Suitability of commercially important Indian pomegranate (*Punica granatum*) cultivars for minimal processing

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ABSTRACT

An experiment was conducted during 2011-2012 to select the most suitable pomegranate (*Punica granatum* L.) cultivars namely Mridula, Kandhari and Bhagwa for minimal processing. The effect of cultivars on quality parameters of minimally processed pomegranate arils packaged in 50 micron polypropylene bags were evaluated over 15 days during cold storage (5 ± 2 °C and 85 ± 5% relative humidity). Aril quality was determined by measuring colour attribute (L* value), respiration rate, TSS, PLW, aril firmness, microbial growth and sensory analysis. Based on microbial limit (7 log cfu/g) and acceptance score (5), minimally processed pomegranate arils prepared from Mridula showed longer shelf-life than Bhagwa. At the end of storage (15th day), arils from Mridula cultivar showed the least browning (>L value), respiration rate, weight loss and microbial count with the highest acceptability score compared to Kandhari and Bhagwa.

Key words: Browning, Cultivars, Colour, Microbial count, Pomegranate arils, Respiration rate

Pomegranate (*Punica granatum* L.) is one of the most important fruit crops in India because of its adaptable nature, high profitability and being cultivated on a commercial scale in temperate, tropical and subtropical regions of country (Kumar et al. 2012). Its fruits are good source of nutrients and bioactive compounds, mainly anthocyanins which exhibit strong chemo-preventive activities such as antimutagenicity, anti hypertension, antioxidative potential and reduction of liver injury (Hertog et al. 1997, Lansky et al. 1998, Lopez-Rubira et al. 2005). The edible part of the pomegranate is called aril which constitutes about 52% of total fruit (w/w), comprising 78% juice and 22% seeds (Kulkarni and Aradhya 2005, Barman et al. 2011).

The hard suture (peel) of pomegranate fruits makes it difficult to extract the arils, thus limiting its consumption as fresh fruit. Therefore, production of pomegranate arils in ‘ready-to-eat’ form would be a convenient and desirable alternative to the consumption of fresh fruits and may further increase pomegranate demand by consumers. Among various factors, selection of variety, ripening stage and storage environment are the major factors that affect storage life of minimally processed produce (Sapers and Miller 1998).

Selection of a wrong variety may yield poor processed product in terms of colour, texture, flavour and overall acceptance (Amiot et al. 1995). Due to varied consumer’s preference, it has become increasingly important to characterize its different varieties to obtain a high quality product with economic proposition. Considering these gaps, the present study was conducted to determine the most suitable cultivars of pomegranate for minimal processing.

MATERIALS AND METHODS

Physiologically mature (Total soluble solids ranging 11-12 °Brix) pomegranate fruits of Mridula, Kandhari and Bhagwa cultivars were harvested from experimental orchard of Mahatma Phule Agricultural University, Rahuri (Southwest part of India) and immediately transported to the postharvest handling laboratory and kept at 5 ± 2 °C and 85 ± 5% RH (relative humidity) until the next day. Pomegranates with defects were discarded and healthy ones uniform in size and appearance were sanitized with 200 µL/L chlorine solution. Husks (peel) were cut at equatorial zone with sharpened knife and arils were manually separated. Separated arils were collected in a tray and gently mixed to assure uniformity. Thereafter, arils were dipped in a solution containing 100 µL/L chlorinated water for 5 min, followed by rinsing with potable tap water at 5 °C (Bhatia et al. 2013) and washed arils were air dried to remove surface water. 100 g of dried arils were placed into 50 micron thickness heat sealed bags made of polypropylene film. Packaged samples were stored at 5 ± 2 °C and 85 ± 5% RH for 15 days and sampling was carried out on 0, 3, 6, 9, 12...
and 15 days of storage. Three packs were analyzed for each cultivar and parameter on sampling days.

Colour characteristics were measured using colour meter (colour tec PCM/PSM, USA). In the CIE (L*, a*, b*) colour space abbreviated CIELAB, the lightness co-efficient, L*, ranges from black (0) to white (100).

Post-storage respiration rate was measured by placing arils in 150 ml capacity container hermetically sealed with a silicone rubber septum for 1 hr. After specified time, the head-space gas was sucked through a hypodermic hollow needle and the respiration rate was quantified by using auto gas analyzer (model: Checkmate 9900 O2/CO2, PBI Dansensor, Denmark). The rate of respiration was expressed as ml CO2/kg/hr.

The total soluble solids of samples were estimated using Fisher Scientific hand Refractometer. The results were expressed as °Brix at 20 °C.

Arils were weighted during storage at regular sampling intervals with the help of an electronic balance. Physiological loss in weight (PLW) was measured by subtracting the initial weight from final weight and expressed as per cent.

Aril firmness was determined by a texture analyzer (model: TA+Di, Stable Micro Systems, UK) using cylindrical probe (75 mm diameter) by programmed settings as follows: pre test speed 5 mm/second, test speed 2 mm/second, post test speed 10 mm/second, compression distance 80 %. First peak force (N) in the force-time curve obtained from a texture analyzer was taken as firmness of the sample.

A total of 10 g arils aseptically weighted and sample was used to make serial dilutions in 0.8 per cent saline blanks. Appropriate dilutions were plated onto triplicate plates of selected medium. The enumeration of mesophilic bacteria, yeast and mold count was performed by using plate count agar (PCA), malt-extract glucose-yeast extract-peptone (MGYP) Agar and Rose Bengal agar, respectively. Plates were incubated at 30 °C for 48 hr for mesophilic bacteria and 5 days for yeast and mold. The colonies were counted and microbial counts were expressed as log cfu/g (colony forming units per gram of sample). Samples for analysis were taken on days 0 (processing day), 3, 6, 9, 12 and 15.

Sensory evaluation of minimally processed pomegranate arils obtained from three cultivars was performed during storage using 9-point hedonic scale with 1, dislike extremely; 2, dislike very much; 3, dislike moderately; 4, dislike slightly; 5, neither like nor dislike; 6, like slightly; 7, like moderately; 8, like very much and 9, like extremely. Scores of 5 and above were considered as acceptable for commercial purposes. The evaluated parameters were colour, taste, texture, juiciness and overall acceptability.

Data for the analytical determination were pooled and subjected to two-way analysis of variance (ANOVA) by taking cultivars and storage days as the two sources of variations and the significant effects were noted. Further, it was subjected to multiple range comparison procedure to identify the pair-wise significant difference between the effects. Results were given as mean ± standard deviation of three independent determinations. Differences were considered to be significant at P = 0.05 (95% confidence level). All analyses were performed with SAS software package, version 9.3.

RESULTS AND DISCUSSION

Colour

There were significant effects of cultivar, storage days and cultivar × storage days interaction on L* (lightness) value (P = 0.05). Regardless of cultivars L* value of minimally processed pomegranate arils declined during storage, showing decrease in brightness of arils (Table 1). Mridula cultivar showed the least change in L* among 3 cultivars indicating lower browning intensity of arils from Mridula cultivar than that of Kandhari and Bhagwa.

In the present study, all the three pomegranate cultivars have shown varied response in respect to L* value of fruit arils. During 15 days storage period, L* value was significantly decreased which indicates progression in aril browning. The previous works carried out by several researchers have also shown colour declining of fresh cut fruits during storage (Dong et al. 2000, Gorny et al. 2000). Ayhan and Esturk (2009) and Gil et al. (1996) also reported significant differences in L* value of ready to eat pomegranate arils during cold storage. Tehranifar et al. (2010) also reported in their study that twenty varieties of Iranian pomegranate varied significantly in their anthocyanin and total phenol content. The variation in aril browning potential of studied pomegranate cultivars may be attributed to difference in phenolics contents, enzymatic activities and juice pH values (Macheix et al. 1990, Oz and Ulukanli 2011).

Table 1 Effect of cultivars and storage period on (L* value) of minimally processed pomegranate arils during cold storage (5 ± 2 °C and 85 ± 5% RH)

<table>
<thead>
<tr>
<th>Cultivars (Storage)</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
<th>15</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mridula</td>
<td>28.15±0.98 bcd</td>
<td>27.91±0.86 cde</td>
<td>27.02±0.66 defg</td>
<td>26.58±0.36 efgh</td>
<td>25.42±0.66 hi</td>
<td>26.58±0.36 efgh</td>
<td>24.83±0.16 i</td>
</tr>
<tr>
<td>Kandhari</td>
<td>27.15±0.77 def</td>
<td>22.84±0.46 j</td>
<td>22.20±0.30 j</td>
<td>22.39±0.77 j</td>
<td>20.30±0.67 k</td>
<td>19.73±0.84 k</td>
<td>22.43±0.6</td>
</tr>
<tr>
<td>Bhagwa</td>
<td>30.82±0.62 a</td>
<td>29.48±1.23 ab</td>
<td>29.01±0.93 bc</td>
<td>28.32±0.56 bcd</td>
<td>26.09±0.44 ghk</td>
<td>25.55±0.11 ghk</td>
<td>28.21±0.44</td>
</tr>
<tr>
<td>Mean</td>
<td>28.71</td>
<td>26.74</td>
<td>26.08</td>
<td>25.76</td>
<td>23.94</td>
<td>23.37</td>
<td></td>
</tr>
</tbody>
</table>

L* = lightness; Values are expressed as mean ± standard deviation; Means with same superscript are homogeneous.
Respiration rate

Respiration rate of arils was found to be significantly affected by cultivars, storage days and their interaction (Fig 1). Among the cultivars, minimally processed arils from Mridula showed lowest mean respiration rate followed by arils obtained from Kandhari and Bhagwa. Irrespective of cultivars, respiration rate of arils showed progressive increase during the entire storage period.

![Fig 1 Effect of cultivars on respiration rate of minimally processed pomegranate arils during cold storage (5 ± 2 °C and 85 ± 5% RH)](image)

The respiration rate is an indication of the rapidity with which compositional changes occur within produce and, hence related to potential shelf-life of produce (Church and Parsons 1995). Odriozola-Serrano et al. (2008) found significant difference in respiration rate of fresh-cut tomatoes prepared from different cultivars. Kim et al. (2010) also reported that differences in cultivars affect the respiration rate of sweet persimmon. Progressive increase in respiration rate during storage of fresh cut pear slices, tomatoes and zucchini has also been reported earlier (Gorny et al. 2000, Odriozola-Serrano et al. 2008, Lucera et al. 2010). Increase in respiration rate of fresh-cut produce attributed to increase in metabolic activities due to minimal processing operations (Rivera-Lopez et al. 2005, Allende et al. 2006).

### Total soluble solids

The effect of cultivars on TSS of minimally processed pomegranate arils during cold storage has been depicted in Table 2. There was significant effects of cultivar, storage day and cultivar × storage day interaction on TSS (P < 0.05). Regardless of storage days, among the cultivars Bhagwa exhibited maximum TSS value followed by Kandhari and Mridula. The mean TSS value of minimally processed pomegranate arils during cold storage from Mridula, Kandhari and Bhagwa were 14.85 °Brix, 15.54 °Brix and 17.09 °Brix respectively.

In our study, minimally processed arils obtained from three cultivars were also found to differ in their TSS content. As general trend, TSS content of arils increased initially till 6th day and then showed the declining trend for rest of storage period. This fluctuating phenomenon in the stored fruit aril may be explained by faster water loss (in early days of storage) due to higher water activity (data not shown) and varied metabolic activities of individual genotypes. Previous researchers have also reported that pomegranate cultivars differ in terms of TSS content (Martinez et al. 2012, Zaouay et al. 2012). The reduction in TSS content has been reported with the progression of ripening in pomegranate during storage (Sepulveda et al. 2000, Ayhan and Esturk 2009). This might be attributed to the conversion of sugars into other organic acids (citric, malic, oxalic and succinic acid) during advanced stage of fruit storage.

### Physiological loss in weight

Results obtained in this study clearly indicate that there were significant (P < 0.05) effects of cultivar and storage days on PLW (Table 2). Minimally processed pomegranate arils from all cultivars showed progressive weight loss throughout the storage period. Irrespective of storage days, PLW remained lower in minimally processed arils obtained from Mridula cultivar followed by Bhagwa and Kandhari. The data obtained from the Mridula cultivar at 15th day of storage revealed the lowest weight loss (0.064 per cent), followed by Bhagwa (0.075 per cent) and Kandhari (0.083 per cent).

### Table 2

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Storage period (days)</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
<th>15</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSS Mridula</td>
<td></td>
<td>15.13±0.41&lt;sup&gt;f&lt;/sup&gt;</td>
<td>15.23±0.05&lt;sup&gt;f&lt;/sup&gt;</td>
<td>15.60±0.2&lt;sup&gt;e&lt;/sup&gt;</td>
<td>15.13±0.2&lt;sup&gt;f&lt;/sup&gt;</td>
<td>14.33±0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.70±0.1&lt;sup&gt;i&lt;/sup&gt;</td>
<td>14.85&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Kandhari</td>
<td></td>
<td>14.66±0.15&lt;sup&gt;gh&lt;/sup&gt;</td>
<td>15.23±0.15&lt;sup&gt;f&lt;/sup&gt;</td>
<td>16.53±0.47&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.00±0.20&lt;sup&gt;de&lt;/sup&gt;</td>
<td>15.63±0.15&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>15.20±0.2&lt;sup&gt;f&lt;/sup&gt;</td>
<td>15.54&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bhagwa</td>
<td></td>
<td>16.73±0.25&lt;sup&gt;c&lt;/sup&gt;</td>
<td>17.56±0.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.17±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.63±0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.43±0.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.00±0.2&lt;sup&gt;de&lt;/sup&gt;</td>
<td>17.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>15.51&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.46&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.96&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

| PLW Mridula   |                       | 0.004±0.0001<sup>a</sup> | 0.016±0.0001<sup>b</sup> | 0.033±0.0001<sup>d</sup> | 0.052±0.0001<sup>f</sup> | 0.064±0.0001<sup>e</sup> | 0.034<sup>a</sup> |
| Kandhari      |                       | 0.026±0.0040<sup>c</sup> | 0.037±0.0002<sup>d</sup> | 0.056±0.0002<sup>f</sup> | 0.073±0.0001<sup>b</sup> | 0.083±0.0005<sup>c</sup> | 0.055<sup>c</sup> |
| Bhagwa        |                       | 0.017±0.0020<sup>b</sup> | 0.024±0.0002<sup>c</sup> | 0.044±0.0002<sup>e</sup> | 0.064±0.0002<sup>b</sup> | 0.075±0.0002<sup>b</sup> | 0.045<sup>b</sup> |
| Mean          |                       | 0.016<sup>a</sup> | 0.025<sup>b</sup> | 0.044<sup>c</sup> | 0.063<sup>d</sup> | 0.074<sup>e</sup> |

Values are expressed as mean ± standard deviation; Means with same superscript are homogeneous.
Loss of weight in stored pomegranate fruit arils is mainly due to evaporation of water and respiration activity. The lower weight loss in Mridula fruit arils would be attributed to distinct cell integrity, permeability of tissues and respiratory attributes (Kappel et al. 2002, Kim et al. 2010). Elyatem and Kader (1984) also established a strong relation between pomegranate respiration rate and loss in weight during storage. Furthermore, minimal processing operations like peeling of fruits and extraction of arils may have led to increase in water transpiration rate due to exposure of ruptured arils (Oz and Ulukanli 2011).

Aril firmness

Firmness of minimally processed pomegranate arils was found to be significantly (P = 0.05) affected by cultivars, storage days and cultivars × storage days interaction (Fig 2). Minimally processed arils of Mridula cultivar exhibited lowest firmness followed by Bhagwa and Kandhari throughout the storage period. As a general trend, firmness of minimally processed pomegranate arils from all three cultivars declined during 15 days of storage period.

Loss of firmness during storage and distribution is a fundamental problem in shelf-life extension of minimally processed fruits due to action of endogenous enzymes related to cell wall degradation and microorganisms activation (Huxsoll and Bolin 1989). The response of three cultivars to firmness in the present study varied. During storage, variation in firmness of pomegranate arils could be ascertained to difference in cell turgor pressure and cell softening enzymes activities present in the aril tissues (Varoquaux 1990, Bailey 2002). Variation in firmness during storage also happens due to alteration of histology that affects the cellular density of the pericarp tissue (Vincent 1989, Lahaye 2013). Earlier workers have also reported significant variation in textural properties (physiological and anatomical parameters) of pomegranate and apple fruits obtained from various cultivars (Al-Said et al. 2009, Ghaifir et al. 2009).

Microbial count

The cultivar, storage days and cultivar × storage days interaction has significantly (P = 0.05) affected the mesophilic bacteria count of minimally processed pomegranate arils (Fig 3). Yeast and mould count of minimally processed pomegranate arils during entire storage period were below the detection limit. Irrespective of cultivars, total mesophilic bacterial count of minimally processed pomegranate arils increased during the entire storage period. Minimally processed arils from Mridula cultivar observed the lowest mesophilic count followed by Kandhari and Bhagwa.

The microbial count of arils from Bhagwa cultivar (7.26 log cfu/g) on 15 day of storage exceeded the maximum limit of 7 log cfu/g set by Spanish legislation (Lopez-Rubira et al. 2005). Taking this limit into consideration, shelf-life of arils from Bhagwa cultivar was limited to 12 days whereas arils from Mridula and Kandhari cultivar exhibited shelf-life of 15 days. The difference in microbial count among three cultivars may be attributed to varied level of aril juice organic acid and tannin content. Martinez et al. (2012) also reported that large difference exists in six Moroccan pomegranate cultivars in terms of titratable acidity. Titratable acidity in ‘Bhagwa’ was found to be 0.32% while Mridula and Kandhari showed titratable acidity of 0.78% and 0.56% respectively (Mir et al. 2007, Fawole et al. 2011).

Sensory evaluation

The effect of cultivars on sensory parameters and acceptance of minimally processed pomegranate arils during cold storage are shown in Fig 4 (15th day). Sensory score of 5 and above out of 9 is the limit of acceptance in terms of product attributes such as aril colour, texture, sweetness and juiciness. The sensory score study performed on 15th day of storage showed that score given in respect to color, texture, sweetness, juiciness and overall acceptance was much higher in minimally processed arils obtained from Mridula cultivar compared to Kandhari and Bhagwa.
Minimally processed arils of Bhagwa cultivar lost their sensory attributes (color and acceptance) much faster as compared to Mridula and Kandhari.

Sensory quality of pomegranate arils depends on cultivar, climatic conditions during fruit maturation and ripening (Borochov-Neori et al. 2009). According to microbial count limit and acceptance score, the shelf-life of minimally processed arils from Mridula cultivar was observed to be 15 days. The reasons for higher score in case of Mridula may be due to low water loss, better visual and organoleptic quality. The sensory score for colour and overall acceptance for Bhagwa cultivar was 4.8 at 15th day of storage which was lower than the acceptance score level for commercial purpose of 5, limiting its acceptance to 12 days. In their study, Ayhan and Esturk (2009) reported that minimally processed arils in passive and active modified atmospheres showed commercial acceptance for 18 days, whereas Lopez-Rubira et al. (2005) found that the shelf-life of minimally processed pomegranate arils from late harvested and early harvested fruits as 10 days and 14 days respectively. The variation in consumer acceptance of stored pomegranate arils may be due to varietal difference, crop growing conditions and packaging environment.

Within a specific fruit crop, the different cultivars have varied response to the shelf life and quality attributes. In our study, arils from Mridula cultivar showed least browning (>L value), respiration rate, weight loss, microbial count coupled with highest acceptability score as compared to Kandhari and Bhagwa. Based on the microbial count and acceptance score, minimally processed pomegranate arils prepared from Mridula showed longer shelf-life than those from Bhagwa; thus Mridula appeared most suitable cultivar for minimal processing.

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