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Chemical changes and antioxidant activity in pomegranate arils during fruit development

Anand P. Kulkarni, Somaradhya Mallikarjuna Aradhya *

Fruit and Vegetable Technology Department, Central Food Technological Research Institute, Mysore 570 013, India

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Abstract

The present research work describes the major chemical changes and antioxidant activity and their significance during development of pomegranates. Pomegranate arils showed a significant ($P \leqslant 0.05$) increase in total soluble solids, total sugar and reducing sugar contents up to 100 days of fruit development, followed by a steady-state in their rate of accumulation. The highest anthocyanin pigment content (138 mg/100 g) was observed in 100 day-old fruit. Significant ($P \leqslant 0.05$) decreases, of 76.2% and 71.1% in the concentration of ascorbic acid and total phenolics, respectively, were recorded from 20 to 100 days of fruit development. The equilibrium concentration of these chemicals on the 100th day marked the attainment of optimum maturity and onset of ripening of pomegranate fruit. After an initial rapid decrease (by 66.9%) in total protein content, pomegranate arils showed a significant ($P \leqslant 0.05$) increase (by 58.7%) during the late-developmental stages (80–120 days). The high antioxidant activity (71.2%) of arils recorded in 20 day-old fruit decreased significantly (by 13%) up to 60 days, concomitant with a decrease in ascorbic acid and total phenolics by 68.4% and 63.9% respectively. An increase in antioxidant activity by 10.6% in the late-developmental stage was due to a build up of anthocyanins. The trend in accumulation and depletion of the above mentioned chemicals marked the different stages of fruit development, maturity and onset of ripening. A decrease in anthocyanin pigment concentration (9.3%) from 100 days onwards, as well as a significant decrease in acidity was found to be the major chemical factor for increased incidence of internal browning in over-ripe fruits.

Keywords: Pomegranate arils; Fruit development; Chemical changes and antioxidant activity

1. Introduction

Pomegranate (*Punica granatum* Linn.) is a widely grown horticulture crop in many tropical and subtropical countries. It is one of the hardest fruit crops and thrives well under arid and semi-arid climatic conditions. The fruits are generally harvested when fully ripe and possess a waxy shining surface of reddish yellow (Biale, 1981). The pomegranate fruits have a low respiration rate and a non-climacteric respiratory pattern (Ben-Arie, Segal, & Guelfat-Reich, 1984).

The edible part of the fruit is called arils and constitutes 52% of total fruit (w/w), comprising 78% juice and 22% seeds. The fresh juice contains 85.4% moisture and considerable amounts of total soluble solids (TSS), total sugars, reducing sugars, anthocyanins, phenolics, ascorbic acid and proteins (El-Nemr, Ismail, & Ragab, 1990) and has also been reported to be a rich source of antioxidants (Gil, Tomas-Barberan, Hess-Pierce, Holcroft, & Kader, 2000; Kulkarni, Aradhya, & Divakar, 2004).

Incidence of internal browning of arils is one of the major problems in pomegranates, which usually occurs in over-ripe fruits (Waskar & Roy, 2000). Prabhu Desai (1989) reported that TSS, acidity, ascorbic acid and reducing sugar contents were low, whereas non-reducing sugars and tannins were high in affected arils. Browning

* Corresponding author. Tel.: +91 821 2515653; fax: +91 821 2517233.
E-mail address: aradhyasm@yahoo.co.in (S.M. Aradhya).

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of tissues is generally attributed to oxidation of phenolics. Harvest maturity is also known to influence quality and the nature of disorders during storage life of fresh fruits (Prabhu Desai, 1989). Early harvest may impede the development of the characteristic colour, taste and aroma of pomegranates, while late-harvested fruits exhibit a reduced shelf life; early deterioration was observed in apple, mango and other tropical fruits (Fellman, Rudell, Mattinson, & Mattheis, 2003; Medlicott, Reynolds, New, & Thompson, 1988). There is an increased concern about the quality of fruit during development and prior to harvest aimed at minimizing post-harvest deterioration. The present study was undertaken to investigate changes in the major chemical composition, along with antioxidant activity, in pomegranate arils during different stages of fruit development and maturation.

2. Materials and methods

2.1. Experimental material

Pomegranate fruits, var. Ganesh, at different developmental stages, namely, 20-, 40-, 60-, 80-, 100-, 120- and 140-day old from the day of fruit set, were harvested from a pomegranate orchard located in the Bagalkot district, India, and brought immediately to the Central Food Technological Research Institute, Mysore. Next day the arils were analyzed for major chemical composition and antioxidant activity. Time delay, from harvesting to analysis, was about 24 h. Four replicates were maintained for each analysis, each replicate indicating a single pomegranate fruit.

2.2. Compositional changes

The pomegranate arils were squeezed, in two-layered muslin cloth, to extract the complete juice. For each chemical analysis, juice was freshly extracted. Prior to estimation of total phenolics, total protein content and antioxidant activity, the juice was centrifuged at 5000 rpm for 10 min at 4 °C. Total soluble solids (TSS) in the juice was measured using a digital refractometer (ATAGO RX-5000, calibrated using distilled water). Total sugars, reducing sugars, titrable acidity and total anthocyanin pigments were estimated according to the method described by Ranganna (2001). Total phenol was determined by Folin–Ciocalteau method (Taga, Miller, & Pratt, 1984). The protein dye binding procedure of Bradford (1976) was used for total protein determination. Ascorbic acid was determined by an HPLC method, modified from Wimalasiri & Wills (1983) as follows: about 5*g* of pomegranate arils were blended in 3% (w/v) metaphosphoric acid, centrifuged at 10,000 rpm for 10 min at 4 °C and the volume was made up to 10 ml. HPLC analysis was carried out on an analytical liquid chromatograph LC-10A (Shimadzu, Singapore), equipped with a Rheodyne 7725i injection valve, fitted with a 20 l sample loop and a 250×4.6 mm, i.d. 5 μm, SS Excil Amino column (SGE, Australia). The sample (10 μl) was eluted with an isocratic solvent mixture comprising 0.1 M citrate–phosphate buffer (pH 2.6): acetonitrile (1:3 v/v) with a flow rate at 1.5 ml/min. The UV detection was carried out at 254 nm with a Shimadzu diode array detector, series SPD-M10 Avp, Shimadzu (Singapore).

2.3. Antioxidant activity

Antioxidant activity of pomegranate juice was determined by the DPPH method described by Moon & Terao (1998). Fresh pomegranate juice (0.1 ml) 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 mixture was shaken vigorously and left to stand for 30 min. Absorbance of the resulting solution was measured at 517 nm by a UV–Visible spectrophotometer (UV-160A, Shimadzu Co. Japan). The reaction mixture without DPPH was used for the background correction. The antioxidant activity was calculated using the following equation:

$$\text{Antioxidant activity } (\%) = \left(1 - \frac{A_{\text{Sample (517 nm)}}}{A_{\text{Control (517 nm)}}}\right) \times 100.$$

2.4. Statistical analysis

The data from four replicates were processed by one-way ANOVA, using the least significant test to determine the level of significance at $P \leqslant 0.05$.

3. Results and discussion

3.1. Compositional changes

3.1.1. Total soluble solids, total sugars and reducing sugars

Pomegranate arils showed an increase in concentration of TSS, total sugars and reducing sugars during fruit development (Figs. 1–3). The lowest TSS (13%), total sugar (12.6%) and reducing sugar (12.2%) contents were recorded in 40 day-old fruit. A significant increase in all of the above three constituents was recorded after the 80th day of fruit development and the highest TSS (15.3%), total sugar (16.6%) and reducing sugar (15.7%) contents were recorded in 140 day-old fruit. The increases in TSS, total sugar and reducing sugar may be attributed to hydrolysis of starch into simple sugars (Biale, 1960), which is very much desired in pomegranate fruit. A similar trend has been reported in fruits such as mango and banana (Bashir & Abu-Goukh, 2003).

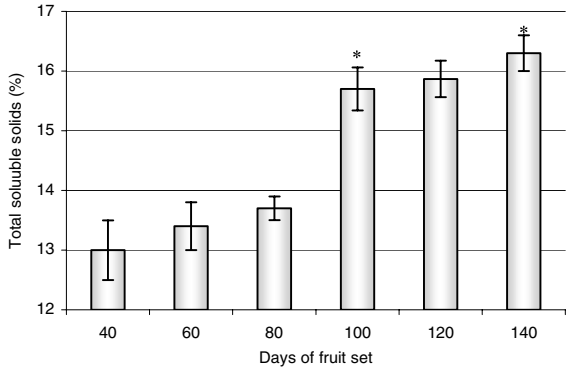

Fig. 1. Total soluble solids (°Brix) of pomegranate arils during fruit development and maturation. 1. Data shown are the means of four replicates vertical bar represents ± standard error. 2. Values followed by an asterisk denote significant difference (at $P \leqslant 0.05$) compared to previous sample.

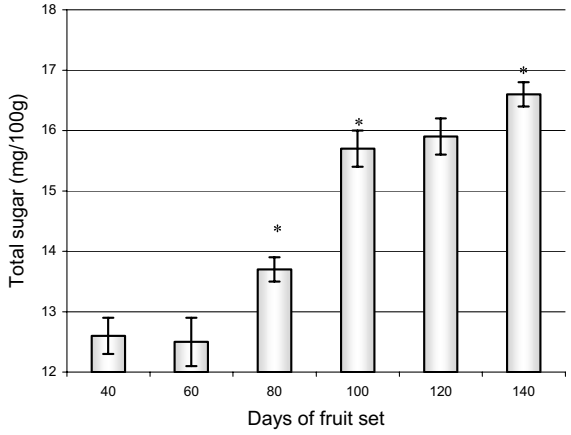

Fig. 2. Total sugar content of pomegranate arils during fruit development and maturation. 1. Data shown are the means of four replicates vertical bar represents ± standard error. 2. Values followed by an asterisk denote significant difference (at $P \leqslant 0.05$) compared to previous sample.

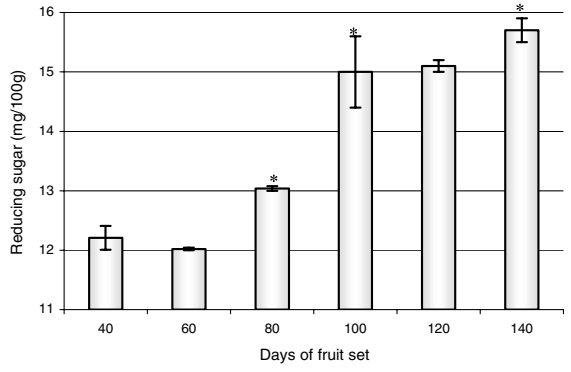

Fig. 3. Reducing sugar content of pomegranate arils during fruit development and maturation. 1. Data shown are the means of four replicates vertical bar represents ± standard error. 2. Values followed by an asterisk denote significant difference (at $P \leqslant 0.05$) compared to previous sample.

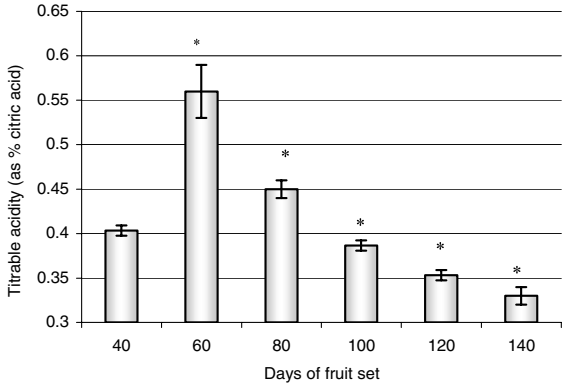

Fig. 4. Titratable acidity of pomegranate arils during fruit development and maturation. 1. Data shown are the means of four replicates vertical bar represents ± standard error. 2. Values followed by an asterisk denote significant difference (at $P \leqslant 0.05$) compared to previous sample.

3.1.2. Titrable acidity

Organic acids found in the pomegranate include citric, malic, acetic, fumaric, tartaric and lactic acids; however, the major acid accounting for titrable acidity in pomegranate arils is citric acid (Melgarejo, Salaza, & Artes, 2000). The highest titrable acidity (0.56 as % citric acid) was recorded in 60 day-old pomegranate fruit arils. This was followed by a continuous, but significant decrease in titrable acidity to the lowest concentration of 0.33 (as % citric acid), which was recorded in 140 day old fruit (Fig. 4). The decrease in acidity coincided with the increase in sugar concentration. A gradual decrease in acidity, concomitant with increased TSS and total sugar content, is an inherent process during ripening of pomegranate to impart the characteristic flavour. However, it also induces discoloration of anthocyanin pigments (Cabrita, Fossen, & Andersen, 2000; Cordenunsi, Nascimento, & Lajolo, 2003).

3.1.3. Anthocyanin pigments

A rapid increase (100%) in the anthocyanin pigment concentration was observed in pomegranate arils between 20 and 80 days of fruit development (Fig. 5). The highest concentration of anthocyanins (138 mg/100 g) was recorded in 100 day-old fruit, which was followed by a slight, but significant decrease of 9.3% up to 140 days of fruit development (Fig. 5). This decrease in the anthocyanin content after 100 days of fruit development may be attributed to a decrease in acidity (Fig. 4). The anthocyanin pigments undergo reversible structural transformation with a change in the acidity (Cabrita et al., 2000).

3.1.4. Total phenolics

Pomegranate arils showed a rapid and significant ($P \leqslant 0.05$) depletion (by 54.5%) in total phenolics during the initial stage of fruit development from 20 to 40 days;

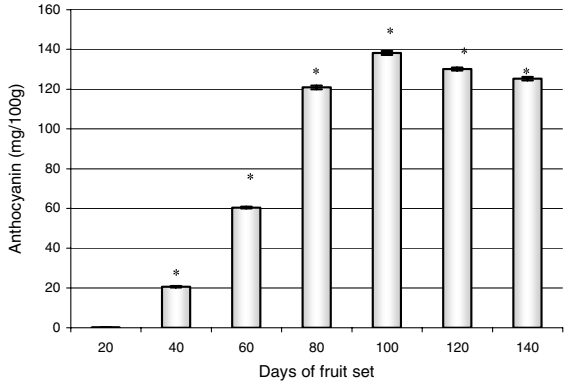

Fig. 5. Total anthocyanin pigment content of pomegranate arils during fruit development and maturation. 1. Data shown are the means of four replicates vertical bar represents ± standard error. 2. Values followed by an asterisk denote significant difference (at $P \leqslant 0.05$) compared to previous sample.

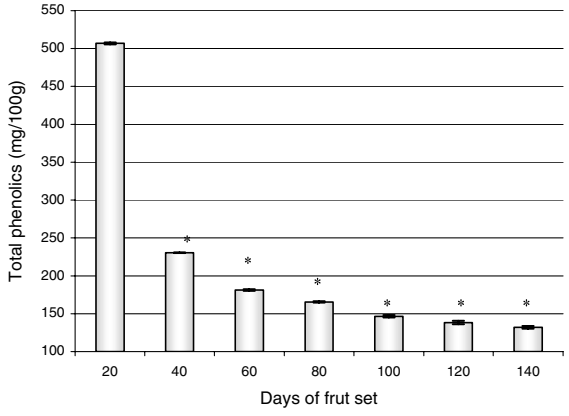

Fig. 6. Total phenolics of pomegranate arils during fruit development and maturation. 1. Data shown are the means of four replicates vertical bar represents ± standard error. 2. Values followed by an asterisk denote significant difference (at $P \leqslant 0.05$) compared to previous sample.

later, the decrease was gradual but significant upto 140 days (Fig. 6). The highest phenolic content (506 mg/100 g arils) was recorded in 20 day-old fruit. There was a nearly 73.9% reduction in total phenolics from 20 to 140 days of fruit development. A decrease in the total phenolics reduces the astringency of fruit (Goldstein & Swain, 1963; Ozawa, Lillas, & Haslam, 1987), which is a desirable sensory attribute in pomegranate. A decrease in phenolic compounds with maturation and ripening has also been reported in banana (Goldstein & Swain, 1963) and guava fruits (Bashir & Abu-Goukh, 2003). Some phenolics are substrates for enzymatic browning. A reduction in the phenolic content with development may reduce the incidence of enzymatic browning. A decrease in phenolics (Fig. 6) also coincided with an increase in anthocyanin pigment content (Fig. 5) after 80 days, after the former may contribute to the biosynthesis of the flavylium ring of anthocyanins.

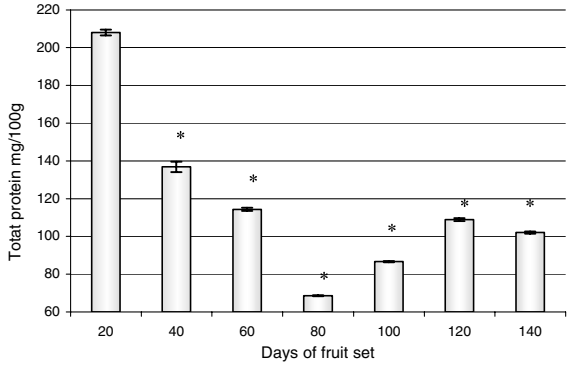

Fig. 7. Total protein content of pomegranate arils during fruit development and maturation. 1. Data shown are the means of four replicates vertical bar represents ± standard error. 2. Values followed by an asterisk denote significant difference (at $P \leqslant 0.05$) compared to previous sample.

3.1.5. Total protein

Pomegranate arils showed the highest total protein (209 mg/100 g) in 20 day-old fruit followed by a rapid decrease (66.9 %) in total protein up to 80 days of fruit development (Fig. 7). The decrease in total protein content may be a consequence of a reduction in demand of endogenous enzymes associated with anabolic activities, which decrease with fruit development and maturity (Frenkel, Klein, & Dilley, 1968). An increase in the total protein content (by 58.7%) between 80 and 120 days might be due to an acceleration of ripening changes that initiate the array of enzyme activities. A slight but significant decrease (6.3%) in protein content after 120 days might be attributed to breakdown of proteins, which is normally observed during senescence of fruits (Abu-Goukh & Abu-Sarra, 1993; Bashir & Abu-Goukh, 2003).

3.1.6. Ascorbic acid content

Ascorbic acid is abundantly present in all plant cells and has many biological functions. As an antioxidant, it prevents browning of tissue, which is an oxidation reaction, directly and indirectly (Smirnoff, 1996). However, ascorbic acid losses during the development of fruits and vegetables have often been reported. Pomegranate arils also showed a similar trend with rapid depletion (63.1%) in the ascorbic acid content during the initial stages, from 20 to 40 days of fruit development. This was followed by a gradual but significant decrease up to 140 days (Fig. 8). The highest ascorbic acid content (360 mg/100 g) was recorded in 20 day-old fruit. A similar trend was reported in mango and guava fruits (Bashir & Abu-Goukh, 2003). A decrease in ascorbic acid (Fig. 8) and phenolics (Fig. 6) contents and an increase in total sugar (Fig. 2) and anthocyanin (Fig. 5) levels during 80 days of fruit development marked the shift in the metabolic activity toward biosynthesis of anthocyanins, wherein polymerization of phenolics and

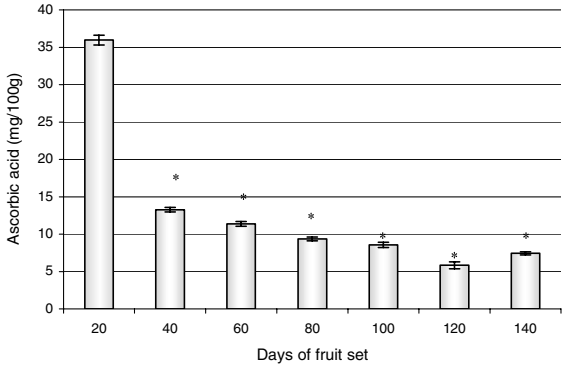

Fig. 8. Ascorbic acid content of pomegranate arils during fruit development and maturation. 1. Data shown are the means of four replicates vertical bar represents ± standard error. 2. Values followed by an asterisk denote significant difference (at $P \leqslant 0.05$) compared to previous sample.

further glycosylation lead to the formation of anthocyanin pigments.

3.2. Antioxidant activity

Antioxidant activity of pomegranate juice was measured in terms of its radical scavenging potential. DPPH$^{\cdot}$ is a stable free radical and the assay can accommodate a large number of samples in a short period of time. The assay is sensitive enough to detect active principles at low concentrations. The pomegranate arils showed a rapid decrease in antioxidant activity (by 13%) from 20 to 60 days of fruit development, which immediately replenished to its peak activity with 10.6% increase on the 80th day (Fig. 9). The lowest antioxidant activity (61.6%), recorded in 60 day-old fruit, might be due to a reduced concentration of total phenolics and ascorbic acid in arils, by 73.9% and 80.1%, respectively (Figs. 6 and 8). The surge in antioxidant activity from the 80th day onwards might be attributed to an increased concentration of anthocyanin pigments (Fig. 5). Anthocyanin, ascorbic acid and phenolic acids, either alone or in combination, are responsible for the antioxidant activity of pomegranate arils (Miller & Rice-Evans, 1997; Scalzo, Iannoccari, Summa, Morelli, & Rapisarda, 2004). The major phenolic acids reported from pomegranate fruit include punicalagin, punicalin, gallagic acid, ellagic acid and gallic acids (Bouchet, Barrier, & Fauconneau, 1998; Chen & Ho, 1997; Kulkarni et al., 2004).

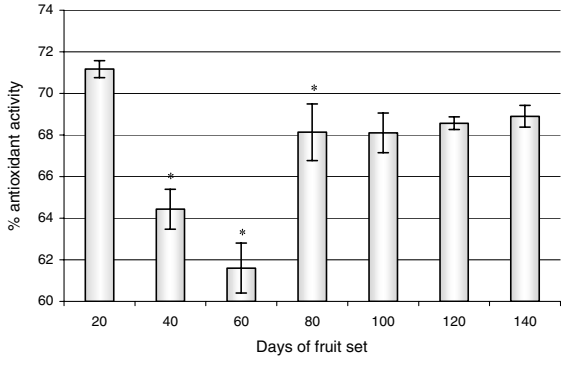

Fig. 9. Antioxidant activity of pomegranate arils during fruit development and maturation. 1. Data shown are the means of four replicates vertical bar represents ± standard error. 2. Values followed by an asterisk denote significant difference (at $P \leqslant 0.05$) compared to previous sample.

4. Conclusions

The changes in the chemical profile of pomegranate arils, from 20 to 140 days, clearly explained their growth, development, maturation and ripening stages. The developmental period of arils was extended up to 80 days from fruit set, which was associated with a continuous increase in concentration of TSS, total sugar, reducing sugar and anthocyanin pigments. This was accompanied by a significant ($P \leqslant 0.05$) reduction in phenolics, ascorbic acid and titrable acidity up to 80 days, followed by a steady-state. This clearly indicates that the increase in anthocyanin content and decrease in phenolics were related to each other; phenolics were being used up in the biosynthesis of the flavylium ring during anthocyanin pigment formation, leading to a reduction in their content. A sharp and significant increase of TSS, total sugar and reducing sugar between 80 and 100 days, along with a very slow decrease of total phenolics and ascorbic acid during the same period, was also observed. The equilibrium concentration of these chemicals on the 100th day may mark the attainment of optimum maturity and onset of ripening. A further increase in TSS, total sugars and reducing sugars was due to progress in ripening in pomegranate fruit. A slight but significant decrease (9.3%) in anthocyanin pigment content was observed after 100 days. This early initiation of anthocyanin discoloration, associated with a decrease in acidity, may be a cause for the internal breakdown of arils in over-ripe pomegranate fruits.

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