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Developmental dynamics in chemical composition during fruit ripening of pomegranate

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Abstract

Aim: The present study was undertaken to investigate the influence of pomegranate fruit developmental and maturity stages on the major chemical composition of pomegranate juice to identify the appropriate time of maturity of pomegranate cv. "Mridula".

Methodology: Fresh fruits of eight-year-old plants of pomegranate cv. 'Mridula' were picked manually in the morning at different developmental stages, viz., 30 to 150 DAFB at ten day interval. Harvested fruits were analysed for physico-chemical analysis.

Results: Pomegranate arils juice showed a significant ($p < 0.05$) increase in sugars and soluble solids content during fruit development. However, titratable acidity and total phenolics of aril juice decreased with advancement of fruit maturity. There was 11.32 times increase in Vitamin C content of juice during fruit development, whereas about 2.38 fold decrease in juice acidity was recorded. The reducing sugars content in aril juice was about 76.35 % - 81.93 % of total sugars. There was overall depletion of total phenolics content during course of fruit development. The results revealed that soluble solids content, SSC/TA ratio, juice pH, reducing sugars, non-reducing sugars, total sugars and Vitamin C of juice was abundant between 140-150 days after full bloom (DAFB). Whereas, TA and TP declined and reached minimum between 140-150 DAFB. SSC/TA ratio showed positive correlation with pH, sugars and Vitamin C, but negatively correlated with TA and TP. The principal components analysis showed that full ripened fruit at 140-150 DAFB recorded higher positive score along component 1 and indicate optimum time of fruit harvest.

Interpretation: In recent years, the search for a easy, reliable and simple determination of maturation has employed the attention of countless pomegranate growers in different pomegranate production regions. The change in the chemical profile of pomegranate from 30 DAFB to 150 DAFB of arils juice clearly explained their growth, development and maturity stages. This information would help pomegranate growers to access the maturity of 'Mridula' cultivars of pomegranate.

Introduction

Pomegranate fruits are important for human health because of their high antioxidant capacity and high polyphenols and anthocyanins content (Gil *et al.*, 2000). The quality of pomegranate fruits is strongly dependent on the cultivars. The acceptability of pomegranate to the consumer and processor depends on aggregate of certain quality attributes that are related to the physico-chemical properties inclusive of size, skin colour, sugar content, TA and flavour (Al-Said *et al.*, 2009). The pomegranate fruit is non-climateric in nature and the timing of harvest is of at most importance if fruit, either for immediate fresh market or for storage, are to reach the customer in prime condition. Review displays the effects of cultivar variation, growing region and physico-chemical compositional status of fruits such as fruit size, fruit weight, fruit colour, juice content, pH of juice, TSS, ascorbic acid content of juice and total phenolics conclude the pomegranate fruit maturity (Al-Mainman and Ahmad, 2002).

In pomegranate fruit, early harvesting of fruit causes depletion of organoleptic properties and browning of internal husk. Whereas, over-ripen fruits are susceptible to physiological disorders and fungal infections during storage. Inadequate literature is available on how fruit peculiarity and components relate to changes in fruit size in pomegranate cv. 'Mridula'. There is an increased concern about the quality of fruit during development and proceeding to harvest aimed at curtail post-harvest devaluation. The present study aimed to investigate developmental dynamic in chemical properties during advancement in fruit growth of pomegranate in sub-tropical conditions.

Materials and Methods

Sample collection : Fresh fruits of eight year old plants of pomegranate cv. 'Mridula' were picked manually in the morning from Fruit Research Farm, Department of Fruit Science, Punjab Agricultural University, Ludhiana (India) at different

developments stages, viz., 30 to 150 DAFB at ten day interval. Harvested fruits were then transported to laboratory in plastic bag for physico-chemical analysis. Nine pomegranate fruits sampled and three replicates were maintained for each analysis and each replicate included three fruits.

Chemical changes : Juice of pomegranate arils of nine fruit were completely extracted by squeezing the aril in muslin cloth. Collected fresh juice was analysed for different chemical compositional changes. Soluble solids content (SSC) were determined with digital refractometer (ATAGO, PAL-1, Model 3810, Japan) at room temperature, expressed in °Brix and subsequent corrections were made at 20 °C (AOAC, 2000). The titratable acidity (TA) content was determined by titrating juice across 0.1 N sodium hydroxide, applying phenolphthalein as indicator and sugars content by the methods of AOAC (2000) and results were expressed in percentage. The pH of juice was determined by digital pH meter (Eutech, Malaysia). Ascorbic acid content was determined according to AOAC (2005) with 2,6-dichloro-indophenol titrimetric method and the result were expressed as mg per 100 ml of fruit juice. Total phenolics were determined in triplicates by the Folin-Ciocalteu (Folin-C.) colorimetric method (Makker, 2000) and the results were expressed as gallic acid equivalents as (GAE) per litre of pomegranate juice.

Statistical analysis : The data for nine replication was analyzed for analysis of variance (ANOVA) and significant difference among the means were determined by LSD at $p < 0.05$ using the SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). Pearson correlation was used to investigate the relationship between the selected attributes associated with fruits maturity and in order to find the inter-relationship among the investigated fruit parameters 'principal component analysis' (PCA) was used.

Results and Discussion

A gradual and significant ($p < 0.05$) increase in soluble solids contents of the aril juice were detected with the

Table 1 : Pearson correlation coefficients (r) among chemical indices associated during fruit developments

	SSC	TA	SSC/TA	pH	Total sugars	Reducing sugars	Non-reducing sugars	Vitamin C	Total phenolics
SSC	1								
TA	-0.958	1							
SSC/TA	0.950	-0.987	1						
pH	0.930	-0.981	0.990	1					
Total sugars	0.873	-0.929	0.954	0.955	1				
Reducing sugars	0.888	-0.937	0.963	0.960	0.999	1			
Non-reducing sugar	0.786	-0.865	0.892	0.904	0.975	0.962	1		
Vitamin C	0.862	-0.925	0.962	0.960	0.910	0.918	0.849	1	
Total phenolics	-0.969	0.962	-0.953	-0.944	-0.917	-0.925	-0.856	-0.870	1

Significance at $p < 0.05$

Fig. 1 : Stage progression changes in (a) soluble solids content and (b) titrable acidity. Mean $\pm$ SE, presents the same letter(s) on vertical bar indicate statistically non-significant difference compared to previous sample ($p < 0.05$)

advancement in fruit maturity (Fig. 1a). During early and mid-season of fruit development the SSC increased significantly from 30 DAFB (5.17 °Brix) to 100 DAFB (12.10 °Brix), however at later stages increment in soluble solids content was very slow. Minimum Soluble solids content were recorded at 30 DAFB (5.17 °Brix) and maximum at 150 DAFB (12.61 °Brix). However, the rate of change of soluble solids content was maximum from 30 DAFB (5.17 °Brix) to 50 DAFB (7.95 °Brix), and minimum from 140

DAFB (12.53 °Brix) to 150 DAFB (12.61 °Brix).

Pomegranate juice contains citric acid, maleic acid, fumaric acid, tartaric acid and lactic acids but major acid accounting for titratable acidity is citric acid (Melgarejo *et al.*, 2000). TA content of aril juice followed a decreasing trend as the fruit approached towards maturity stage (Fig. 1b). A significant ($p < 0.05$) decrease in TA content of fruit occurred between 30 DAFB

Fig. 1 : Stage progression changes in (c) soluble solids content / titrable acidity and (d) juice pH. Mean $\pm$ SE, presents the same letter(s) on vertical bar indicate statistically non-significantly different compare to previous sample ($p < 0.05$)

to 60 DAFB. Subsequently, a non-significant decrease in TA between adjacent intervals was noted till 150 DAFB. The maximum TA content was recorded as 0.76% at earlier stage of fruit development (30 DAFB) and minimum was recorded as 0.32% at 150 DAFB. During fruit growth and development about 2.38 fold decreases in TA between 30 DAFB (0.76%) to 150 DAFB (0.32%) was noticed. However, the rate of change in juice TA was recorded maximum between 30 DAFB (0.76%) to 40 DAFB

(0.66%) and minimum between 140 DAFB to 150 DAFB.

A significant increase in SSC/TA ratio was recorded as fruit progressed towards the maturity (Fig. 1c). The rate of increase in SSC/TA ratio was recorded maximum from 80 DAFB (20.87) to 90 DAFB (24.72), closely followed by 90 to 100 DAFB i.e., from 24.72 to 28.56, respectively, and the minimum change was recorded from 140 DAFB (38.02) to 150 DAFB (39.25). Decrease in the

Fig. 1 : Stage progression changes in (e) total sugar and (f) reducing sugar. Mean $\pm$ SE, presents the same letter(s) on vertical bar indicate statistically non-significantly different compare to previous sample ($p < 0.05$)

content of juice TA with a gradual increase in SSC during fruit advancement may be resulted into the elevated TSS/TA ratio.

The augment in SSC in pomegranate could be explained by the augmentation of hexose sugars (Shulman *et al.*, 1984). Similar trend in SSC during fruit development was also recorded earlier workers (Borochoy-Neori *et al.*, 2009 and Zarei *et al.*,

2011) in pomegranate. A gradual decrease in TA, concomitant with increased in the SSC and total sugars content is an inherent process during ripening of pomegranate to impart the characteristic flavour (Kulkarni and Aradhya, 2005). Prasad *et al.*, (1999) reported that the increase in SSC/TA ratio in pomegranate juice is due to increase in sugar content in juice and reduction in acidity with maturity.

Fig. 1 : Stage progression changes in (g) non-reducing sugar and (h) Vitamin C. Mean $\pm$ SE, presents the same letter(s) on vertical bar indicate statistically non-significantly different compare to previous sample ($p < 0.05$)

The pH of aril juice increased significantly and was static till complete aril development (Fig. 1d). The minimum value of juice pH was recorded as 3.51 during 30 DAFB and maximum was 3.9 at 150 DAFB. The rate of change in pH was maximum from 40 DAFB - 50 DAFB and the minimum was recorded between 140 DAFB and 150 DAFB. Decrease in juice TA may have resulted into increment in juice pH throughout the fruit growth and hence, both the parameters were inversely

correlated. This is in agreement with the result of Borochoy-Neori *et al.* (2009); Zarei *et al.* (2011). However, the juice pH of 'Bhagwa' cultivar of pomegranate decreased from early immature to early half-ripe stage, but further changes were in significant until the full ripe stage (Fawole and Opara, 2013c).

Fig. 1e-g reveals that the total sugars content of fruit juice continued to increase significantly ($p < 0.05$) throughout the

Fig. 1 : Stage progression changes in (i) total phenolics. Mean $\pm$ SE, presents the same letter(s) on vertical bar indicate statistically non-significantly different compare to previous sample ($p < 0.05$)

course of study. The level of total sugars, reducing sugar and non-reducing sugars was recorded lowest on the first sampling (40 DAFB) as 2.72%, 2.07 % and 0.61 %, respectively and recorded highest during final sampling (150 DAFB) as 10.28 %, 8.15 % and 2.02 %. The rate of change in total sugars and reducing sugars was maximum recorded from 130 DAFB (8.11 % and 6.46 %, respectively) to 140 DAFB (9.46 % and 7.54 %, respectively). Whereas, the increase in non-reducing sugars was highest from

Fig. 2 : Scree plot of variance explained by each factor of principal components

120 DAFB (1.18 %) to 130 DAFB (1.57 %), but was recorded minimum between 90 DAFB (1.09 %) and 100 DAFB (1.10 %).

The present investigation of total sugars increases during fruit advance towards the ripening, confirms the findings of Legua *et al.* (2000), Kulkarni and Aradhya (2005). A rapid increase in

Fig. 3 : Principal component analysis of the first two factors (F1 and F2) showing dispersion of 'Mridula' pomegranate fruit cultivar based on the measured parameters at fruit maturity stages; components pattern (A) and component score (B)

total sugars in pomegranate from immature to early half-ripe stage was due to intense fruit expansion during maturity stages followed by a comparative delay, but significant accretion of sugars until full-ripening stage (Fawole and Opera, 2013b).

A significant increase in Vitamin C content of aril juice was acclaimed during the fruit development of pomegranate fruit (Fig. 1h). Vitamin C content of juice increased sharply from 5.93 mg 100 ml⁻¹ (40 DAFB) to 67.07 mg 100 ml⁻¹ (150 DAFB). There was slow increase in Vitamin C from 40 DAFB (5.93 mg 100 ml⁻¹ to 70 DAFB (9.26 mg 100 ml⁻¹) afterward a sudden and significant ($p < 0.05$) increase in Vitamin C from 70 DAFB (9.26 mg 100 ml⁻¹) to 120 DAFB (63.33 mg 100 ml⁻¹) was noted. Overall, 11.32 times increase in Vitamin C content of aril juice was noted during fruit development. A similar trend in increase in Vitamin C content was reported by Shwartz *et al.* (2009) in pomegranate cv. Wonderful. However, Vitamin C content decreased during fruit advancement toward maturity (Kulkarni and Aradhya, 2005) for Ganesh cultivar, Al-Maiman and Ahmad (2002) for Taifi cultivar and Zarei *et al.* (2011) in Rabbab-e-Fars cultivar of pomegranate, respectively.

Pomegranate juice is rich in phenolic compounds which have synergistics and additive effects on its pharmacological activities (Seeram *et al.*, 2005). Total phenolics content of the aril juice followed a decreasing trend as the pomegranate fruit approached maturity stage (Fig. 1i). The highest total phenolics content in aril juice was recorded at early immature stage (40 DAFB, 2029.57 mg GAE/L), but least recorded at 140 DAFB (496.76 mg GAE/L). Overall, depletion in total phenolics was 70.98% during fruit development. A higher rate of depletion in total phenolics was recorded between 60 DAFB (1681.96 mg GAE/L) and 70 DAFB (1292.48 mg GAE/L) whereas minimum recorded between 100 DAFB (852.74 mg GAE/L) to 110 DAFB (815.05 mg GAE/L). The loss of astringency is one of the desirable changes that occur during maturation and ripening of pomegranate and is primarily due to decrease in phenolic compounds during fruit maturation (Kulkarni and Aradhya, 2005; Shwartz *et al.* 2009; Labbe *et al.*, 2010). Reduction in TP content in the present study is similar the finding of Weerakkody *et al.* (2010), where reduced total phenolics was recorded in Wonderful pomegranate cultivar during the early season.

Pearson correlation was used to investigate the relationship between selected attributes associated with fruit maturity (Table 1) of the pomegranate. Soluble solid content showed significant and positive correlation with SSC/TA ($r^2 = 0.950$), pH ($r^2 = 0.930$), total sugars ($r^2 = 0.873$), reducing sugars ($r^2 = 0.888$) and Vitamin C ($r^2 = 0.862$). Also, SSC correlated negatively strong with TP ($r^2 = -0.969$), this relationship reflected the correlations of TA between SSC/TA ($r^2 = -0.987$), pH ($r^2 = -0.981$), total sugars ($r^2 = -0.929$), reducing sugars ($r^2 = -0.937$), non-reducing sugars ($r^2 = -0.865$) and Vitamin C ($r^2 = -0.925$) except TP ($r^2 = 0.962$). This further suggested that SSC/TA was positively correlated with pH, sugars and Vitamin C but negatively correlated with total phenolics. Furthermore, the relationship between total sugar and total phenolics was negatively correlated. Pearson correlation coefficient (r) among the selected indices associated fruit maturity were similar to the study of Fawole and Opara (2013a) in pomegranate cvs. 'Ruby' and

'Bhagwa', respectively.

Furthermore, to access an extensive view on the metabolic shift that exit during fruit advancement, the whole info set was subjected to Principal Component Analysis. The overall variability is interpreted by four factors (F1 - F4), with the first two factors of PCA showing correlation of 97.88 % (Fig. 2). The first factor (F1) was responsible for 93.47 % total variations, however the second factor (F2) elucidated only 4.41 % of total variations, indicating that the maximum variations in fruit maturity indices was interpreted by F1 (Fig. 3A). A general view of the PCA showed that young fruits had higher titratable acid and total phenolics, while fully mature fruits have higher SSC which is correlated with SSC/TA, pH, Vitamin C and sugars of fruits juice. Negative scores along F1 corresponded to late immature fruits at 80 DAFB. Full mature fruits had high positive scores along F1, while low negative scores were half mature at 110 DAFB (Fig. 3B). In the present study, decrease in acidity and TP in immature fruits was also characterized by the shift from right to left, reflecting the beginning of maturity process between 110 DAFB to 130 DAFB. The beginning of ripening process in the cultivar could well ascribed to fruit at 110 DAFB, where consolidation of fruit biochemical indices seemed to be at equilibrium (Fig. 3A and Fig. 3B). These results are similar to those reported by Fawole and Opara (2013b) where maturity of 'Bhagwa' pomegranate fruit could be fixed around 165 DAFB where fruit were characterize by intense chemical changes in fruit juice.

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