chemical properties during pomegranate fruit maturation [18]. In addition, the physical and chemical characteristics of the fruit are highly dependent on the season of development and ripening [19–21].

Data about the importance of pomegranates in human nutrition has increased extensively in the last years. In spite of the various pomegranate cultivars grown (more than 760 original, wild and decorative cultivars) in different regions of Iran, the fruit physical and chemical characteristics of some pomegranate cultivars during fruit ripening have not been evaluated in detail yet. As far as we know, there are no data in the literature about changes in total tannins, condensed tannin and antioxidant activity during growth and development of pomegranate fruit in Iran. Therefore, the objective of our present research was to investigate changes in physicochemical properties of pomegranate fruit cv. 'Rabbab-e-Fars' at three different stages, from fruit set to ripening.

## 2. Materials and methods

Pomegranate fruits cv. 'Rabbab-e-Fars' were selected randomly from different mature trees (14 years old) from the Agricultural Research Center of Yazd province, Iran. Since flowering occurs in three distinct waves, about 500 flowers were marked at full bloom to provide fruit samples. The fruits were harvested at different developmental stages, namely, 20, 80 and 140 days old from the day of fruit set. These times were chosen to find the effects of fruit set, maturation and ripening on fruit characteristics. Fruits were transferred soon after harvest in plastic bags to the laboratory, where pomegranates with defects (sunburn, cracks, cuts and bruises in peel) were discarded. Four replicates were maintained for each analysis; each replicate included five pomegranate fruits. All reagents, solvents and standards used were of analytical reagent grade.

Twenty fruits of each stage were individually analyzed for physical characteristics. Fruits were weighed on a balance with 0.001 g accuracy. Fruit volume was calculated by a liquid displacement method. The density of the fruit was obtained by the ratio of weight to volume. The length and diameter of the fruit and calyx were measured with a vernier caliper. The measurement of fruit length was made on the polar axis, *i.e.*, between the apex and the end of the stem. The maximum width of the fruit, as measured in the direction perpendicular to the polar axis, is defined as the diameter. After whole-fruit size measurements, the arils were manually separated from the fruits, and total arils and peel per fruit were measured as above. The measurements of the peel thickness were made using the vernier caliper. Fruit juice content was measured by extraction of total arils per fruit using an electric extractor (Toshiba 5020). The dry matter (peels, juices and seeds) was determined by drying at 70 °C under vacuum [22]. The fresh juices were analyzed for major chemical composition and antioxidant activity.

Titratable acidity (TA) was determined by titration to pH 8.1 with 0.1 M NaOH solution and expressed as g of citric acid per 100 g of juice [22]. The pH measurements were performed using a digital pH meter (Metrohm model 601) at 21 °C. Total soluble solids (TSS) were determined with a digital refractrometer (Erma, Tokyo, calibrated using distilled water). The results were reported as °Brix at 21 °C. The maturity index (MI) was calculated by dividing TSS by TA. Total sugars were estimated according to the method described by Ranganna [23] and results were expressed as g per 100 g of juice. Ascorbic acid was determined by employing the method described by Ruck [24] and results were expressed as g per 100 g of juice. Total anthocyanins were estimated by the pH differential method using two buffer systems: potassium chloride buffer, pH 1.0 (25 mM), and sodium acetate buffer, pH 4.5 (0.4 M) [25]. The results were expressed as mg of cyanidin-3-glucoside equivalents per 100 g of juice.

Total phenolics were measured using the Folin-Ciocalteau method [26]. Total tannins were determined after adding insoluble PVPP and reacting with Folin Ciocalteau reagent [26]. The results were expressed as mg tannic acid equivalents per 100 g of juice. Condensed tannins were analyzed according to the method of Porter *et al.* [27], and results were expressed as mg catechin equivalents per 100 g of juice.

Antioxidant activity was determined by the DPPH method described by Moon and Terao [28]. Briefly, 0.1 ml of pomegranate juice was mixed with 0.9 mL of 100 mM Tris–HCl buffer (pH = 7.4) to which 1 mL of DPPH (500 µM in ethanol) was added. The control sample was prepared in a similar way by adding 0.1 mL of water instead of pomegranate juice. The mixtures were shaken vigorously and left to stand for 30 min. Absorbance of the resulting solution was measured at 517 nm by a Cecil 2010 UV–visible spectrophotometer. The reaction mixture without DPPH was used for the background correction. The antioxidant activity was calculated using the following equation: antioxidant activity (%) = $[1 - (A_{sample\ 517\ nm} / A_{control\ 517\ nm})] \times 100$.

Data were analyzed by Statistical Analysis System (SAS) software Version 9.1 using analysis of variance (ANOVA) and differences among means were determined for significance at $P < 0.05$ using Tukey's test.

## 3. Results and discussion

The physical properties of pomegranate fruit at each stage showed significant differences, except fruit density and calyx length (*table I*). The mean fruit weight of the three different stages comprised (76.71, 150.32 and 235.09) g, respectively, for 20, 80 and 140 d after fruit set (*table I*). The fruit weight increased progressively during fruit ripening, which is in agreement with Al-Maiman and Ahmad [18]. Shulman *et al.* [29] reported that increase in the fruit weight varied depending on the cultivars and ecological conditions. According to our results, as the fruits matured, fruit volume also increased (*table I*). Thus, there is a close relation between fruit weight and fruit volume. Similar results were also reported for avocado [30]. A significant increase in length and diameter of the fruit was observed during fruit ripening. The highest length (71.15 mm) and diameter (83.16 mm) of the fruit were recorded in 140-day-old fruit

**Table I.**
Changes in physical characteristics of pomegranate fruit during ripening (cv. 'Rabbab-e-Fars', Agric. Res. Cent., Yazd prov., Iran).

| Days after fruit set | Fruit | | | | | | Calyx | | |
|---|---|---|---|---|---|---|---|---|---|
| | Weight (g) | Volume ($cm^3$) | Density ($g \cdot cm^{-3}$) | Length (mm) | Diameter (mm) | [length/ diameter] | Length (mm) | Diameter (mm) | [length/ diameter] |
| 20 | 76.71 ± 4.83 c | 78.71 ± 4.83 c | 0.97 ± 0.01 a | 53.11 ± 1.22 c | 47.06 ± 3.57 c | 1.13 ± 0.11 a | 23.43 ± 1.26 a | 21.72 ± 0.47 c | 1.08 ± 0.05 a |
| 80 | 150.32 ± 14.47 b | 159.20 ± 13.49 b | 0.94 ± 0.03 a | 65.27 ± 1.63 b | 66.64 ± 1.44 b | 0.97 ± 0.01 b | 23.51 ± 0.54 a | 26.09 ± 0.76 b | 0.90 ± 0.01 b |
| 140 | 235.09 ± 9.89 a | 250.15 ± 12.71 a | 0.94 ± 0.01 a | 71.15 ± 2.10 a | 83.16 ± 3.44 a | 0.85 ± 0.01 b | 23.54 ± 1.256 a | 31.43 ± 1.25 a | 0.74 ± 0.01 c |

| Days after fruit set | Peel | | | Aril | | Juice | |
|---|---|---|---|---|---|---|---|
| | Thickness (mm) | Weight (g) | Percentage | Weight (g) | Percentage | Weight (g) | Percentage (of whole fruit) |
| 20 | 5.26 ± 0.19 a | 56.19 ± 1.94 c | 73.33 ± 4.08 a | 20.52 ± 4.18 c | 26.66 ± 4.08 c | 5.80 ± 2.13 c | 7.56 ± 2.64 c |
| 80 | 4.09 ± 0.33 b | 83.93 ± 4.48 b | 56.13 ± 4.88 b | 66.38 ± 13.59 b | 43.86 ± 4.88 b | 50.94 ± 10.22 b | 33.78 ± 4.99 b |
| 140 | 3.05 ± 0.14 c | 98.96 ± 4.22 a | 42.13 ± 2.09 c | 136.13 ± 9.46 a | 57.86 ± 2.09 a | 112 ± 5.31 a | 48.01 ± 1.02 a |

| Days after fruit set | Seed | | Dry matter (%) | | |
|---|---|---|---|---|---|
| | Weight (g) | Percentage | Peel | Juice | Seed |
| 20 | 13.88 ± 3.88 b | 18 ± 1.01 a | 37.27 ± 2.68 b | 8.06 ± 0.16 c | 21.29 ± 1.73 a |
| 80 | 21.19 ± 2.85 a | 14.06 ± 0.83 ab | 41.48 ± 1.87 b | 10.18 ± 0.17 b | 22.34 ± 3.95 a |
| 140 | 24.66 ± 1.12 a | 10.49 ± 0.54 b | 45.89 ± 1.33 a | 12.53 ± 0.17 a | 25.34 ± 2.93 a |

Means of 20 fruits in each column followed by different letters are significantly different ($P < 0.05$); ±, standard deviation.

**Table II.**
Changes in chemical characteristics of pomegranate fruit juice during fruit ripening (cv. 'Rabbab-e-Fars', Agric. Res. Cent., Yazd prov., Iran).

| Days after fruit set | pH | Total soluble solids (°Brix) | Titratable acidity ($g \cdot 100\ g^{-1}$) | Maturity index | Total sugars ($g \cdot 100\ g^{-1}$) |
|---|---|---|---|---|---|
| 20 | 2.65 ± 0.02 c | 10.30 ± 0.39 c | 2.76 ± 0.09 a | 3.73 ± 0.26 c | 7.40 ± 0.05 c |
| 80 | 2.78 ± 0.06 b | 15.17 ± 0.30 b | 1.91 ± 0.07 b | 7.93 ± 0.45 b | 13.20 ± 0.07 b |
| 140 | 3.23 ± 0.02 | 19.56 ± 0.39 a | 1.35 ± 0.04 a | 14.48 ± 0.23 a | 17.88 ± 0.08 a |

| Days after fruit set | Total anthocyanins | Ascorbic acid | Total phenolics | Total tannins | Condensed tannins | Antioxidant activity (%) |
|---|---|---|---|---|---|---|
| | ($mg \cdot 100\ g^{-1}$) | | | | | |
| 20 | 3.68 ± 0.48 c | 25.84 ± 0.84 a | 1938.12 ± 20.25 a | 100.60 ± 2.49 a | 41.72 ± 0.22 a | 66.41 ± 0.10 a |
| 80 | 15.28 ± 0.48 b | 13.09 ± 0.50 b | 1298.19 ± 23.92 b | 61.60 ± 2.49 b | 26.06 ± 0.20 b | 61.44 ± 0.11 b |
| 140 | 24.42 ± 0.41 a | 9.78 ± 0.44 a | 786.20 ± 20.40 c | 32.60 ± 2.49 c | 12.50 ± 0.12 c | 56.84 ± 0.49 c |

Means of 20 fruits in each column followed by different letters are significantly different ($P < 0.05$); ±, standard deviation.

(*table I*). The [length / diameter] ratio of fruit decreased significantly with the advance in fruit development, which was consistent with previous reports [31, 32]. The calyx diameter showed significant increase, but calyx length did not show significant increase during fruit ripening (*table I*).

A significant decrease in peel thickness was observed from 5.26 mm in 20-day-old fruit to 3.05 mm in the 180-day-old fruit (*table I*). It was assumed that this decrease was due to increase in the fruit growth rate, which agrees with the results reported by Gozleski *et al*. [32]. The peel and seed percentage declined significantly, while the percentage of aril and juice (of the whole fruit) increased significantly during fruit maturity (*table I*). The lowest amount of peel (42.13%) and seeds (10.49%) and the highest amount of aril (57.86%) and juice (48.01%) were recorded in 140-day-old fruit. About half of the whole fruit weight during many stages of fruit ripening consisted of the aril. The results were in agreement with the findings reported by Shulman *et al*. [29] and Gozleski *et al*. [32]. Al-Maiman and Ahmad [18] also reported that these differences could be attributed to metabolic changes during ripening. The peel and juice dry matter showed significant increase, but seed dry matter did not show significant increase during fruit ripening (*table I*).

One of the processes occurring during fruit ripening is the hydrolysis of starch that accumulates into simple sugars in the early stages of fruit development. Starch and sucrose change into glucose during fruit ripening [33]. The concentration of total soluble solids and total sugars increased significantly during fruit ripening (*table II*). The highest contents of total soluble solids (19.56%) and total sugars ($17.88\ mg \cdot 100\ g^{-1}$) were observed in 140-day-old fruit. Biale reported that the increase in total soluble solids and total sugars during fruit ripening was due to hydrolysis of starch into sugars [34]. Similar patterns of changes were reported for other pomegranate cultivars [5, 18, 35], blackberry [36] and sweet cherry [37].

Titratable acidity content decreased significantly, while pH value increased significantly during fruit ripening (*table II*). The lowest titratable acidity content ($1.35\ g \cdot 100\ g^{-1}$) and the highest pH value (3.23) were recorded in 140-day-old fruit. Acidity was inversely correlated with pH. The ripe fruit, which had a low acid content, had a correspondingly high pH. Titratable acidity reduction can be used as a standard criterion to detect maturation, like soluble solid content. A reduction in titratable acidity content was also reported for other fruits, such as mango and banana [38], and blackberry [36].

The maturity index [TSS / TA] also appeared to be a good indicator of fruit maturity as it increased significantly during fruit ripening (*table II*). Chace *et al.* reported that titratable acidity content of 1.8% and soluble solid content of 17% could be used as the signs of maturation in some pomegranate cultivars grown in California [39].

Anthocyanins are members of the phenolic compounds that contribute to the red, blue or purple colors of many fruits, including pomegranate juice, and they are well known for their antioxidant activity. The total anthocyanin content increased significantly during fruit ripening, being (3.68, 15.28 and 24.42) mg·100 $g^{-1}$ for (20-, 80- and 140-) day-old fruits, respectively (*table II*). A rapid increase in total anthocyanin concentration during ripening was also reported for the 'Mollar' and 'Ganesh' pomegranate cultivars [5, 18].

Ascorbic acid is abundant and it has many biological functions in fruits; it has some roles such as controlling many aspects of redox (oxidation-reduction) and antioxidant activity [4]. In our data, the ascorbic acid concentration declined significantly during fruit ripening, being a minimum of 9.78 mg·100 $g^{-1}$ in 140-day-old fruit (*table II*), which is in agreement with Kulkarni and Aradhya [4]. A decrease in ascorbic acid level and an increase in total sugar and total anthocyanin contents during fruit ripening are due to the shift in the metabolic activity toward biosynthesis of anthocyanins.

As significant decrease in the level of total phenolics was observed during fruit ripening (*table II*). The lowest total phenolic content (786.20 mg·100 $g^{-1}$) was recorded in 140-day-old fruit. The decline in the total phenolic level may be due to the oxidation of phenolic content by polyphenol oxidase that characterizes these stages of maturity [40]. Similar results were also reported for banana [41], pear [40], strawberry [42], guava [38] and other pomegranate cultivars [5, 18]. A reduction in total phenolics along with the greatest accumulation of total anthocyanins may contribute to the biosynthesis of the flavylium ring of anthocyanins.

Tannins are secondary metabolites which defend plants from herbivores by protein precipitation and increased acidity. It has been reported that tannins play an important role in human health and are implicated with numerous biological properties. Total tannin content reduced significantly during fruit ripening (*table II*). The decline in the soluble tannin level with fruit ripening was also reported in different persimmon cultivars [43]. A decrease in the total tannins reduces the astringency of fruit, which is a desirable sensory attribute in pomegranate.

Condensed tannins are also known as proanthocyanidins, which are polymeric flavonoid molecules that are found in a range of higher plant species. The amount of condensed tannin decreased significantly during fruit ripening (*table II*). The decline in condensed tannin content may be due to increasing enzyme activity such as anthocyanin synthase (AS) and 3-glycosyl transferase (3GT) in the formation of anthocyanins [44].

Antioxidant activity was measured in terms of its radical scavenging potential. Antioxidants react with DPPH, which is a stable free radical, and convert it into α, α-diphenyl-β-picryl hydrazine. The degree of discoloration indicates the scavenging potential of the antioxidant extract. The level of antioxidant activity was reduced significantly during fruit ripening (*table II*), which was in agreement with the results of Kulkarni and Aradhya [4]. The decline in antioxidant activity might be due to a decreased concentration of phenolic compounds and ascorbic acid.

## 4. Conclusions

The changes in the physical and chemical characteristics of pomegranate, from fruit set to ripening, clearly explained its growth, development and ripening stages. These results provide important knowledge of changes in total phenolics, total tannins and condensed tannins during fruit growth and maturity, emphasizing that pomegranate fruit can be a good source of bioactive compounds. In addition, these data are very useful for determination of the fruit quality. However, since there are many pomegranate cultivars in Iran which are consumed fresh or processed, more studies of physical and chemical properties are required for different cultivars.

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## Evaluación de las características físico químicas de los frutos de *Punica granatum* L. durante la maduración.

**Resumen — Introducción**. El fruto de *Punica granatum* es una buena fuente de compuestos bioactivos. A pesar de que en estos últimos años se hayan desarrollado bien los datos sobre la importancia de este fruto en cuanto a nutrición humana, aún no se han estudiado en detalle sus características físicas y químicas durante su maduración en ciertos cultivares iraníes. Por lo tanto, se evaluaron las características físico-químicas de las granadas (cv. Rabbab-e-Fars), en tres etapas diferentes de su desarrollo, desde el cuajado hasta la maduración. **Material y métodos**. Se midieron el peso en estado fresco, el volumen del fruto, así como el porcentaje de corteza, de arilo, de jugo y de semillas en tanto que características físicas; y, se evaluaron los sólidos solubles totales, el pH, la acidez total, el ácido ascórbico, los azúcares totales, las antocianinas, los compuestos fenólicos y los taninos, los taninos condensados y la actividad antioxidante en el jugo en tanto que propiedades químicas. **Resultados**. El porcentaje más alto de arilo (57.86%) y de jugo (48.01%) y el porcentaje más bajo de corteza (42.13%) y de semillas (10.49%) se observaron en los frutos en fase de 140 días después de cuajado. Se registró un crecimiento importante de concentración de sólidos solubles totales y de azúcares totales durante la maduración del fruto. El contenido de ácido ascórbico disminuyó de manera significativa, mientras que la cantidad de antocianinas totales aumentó significativamente con la madurez del fruto. El fruto maduro con una escasa acidez total ($1.35\ g \cdot 100\ g^{-1}$ de jugo del fruto) mostró asimismo un pH elevado (3.23). Los contenidos en compuestos fenólicos totales, taninos totales, taninos condensados y la actividad antioxidante disminuyeron significativamente durante la maduración del fruto. **Conclusión**. Nuestros resultados proporcionan informaciones importantes sobre los cambios de las propiedades físicas y químicas del fruto de *P. granatum* durante su maduración; éstas son muy útiles para determinar la calidad del fruto.

**Iran República Islámica / *Punica granatum* / frutas / composición aproximada / propiedades fisicoquímicas / antioxidantes**