Effect of Salicylic acid Pre-treatment on Functional and Sensory Quality of Minimally Processed Pomegranate (*Punica granatum* L) Arils

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The present study was conducted to investigate the effect of salicylic acid pretreatment on functional and sensory quality of minimally processed pomegranate arils. ‘Mridula’ pomegranate arils were treated with aqueous solutions of salicylic acid (1 mM, 2 mM and 3 mM) by dipping them for 60 s. After treatment, arils were dried to remove surface water and 100 g of arils were packed in polypropylene (PP). Packaged samples were stored at 5±2 ºC and 85±5% relative humidity for 19 d. Minimally processed pomegranate arils treated with SA (2 mM) retained better colour (L* value), showed highest aril firmness total phenols, anthocyanins and antioxidant activity. The sensory scores and overall acceptance for 2 mM SA treated arils were higher than other pre-treatments (control, 1 mM SA and 3 mM SA) during storage. Conclusively, SA (2 mM) pre-treatment may be used to maintain desirable quality characteristics of minimally processed pomegranate arils.

**Keywords**: Pomegranate Arils, Minimal Processing, Salicylic Acid, Phenols, Antioxidant

**Introduction**

Minimally or lightly processed products were developed to supply hotels, restaurants, catering services and other institutions. Consumers demand safe, nutritious and ready-to-eat/use food products which have made minimal processing as integral part of rapidly growing horticulture industry. Many terms are applied to fruits and vegetables cleaned and prepared in fresh form viz. lightly processed, minimally processed, prepared, precut, fresh-processed, and partially processed. Minimal processing techniques have emerged to meet the challenge of replacing traditional methods of preservation while retaining nutritional and sensory quality. The recent interest for pomegranate fruit is not only because of the exceptional unique sensory quality, but also due to the scientific evidences that suggest its therapeutic activity. The health benefits and high bioactive components from pomegranate fruit are appreciated due to its strong chemopreventive activities such as antimutagenicity, antihypertension, antioxidative potential and reduction of liver injury1. However, its consumption is not very common mainly because of the difficulty to peel it and extracting the arils, which is why ready-to-eat pomegranate arils could be a good way to increase the consumption. In recent years there has been considerable pressure by consumers to reduce or eliminate chemically synthesized additives in foods. Thus, efforts are conducted to find natural alternatives to the currently used additives to maintain the quality of minimally processed fruits. Hence, naturally occurring compounds such as phenols, aldehydes, and organic acids have been tested to prove their effectiveness in fresh-cut fruits. Salicylic acid (SA), natural and safe phenolic has shown beneficial effect on human health. SA is a plant growth regulator and also effects post-harvest decay, disease resistance, oxidative stress, fruit ripening, ethylene biosynthesis and action, fruit firmness, respiration, antioxidant systems and nutritional quality of horticultural crops2. Previous studies have been reported on the effect of SA on whole fruits3,4,5. However, only one study has been done to check the efficiency of SA in controlling browning of fresh cut water chest nut and result showed that treatment with SA effectively controlled discoloration and maintained quality of fresh cut water chest nut during storage6. The objective of this study was to further investigate effects of SA treatment on functional and sensory quality of minimally processed pomegranate arils.

**Materials and methods**

**Plant material and experimental design**

Pomegranate fruits of Mridula cultivar harvested at Physiological maturity (Total Soluble Solids ranging
from 11 to 12 °Brix) from experimental orchard of Mahatma Phule Agricultural University, Rahuri and immediately transported to the postharvest handling laboratory and kept at 5±2 °C and 85±5% 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⁻¹ 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 treated with aqueous solutions of salicylic acid (1 mM, 2 mM and 3 mM) by dipping them for 60 s and treated arils were air dried to remove surface water. Dried arils with sample size of 100 g were packed in polypropylene (PP). Packaged samples were stored at 5±2 °C and 85±5% relative humidity for 19 d and sampling was carried out on 0, 5, 10, 13, 16 and 19 d of storage. Three packs were analyzed for each pre-treatment and parameter on scheduled sampling day.

Colour

Colour characteristics were measured using colour meter (Colour Tec PCM/PSM, USA), the instrument generates a set of Cartesian coordinate which pin point the measured colour in a three dimensional colour space. In the CIE (L*, a*, b*) colour space abbreviated CIELAB, the lightness co-efficient, L*, ranges from black (0) to white (100).

Aril firmness

Aril firmness was determined by a texture analyzer (model: TA+Di, Stable Micro Systems, UK) using compression test. The sample was compressed using a cylindrical probe (75 mm diameter) by programmed settings as follows: pre test speed 5 mm s⁻¹, test speed 2 mm s⁻¹ and post test speed 10 mm s⁻¹ and compression distance 80%. First peak force (N) in the force-time curve obtained from a Texture Analyzer during the compression of pomegranate arils by the cylinder probe was taken as firmness of the sample.

Respiration rate

Post-storage respiration rate was measured by placing arils in 150 mL capacity container hermetically sealed with a silicone rubber septum for 1 h. 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⁻¹ h⁻¹ at 219878.028 N m⁻² pressure and 25 °C.

Total phenols

The phenolics content of the minimally processed pomegranate arils was determined by the Folic-Ciocalteu method with some modifications, using gallic acid as standard. The absorbance was measured at 750 nm using 1 cm cuvette. Total phenolics content was expressed in µg gallic acid equivalent g⁻¹.

Total anthocyanins

Total anthocyanins content was determined by the pH differential method using two buffer systems-potassium chloride buffer, pH 1.0 (0.025 M) and sodium acetate buffer, pH 4.5 (0.4 M). Two gram of arils were crushed in ethanol and centrifuged at 10,000 rpm for 600 s at 18 °C. The supernatant were diluted in pH 1.0 and pH 4.5 buffers, and absorbance were measured at 510 nm and 700 nm using 1.0 cm path length cuvettes. The pigment content was calculated and expressed as mg equivalent cyanidin-3-glucoside 100 g⁻¹ aril fresh weight, using an extinction coefficient of 26900 L cm⁻¹ M⁻¹ and a molecular weight of 449.2 g.

Antioxidant activity

Antioxidant activity was measured by cupric reducing antioxidant capacity (CUPRAC) method and results were expressed as µM equivalent trolox g⁻¹.

Sensory evaluation

Sensory evaluation of minimally processed pomegranate arils pretreated with SA (1 mM, 2 mM and 3 mM) 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.

Statistical analysis

Data for the analytical determination were subjected to two-way analysis of variance (ANOVA) by taking pre-treatments 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. Differences were considered to be significant at \( P \leq 0.05 \) (95% confidence level). All analyses were performed with SAS software package, version 9.3.

**Results and Discussion**

**Colour**

L* value was found to be significantly (\( P \leq 0.05 \)) affected by SA pre-treatments (Figure 1(a)). Irrespective of pre-treatments there was decrease in L* value of minimally processed pomegranate arils with progression of storage period. Rate of reduction of L* value was found to be highest for control arils followed by higher concentrations of SA (3 mM). SA 2 mM showed highest mean L* value (19.67) followed by SA 1 mM (19.06) and SA 3 mM (18.91). The present study reveals that, all the pre-treatments showed decrease in L* value with the advancement of storage period of 19 d. Decrease in L* value is a useful indicator of darkening during storage, either resulting from oxidative browning reactions or from increasing pigment concentration. In present study, irrespective of pre-treatments the decrease in L* value indicated the progression of aril browning with advancement of storage period. Among SA pre-treatments, 2 mM SA was most effective in controlling browning followed by 1 mM and 3 mM SA pre-treatment. Earlier researcher also reported that increasing SA concentration (from 0 to 4 mM) enhanced browning inhibition in fresh-cut water chest nut. This indicated reduction in red colour attributed to breakdown of anthocyanins and other phenol compounds and development of brown pigment. Control arils exhibited faster development of browning whereas; SA pre-treatments delayed the browning of arils during storage.

**Aril firmness**

Firmness of minimally processed pomegranate arils was significantly (\( P \leq 0.05 \)) influenced by SA pre-treatments and storage days (Figure 1(b)). The result revealed that, there was continuous gradual decline in aril firmness under all the pre-treatments with advancement of storage period. The firmness loss rate was found significantly higher in control arils as compared to arils pre-treated with various concentrations of SA. Up to 5 d of storage, no significant difference in aril firmness was observed among the pre-treatments. After that arils treated with SA 2 mM showed much slower decline in aril firmness as compared to other pre-treatments. At the end of storage period, highest firmness (115.89 N) was retained with SA 2 mM pre-treatment, while it was lowest (96.46 N) for control arils. Firmness is an important characteristic which governs the postharvest life of fresh cut horticultural produce. In present study all the pretreatment showed declining trend in aril firmness, similar trend was also observed in previous studies on minimal processing of pomegranate arils. The higher aril firmness in SA treated arils during storage may be ascribed to reduced activities of softening enzymes such as pectin methyl estrase (PME) and polygalacturonase (PG). Retention of firm fruitiness as the result of SA treatment has been reported in several whole fruits like peach, kiwifruit, strawberry and pomegranate. Some biochemical changes are also responsible for loss of firmness due to action of endogenous enzymes related to cell wall degradation, microorganisms activation, transformation of proteopectin to water soluble pectin, decrease in cellulose crystallinity, thinning of cell walls, diffusion of sugar to the intercellular spaces. The effect of pre-treatments on respiration rate revealed that, SA pre-treatments were highly effective in reducing the respiration rate of minimally processed pomegranate arils (Figure 1(c)). Irrespective of pre-treatments, respiration rate of arils showed inclined pattern throughout storage. As compared to SA pre-treatments, respiration rate of control arils increased rapidly and was observed to be highest during 19 d of storage period. Finally after 19 d of storage, highest respiration rate (101.96 mL CO\(_2\) kg\(^{-1}\) h\(^{-1}\)) was observed in control arils, while it was lowest (72.48 mL CO\(_2\) kg\(^{-1}\) h\(^{-1}\)) for 2 mM SA treated arils. Progressive increase in respiration rate during storage of fresh cut pear slices, tomatoes and zucchini has also been reported earlier. Increase in respiration rate of fresh-cut produce may be due to increase in metabolic activities accelerated by minimal processing operations. In present study, minimally processed pomegranate arils treated with SA showed significantly lower respiration rate as compared to control. Further, the impact of lower dose (SA 2 mM) was found better than higher dose (SA 3 mM). The reduction in respiration rate of SA treated pomegranate arils may be ascribed to delay in metabolic activities. Salicylic acid as an electron donor produces free radicals which prevents normal...
respiration. Previous studies on plum\textsuperscript{15} and pomegranate\textsuperscript{16} also showed reduced respiration rate in SA treated whole fruits.

**Total phenols**

Phenolic compounds are the secondary metabolites, responsible for the flavour and colour development in the fruits. Irrespective of pre-treatment, gradual increase was observed in total phenols up to 13 d followed by declining trend during entire storage period of 19 d (Table 1). SA treated arils showed higher phenol content as compared to control arils. Mean value of phenol content was found to be higher for 2 mM SA (207.79 µg gallic acid equivalent g\textsuperscript{-1}) while lowest (188.46 µg gallic acid equivalent g\textsuperscript{-1}) value was recorded for control arils. The increase in phenol content was also observed in previous studies on pomegranate arils and fresh cut grapes\textsuperscript{16,17}. The physical damage of plant tissue during minimal processing can increase phenylalanine ammonialyase (PAL) activity to initiate repair process, which leads to an increase in phenolic compounds. Higher concentration of phenol content in SA treated arils might be attributed to higher activity of PAL enzymes and lower activity of polyphenol oxidase (PPO). Here in control arils, browning was observed while, it was significantly lower in SA treated arils. This aril browning indicated the higher activity of PPO enzymes in control, which subsequently lowered content of phenolic compounds.

**Total anthocyanins**

There was a gradual decline in total anthocyanins in all the pre-treatments with the advancement of storage period (Table 1). Control pomegranate arils showed rapid decrease in total anthocyanin content up to 19 d of storage. However, arils treated with SA showed slower decrease in total anthocyanins during entire storage. After 19 d of storage period, highest total anthocyanins content (22.26 mg equivalent cyanidin-3-glucoside 100 g\textsuperscript{-1}) was found in SA 2 mM treated arils while, it was lowest (16.86 mg equivalent cyanidin-3-glucoside 100 g\textsuperscript{-1}) in control. Control arils exhibited faster degradation of total anthocyanins, while SA pre-treatments retarded the degradation during cold storage of 19 d. Previous work has also showed similar trend of declining anthocyanin content in minimally processed pomegranate arils during storage\textsuperscript{16,18}. Declining trend in anthocyanin content may be due to increasing pH. In present study, arils of all the pre-treatments showed decrease in titratable acidity (data not shown) which may have led to increased pH hence affecting anthocyanin content during storage. Arils of SA (2 mM) pre-treatment showed higher content of anthocyanins as compared to other pre-treatment during entire storage. These results were in agreement with L* value recorded previously.

| Table 1 — Effect of salicylic acid pre-treatments and storage period (5±2 °C and 85±5% relative humidity) on total phenols (µg gallic acid equivalent g\textsuperscript{-1}), total anthocyanins (mg equivalent cyanidin-3-glucoside 100 g\textsuperscript{-1}) and total antioxidants (µM equivalent trolox g\textsuperscript{-1}) of minimally processed pomegranate arils. |
|---------------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| **Pre-treatments**              | **D 0**           | **D 5**           | **D 10**          | **D 13**          | **D 16**          | **D 19**          |
| **Total phenols**               |                   |                   |                   |                   |                   |                   |
| Control                         | 158.36\textsuperscript{a} | 170.90\textsuperscript{m} | 182.60\textsuperscript{i} | 196.22\textsuperscript{d} | 207.01\textsuperscript{gh} | 215.70\textsuperscript{f} | 188.46\textsuperscript{d} |
| SA 1 mM                         | 158.65\textsuperscript{a} | 176.96\textsuperscript{i} | 188.33\textsuperscript{j} | 207.61\textsuperscript{f} | 221.72\textsuperscript{d} | 242.9\textsuperscript{h} | 199.37\textsuperscript{b} |
| SA 2 mM                         | 158.62\textsuperscript{e} | 181.06\textsuperscript{k} | 195.01\textsuperscript{i} | 214.50\textsuperscript{e} | 235.53\textsuperscript{c} | 262.03\textsuperscript{a} | 207.79\textsuperscript{d} |
| SA 3 mM                         | 158.20\textsuperscript{e} | 175.93\textsuperscript{l} | 187.35\textsuperscript{j} | 205.34\textsuperscript{h} | 218.31\textsuperscript{e} | 236.73\textsuperscript{c} | 196.98\textsuperscript{c} |
| Mean                            | 158.46\textsuperscript{f} | 176.21\textsuperscript{e} | 188.32\textsuperscript{d} | 205.91\textsuperscript{c} | 220.64\textsuperscript{b} | 239.35\textsuperscript{a} |                   |
| **Total Anthocyanins**          |                   |                   |                   |                   |                   |                   |                   |
| Control                         | 24.34\textsuperscript{a} | 22.67\textsuperscript{def} | 21.33\textsuperscript{ghi} | 19.36\textsuperscript{d} | 18.48\textsuperscript{k} | 16.86\textsuperscript{d} | 20.5\textsuperscript{c} |
| SA 1 mM                         | 24.26\textsuperscript{b} | 23.38\textsuperscript{abcd} | 23.06\textsuperscript{de} | 22.68\textsuperscript{def} | 21.66\textsuperscript{gh} | 20.81\textsuperscript{h} | 22.64\textsuperscript{b} |
| SA 2 mM                         | 24.46\textsuperscript{a} | 24.01\textsuperscript{bc} | 23.61\textsuperscript{abc} | 23.16\textsuperscript{bcd} | 22.81\textsuperscript{def} | 21.91\textsuperscript{ghi} | 23.3\textsuperscript{a} |
| SA 3 mM                         | 24.25\textsuperscript{a} | 23.03\textsuperscript{de} | 22.62\textsuperscript{ef} | 21.99\textsuperscript{fg} | 21.08\textsuperscript{h} | 20.17\textsuperscript{j} | 22.19\textsuperscript{b} |
| Mean                            | 24.33\textsuperscript{a} | 23.27\textsuperscript{b} | 22.65\textsuperscript{c} | 21.80\textsuperscript{d} | 21.01\textsuperscript{e} | 19.94\textsuperscript{f} |                   |
| **Total Antioxidants**          |                   |                   |                   |                   |                   |                   |                   |
| Control                         | 16.38\textsuperscript{gh} | 16.74\textsuperscript{dfe} | 17.80\textsuperscript{hde} | 16.44\textsuperscript{fgh} | 15.99\textsuperscript{hi} | 14.90\textsuperscript{g} | 16.37\textsuperscript{c} |
| SA 1 mM                         | 16.51\textsuperscript{gh} | 17.94\textsuperscript{bcde} | 18.60\textsuperscript{abc} | 17.85\textsuperscript{bcde} | 17.06\textsuperscript{defgh} | 16.36\textsuperscript{gh} | 17.39\textsuperscript{b} |
| SA 2 mM                         | 16.67\textsuperscript{gh} | 18.67\textsuperscript{bc} | 19.62\textsuperscript{a} | 19.00\textsuperscript{b} | 18.37\textsuperscript{abc} | 17.43\textsuperscript{abc} | 18.29\textsuperscript{a} |
| SA 3 mM                         | 16.99\textsuperscript{gh} | 17.58\textsuperscript{de} | 18.23\textsuperscript{cd} | 17.55\textsuperscript{de} | 16.90\textsuperscript{gh} | 16.18\textsuperscript{hi} | 17.19\textsuperscript{b} |
| Mean                            | 16.56\textsuperscript{de} | 17.73\textsuperscript{b} | 18.56\textsuperscript{e} | 17.71\textsuperscript{bc} | 17.08\textsuperscript{cd} | 16.22\textsuperscript{e} |                   |

Note: Means with same superscript are homogeneous
Antioxidant activity

All the pre-treatments significantly influenced the antioxidant activity of pomegranate arils, during cold storage of 19 d (Table 1). Rapid increase in antioxidant activity was observed up to 10 d of storage, followed by declining trend. At end of storage, control arils showed lowest (14.90 μM trolox equivalent g⁻¹) antioxidant activity as compared to SA pre-treated arils. Among pre-treatments, highest mean antioxidant activity (18.29 μM trolox equivalent g⁻¹) was found in minimally processed arils which were treated with SA 2 mM. Antioxidant contributing property of phenolic compounds has been reported earlier in pomegranate19. Higher antioxidant capacity in SA treated arils might be attributed to higher anthocyanins and phenol content. These findings are in agreement with earlier worker4 who reported acetyl salicylic acid treatment maintained higher antioxidant capacity in pomegranate fruits. The antioxidant capacity decreased after 13th d of storage in all the pre-treatments. Depletion in antioxidant capacity can be linked with decline in phenolics and anthocyanins, with progressive increase in storage period. Previous researcher20 also observed declining trend in the antioxidant capacity of minimally processed pomegranate arils during storage. Fresh-cut tissues are primarily subjected to oxidative stress, presumably causing membrane damage and altering the composition and content of antioxidant compounds, resulting in changes in the total antioxidant activity of the tissue.

Sensory evaluation

The sensory score was rated by panelists in relation to colour, sweetness, texture, juiciness and overall acceptability. Changes in sensory attributes of minimally processed arils as influenced by various pre-treatments were evaluated during storage for 19 d (Figure 1(d)). SA (2 mM) pre-treatment had better score than control and other pre-treatments. The sensory score for quality parameters and overall acceptance for control arils were < 5 on 19th d of storage which was lower than the commercial acceptance level score of 5. Minimally processed arils pre-treated with SA (2 mM) showed highest overall acceptance score (7.8) at the end of storage period followed by 1 mM SA (6). This study revealed that, overall acceptability of minimally processed pomegranate arils after 19 d of storage was highly influenced by SA pre-treatments. Among the pre-treatments, SA 2 mM treated arils had better overall acceptability score primarily because, these arils had attractive appearance, colour and aroma. While in case of control, appearance and other parameters were highly affected due to browning. But, arils treated of SA (2 mM) showed lower incidence of aril browning, thus it had retained better appearance, aroma, taste and texture, leading to higher acceptability of arils.

Conclusions

SA pre-treatment proved effective in maintaining quality parameters during storage up to 19 d. Comparing to the higher doses and control, lower doses of SA (2 mM) can be gainfully utilized for quality retention and shelf-life extension of minimally processed pomegranate arils during storage.

Reference

4. Bhatia K, Asrey R & Varghese E, Correct packaging retained phytochemical, antioxidant properties and increases shelf life


