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Dhinesh Kumar V

Assistant Professor, Agriculture
 Engineering, Mahendra
 Engineering College, Namakkal,
 Tamil Nadu, India

D Ramasamy

Professor and Head, Department
 of Food science and Technology,
 CFDT, Chennai, Tamil Nadu,
 India

Jerish Joyner J

Assistant Professor, Department
 of Food Engineering, CFDT,
 Chennai, Tamil Nadu, India

Effect of active: Modified atmosphere packaging material on biochemical and microbial characteristics of pomegranate arils during storage

Dhinesh Kumar V, D Ramasamy and Jerish Joyner J

Abstract

Minimally processed pomegranate rapidly loses its overall quality because of high water loss and microbial contamination. Pomegranate arils cv. Bhagwa were packed in (Poly propylene, low density poly ethylene and Xtend bags) with different water and gas transmission rate on minimally processed 'Bhagwa' pomegranate arils stored at $5 \pm 2^\circ\text{C}$ and $85 \pm 5\%$ RH for 18 days. During course of storage, phytochemical and antioxidant properties of minimally processed arils were determined at 3 days interval. The results indicated that packaging material influenced ascorbic acid, phenols, anthocyanins, antioxidant and sensory score of minimally processed arils. Arils packed in Xtend bags retained better total phenols, anthocyanins, total phenols, ascorbic acid and antioxidant compared to LDPE and PP packed arils. Among tested packaging materials, Xtend film maintained better quality characteristics of minimally processed arils up to 18 days storage period.

Keywords: *Punica granatum* L., minimal processing, packaging, phytochemical, shelf-life

Introduction

Pomegranate (*Punica granatum* L.) is a highly valuable fruit due to its special sensory, nutrition properties, and bioactive constituents in term of phenolic compounds, anthocyanin content, and antioxidant activity. Pomegranate is also used in a number of systems of medicine for a variety of ailments. Several studies have been focused on anti-carcinogenic, anti-inflammatory and antioxidant properties of pomegranate constituents during last decades (Jurenka, 2008). However, pomegranate consumption, especially in public area is limited because of difficulties in peeling to extract the arils, which lead its consumption to be a time consuming process and phenolic metabolites irritates, and stain the fingers (Caleb, 2012) [18, 19]. Pomegranate (*Punica granatum* L.) belongs to the *Punicaceae* family. It is native to Persia (Iran) and widely cultivated in the Mediterranean region. The edible part (aril) of the fruit is consumed as fresh arils or as processed products such as jam, jelly, wine and beverages. Scientific evidence had linked increasing consumption of pomegranate fruit to improve human health as a result of its active phenolic compounds which have potent pharmacological activities, including, antioxidant, anti-helminthic, anti-mutagenic, anti-hypertension and anti-inflammatory activities (Fawole *et al.*, 2012).

With increasing demand for fresh and natural products without addition of harmful chemicals, packaging film seems to be an ideal tool for preservation of minimally processed fruits, being cheap and easy to apply. Selection of packaging material is very important as combination of horticultural produce and permeability of film results in the passive evolution of an appropriate atmosphere within sealed packages (Jacobsson *et al.* 2004). The modification of carbondioxide and oxygen concentrations in the packages could help to maintain freshness and visual appearance of fresh-cut produce by reducing respiration and ethylene production, and/or physiological and pathological deterioration during storage (Rocha *et al.* 2003). Previous findings indicated that packaging material also influence the nutritional and sensory quality of minimally processed produce (Jacobsson *et al.* 2004 and Shiri *et al.* 2011). As evident from earlier findings that the response of the individual packaging film in relation to shelf-life and quality greatly varied with crop and even within the crop varieties. Looking into these gaps, the present study was undertaken to determine the effect of packaging materials (PP, LDPE and KPA) with different water and gas transmission rate on quality of minimally processed Bhagwa pomegranate arils during cold storage.

Correspondence**Dhinesh Kumar V**

Assistant Professor, Agriculture
 Engineering, Mahendra
 Engineering College, Namakkal,
 Tamil Nadu, India

Materials and Methods

Plant material and experimental design

Physiologically mature (total soluble solids ranging from 11 to 12 Brix) pomegranate fruits of Bhagwa cultivars were purchased from Anna fruit market, koyambedu, Chennai and immediately transported to the Food science and Technology laboratory and kept at $5 \pm 2^\circ\text{C}$ and $85 \pm 5\%$ relative humidity (RH) until the next day. Pomegranates with defects were discarded and healthy ones uniform in size and appearance were sanitized with 200 $\mu\text{L/L}$ 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 $\mu\text{L/L}$ chlorinated water for 5 min, followed by rinsing with potable tap water at 5°C (Gil et al. 1996 b) [16] and washed arils were air dried to remove surface water. Pomegranates with defects were discarded and healthy ones uniform in size and appearance were selected for minimal processing. Thereafter 100 g of minimally processed arils were packed in three different packaging films made up of polypropylene (PP), low density poly ethylene (LDPE) and Xtend film. Packaged samples were stored at $5 \pm 2^\circ\text{C}$ and $85 \pm 5\%$ RH for 15 days and data were recorded on 0, 3, 6, 9, 12, 15 and 18 days of storage.

Ascorbic acid

Ascorbic acid content of pomegranate juice was quantitatively determined by 2, 6-dichlorophenol indophenols visual titration method. 10 ml of sample was made to volume 100 ml with 0.4 per cent oxalic acid and filtered. 10 ml of this filtrate was mixed with 15 ml of 0.4 per cent oxalic acid and titrated with standard dye. The end point was recorded when pink colour persisted for 10-15 seconds. The results were expressed as mg per 100 gm of sample.

$$\text{Ascorbic acid, mg/100g} = \frac{\text{Titre value} \times \text{Dye factor} \times \text{Volume made up}}{\text{Volume taken for titration} \times \text{Weight of the sample}} \times 100$$

Total phenols

The total phenolic contents were determined using the Folin-Ciocalteu method using gallic acid standard described by Cao. About 1ml of sample (previously diluted to 1:9 with distilled water) was mixed with 5 ml of Folin-Ciocalteu (FC) reagent (previously diluted 10-fold with distilled water) and set for 1 h in the dark at room temperature. Then 4 ml sodium carbonate (7.5%) was added to the mixture and reacted for 15 min, and the mixture was immediately measured at 765 nm by a UV spectrophotometer (Plate 11) (UV-1800, Shimadzu Analytical (India) Pvt. Ltd). Previously, Gallic acid standards at different concentration (50-1000mg/100ml) were used to plot the standard curve. The results of sample were interpreted with Gallic acid standard curve and expressed as mg of gallic acid equivalent (GAE) per 100 ml of sample (mg GAE/100ml).

Total anthocyanins

The total anthocyanins measurement was based on the spectrophotometric pH differential method reported by Giusti and Wrolstad (2001). The sample was centrifuged at 12000 rpm for 15 min in a refrigerated centrifuge (Rota 4R-V/Fm, Plasto Crafts Industries Pvt. Ltd., India) (Plate 13) at an ambient temperature ($28 \pm 2^\circ\text{C}$). Then the supernatant was collected for further analysis. The supernatant was diluted to 0.5:4.5 using 0.025 M potassium chloride solution and 0.4 M sodium acetate solution adjusted to pH 1.0 and 4.5 with HCl,

respectively, then equilibrated for 15 min in the dark. The absorbance of each dilution was measured at 510 nm and 700 nm using a UV spectrophotometer (UV-1800, Shimadzu Analytical (India) Pvt. Ltd.), with distilled water as blank. Total anthocyanin content was calculated by the following equation,

$$\text{Total anthocyanins (mg/100ml)} = \frac{A \times M_w \times DF \times 100}{(\epsilon \times l)}$$

Where A = $(A_{510} - A_{700})_{\text{pH}1.0} - (A_{510} - A_{700})_{\text{pH}4.5}$, M_w is the molecular weight of cyanidin-3-glucoside (449.2 g/mol), ϵ is the molar absorptivity (26,900 L/cm/mg), DF=10 (Dilution factor).

Antioxidant activity

Antioxidant activity of pomegranate juice was determined by the DPPH method described by Moon and 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, Kyoto, Japan). The antioxidant activity was calculated using the following equation:

$$\text{Total antioxidant activity (\%)} = 1 - \frac{A_{\text{sample (517nm)}}}{A_{\text{control (517nm)}}} \times 100$$

Sensory evaluation

Sensory evaluation of minimally processed pomegranate arils obtained from 'Mridula' cultivars packed in three different packaging material 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 above 6 were considered as acceptable for commercial purposes. The evaluated parameters were colour, taste, texture, juiciness and overall acceptability.

Statistical analysis

Statistical analysis was carried out to study the effect of different parameters on all the dependent variables. The data were tabulated and subjected to statistical analysis performed using IBM SPSS® 20.0 for Windows® software as per the standard procedure of Snedecor and Cochran (1994). Analysis of variance (ANOVA) was conducted to determine whether significant effect exists on packaging materials, physiological loss in weight, bio active components content and colour value.

Results and Discussion

Ascorbic acid

Effect of packaging material on ascorbic acid content of minimally processed pomegranate arils during storage was found to be significant (Fig. 1). Irrespective of packaging material ascorbic acid content showed progressive declining pattern during entire storage. Declining trend of ascorbic acid was much pronounced in LDPE packed arils. Packaging of arils in Xtend film may cause the better control over pack in atmospheric conditions and resulted in the higher ascorbic acid.

Arendse *et al.* (2014) [3] reported that a gradual decrease, in ascorbic acid content in pomegranate arils during storage. The reduction in the level of ascorbic acid might be the result of delayed biosynthesis or fast degradation of ascorbic acid in MAP stored arils.

The declining trend of ascorbic acid attributed to water loss, cell wall damage, temperature, humidity, and packaging environment. Similar declining trend of ascorbic acid was also reported in minimally processed pomegranate arils during storage.

Ascorbic acid is very sensitive to the enzyme phenolase, temperature, pH, oxygen and light and therefore a reduction of ascorbic acid during storage was expected. Further ascorbic acid is affected and its activity is reduced by the presence of oxygen, alkalinity and high temperatures, Coultate (2007).

Total phenols

The effect of packaging material, storage days and their interaction on total phenol content was found to be significant ($P = 0.05$). Arils packed in Xtend film showed highest phenolics content compared to LDPE and PP during entire course of storage (Table 2). Irrespective of packaging material, progressive increase in total phenol content was observed till 18 day followed by onward declining pattern during storage.

Stress response to wounding (production of stress alleviating phyto-chemicals) during minimal processing operations is well known phenomenon. Similar response to wounding was reported for fresh cut grapes.

Tomás-Barberán and Espín (2001) reported that the increase of the phenolic compounds during the storage could be related to stimulation of the activity of some enzymes involved in

phenolic biosynthesis in cold storage. However, high CO_2 storage can have marked effects on phenolic metabolites and quality, as it can decrease anthocyanin in pomegranate arils. Fawole and Opara (2013) [17] reported that a decline in total phenolic concentration in pomegranate fruit may be related to the breakdown of phenolic compounds as result in enzymatic activity occurring during storage.

Decrease in aril phenol content during later phase of storage (12 day onward) may be attributed to water loss, changes in acidity and TSS content. These changes could have affected total anthocyanins and antioxidant activity and finally resulted into varied phenols content during storage.

Total anthocyanins

Total anthocyanin content was found to be significantly ($P = 0.05$) affected by packaging material, storage days and their interaction (Table 2). It was observed that there was gradual decrease in total anthocyanins with the advancement of storage period. Arils packed in Xtend bags showed highest average anthocyanin content followed by LDPE and PP.

Anthocyanin content decreased as the storage period increased, which could be due to the processing temperature, storage conditions, application post-harvest treatments, the chemical nature of anthocyanins, pH, sugars, exposure to light and metals can affect the stability of anthocyanin content, Jaiswal *et al.* (2010).

The relative amounts anthocyanin in coloured fruits and vegetables depend on the pH and anthocyanin structure. The declining trend of anthocyanin content was reported in minimally processed pomegranate arils during storage, Maghoumi *et al.* (2013).

Table 1: Effect of packaging material and storage on total phenols (μg gallic acid equiv. g), ascorbic acid content, antioxidant activity (%) and total anthocyanins (mg equiv. cyanidin-3-glucoside 100g^{-1}) of minimally processed pomegranate arils during cold storage ($5 \pm 2^\circ\text{C}$ and $85 \pm 5\%$ RH)

	Type of Packaging material	Storage Days						
		0	3	6	9	12	15	18
Total anthocyanins mg /100g	MAP-A	24.16±0.30 ^a	22.96±0.23 ^{ad}	21.58±0.28 ^{bc}	20.72±0.19 ^{cd}	19.64±0.12 ^{ef}	18.78±0.03 ^{fg}	18.02±0.24 ^{gh}
	MAP-B	24.16±0.20 ^a	21.2±0.02 ^a	20.16±0.33 ^a	19.4±0.32 ^a	17.32±0.35 ^a	15.46±0.14 ^a	15.2±0.35 ^{gh}
	MAP-C	24.16±0.49 ^d	21.14±0.22 ^{fg}	20.8±0.17 ^b	17.48±0.37 ^b	15.01±0.12 ^{ad}	13.82±0.12 ^b	13.04±0.31 ^b
Ascorbic acid mg /100g	MAP-A	8.28±0.06 ^a	8.22±0.11 ^a	7.86±0.10 ^{bc}	6.98±0.12 ^{ae}	6.52±0.19 ^{ad}	5.98±0.37 ^a	5.76±0.14 ^a
	MAP-B	8.28±0.03 ^a	7.48±0.11 ^{ae}	6.52±0.05 ^{cd}	6.20±0.05 ^a	5.54±0.02 ^a	5.20±0.02 ^a	5.08±0.12 ^a
	MAP-C	8.28±0.12 ^a	7.10±0.05 ^{bc}	6.22±0.12 ^{cd}	6.10±0.08 ^{de}	5.28±0.11 ^{fg}	4.96±0.05 ^a	4.78±0.08 ^a
Antioxidant activity (%)	MAP-A	56.14±1.34 ^a	56.90±1.42 ^a	57.00±0.83 ^{ac}	57.48±0.53 ^a	57.02±0.10 ^a	55.28±1.35 ^{bc}	54.08±0.19 ^{cd}
	MAP-B	56.14±0.93 ^a	56.94±1.21 ^a	57.18±0.71 ^{bc}	58.10±1.28 ^{cd}	57.32±0.10 ^{fg}	55.04±0.74 ^{gh}	53.24±0.62 ^{ae}
	MAP-C	56.14±0.551 ^a	56.86±0.06 ^a	57.24±0.53 ^{ab}	58.20±0.30 ^{ac}	57.45±0.89 ^{ad}	55.00±0.08 ^{fg}	53.02±0.91 ^{gh}
Total phenols (μg gallic acid equiv.g)	MAP-A	156.92±0.24 ^a	160.47±3.56 ^{ab}	163.24±2.46 ^{cd}	166.82±1.47 ^{ac}	164.24±0.85 ^a	162.15±1.26 ^a	161.08±0.92 ^a
	MAP-B	156.92±2.167 ^a	158.28±0.41 ^a	163.01±2.46 ^{ab}	165.86±0.60 ^{bc}	162.2±1.60 ^{cd}	160.15±0.16 ^{de}	157.08±3.51 ^{ae}
	MAP-C	156.92±0.16 ^a	158.04±1.48 ^a	163.49±1.10 ^{ab}	165.72±3.27 ^{bc}	162.2±4.05 ^{cd}	160.15±0.50 ^{de}	157.08±3.43 ^{ef}

The reduction in anthocyanin content could be attributed to the presence of oxidative enzymes. Synthesis of anthocyanin pigments in fruits during storage at low temperatures had been reported in pomegranate. Furthermore, anthocyanin synthesis and/or degradation might have been affected by CO_2 and O_2 levels, Caleb *et al.* (2013) [17].

Antioxidant Activity

Effect of packaging material, storage days and the interaction on antioxidant activity of minimally processed pomegranate arils was found to be significant ($P = 0.05$). Among the packaging materials, PP packed arils retained the highest antioxidant activity followed by LDPE and PP (Fig. 2). Irrespective of packaging material, antioxidant activity of

minimally processed pomegranate arils increased till 9th day followed by declining pattern during entire storage.

Zheng *et al.* (2008) who stated that there was a positive relationship between antioxidant activity and total phenolic content indicating the effect of polyphenol content on antioxidant activity. The increase in antioxidant activity was probably due to the higher retention of anthocyanin pigment and synthesis of phenolic compounds.

Asrey (2015) found that decrease in antioxidant capacity might be attributed to the O_2 promoted oxidation of the constitutive phenolic compounds.

Higher antioxidant content in Xtend film packed arils could be due to higher retention of anthocyanin pigment and phenolics content. Antioxidant capacity in fresh cut grape

increased up to seven days followed by declining pattern till end of storage period. Appreciation in antioxidant activity may be due to wound induced response during minimal

processing operations. With prolonged aril storage, decrease in antioxidant capacity may attribute to the O₂-promoted oxidation of the constitutive phenolic compounds.

Table 2: Effect of active MAP-A and packaging materials on total mesophilic bacteria and yeast & mold counts of minimally processed pomegranate arils during storage at 5°C (Mean ± SE)

Parameters	Type of MAP	Xtend				PP		LDPE	
		Control	0 Days	9 Days	18 Days	9Days	18Days	9Days	18Days
Total Plate Count log cfu/ml ⁻¹	MAP-A	6.8 ^a	1.0 ^g	5.8±0.05 ^{ab}	5.8±0.02 ^a	5.8±0.14 ^{cd}	6.0±0.04 ^{ac}	5.8±0.12 ^{ab}	6.0±0.08 ^a
	MAP-B	7.4 ^b	1.0 ^g	5.5±0.04 ^{cd}	5.6±0.05 ^{cd}	5.7±0.06 ^a	6.2±0.05 ^{de}	6.2±0.14 ^{ac}	6.6±0.07 ^{ae}
	MAP-C	8.2 ^c	1.0 ^g	5.4±0.05 ^d	5.5±0.02 ^d	5.5±0.15 ^{ab}	6.0±0.03 ^a	6.4±0.06 ^a	6.8±0.15 ^{ca}
Yeast and mold count log cfu/ml ⁻¹	MAP-A	7.4 ^d	0.5 ^d	3.1±0.05 ^c	4.6±0.28 ^a	3.4±0.15 ^{fg}	4.8±0.28 ^c	3.8±0.12 ^b	4.6±0.13 ^g
	MAP-B	8.6 ^e	0.5 ^d	3.8±0.24 ^b	4.8±0.25 ^a	4.0±0.20 ^{gh}	5.0±0.25 ^{cd}	4.4±0.09 ^{bc}	5.0±0.20 ^{fg}
	MAP-C	9.2 ^f	0.5 ^d	3.3±0.13 ^c	4.5±0.35 ^a	3.8±0.27 ^b	4.7±0.10 ^c	4.2±0.17 ^{bd}	4.8±0.15 ^g

Sensory evaluation

Sensory score above 6 out of 9 is the limit of acceptance in terms of product attributes such as aril colour, texture, sweetness and juiciness. The sensory evaluation performed during storage showed that arils packed in PP bags scored highest point with respect to colour, crispness, sweetness, juiciness and overall acceptance as compared to LDPE and PP packed arils (data not shown). The reasons for higher score of arils packed in Xtend film bags attributed to low water loss, better colour retention and organoleptic quality. The sensory score for crispness and overall acceptance for arils packed in LDPE bags were lower than the commercial acceptance level score of above 6 on 15day, limiting its acceptance to 9 days. Minimally processed arils packed in Xtend film and PP scored above the limit set for commercial acceptance on 18th day of storage period. The shelf life of minimally processed pomegranate arils from late harvested and early harvested fruits as 10 days and 14 days respectively.

Conclusions

Packaging film had the most significant impact on the phytochemical retention antioxidant properties and shelf- life of pomegranate arils cultivar (CV‘Bhagwa’) and these properties were significantly higher with Xtend film bags even up to 18 days. This finding highlights the significance of selecting of right kind of packaging films for minimally processed fresh produce handling with special reference to pomegranate cultivar ‘Bhagwa’.

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