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Storage and Packaging Material Effects on Pomegranate (*Punica granatum*) Arils Quality Attributes during Storage

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ABSTRACT

Background: Minimally processed ready-to-eat pomegranate arils have become popular due to their convenience, high value, unique sensory characteristics and health benefits. The main objective of this key research is to understand the effect of packaging materials and the quality profile of pomegranate arils (Bhagwa) storage at 5-7°C, 85-90% RH till 18 days was tested.

Methods: Minimally processed arils at three-day intervals throughout storage, the antioxidant and phytochemical characteristics were assessed. There has been a variable change in the ascorbic acid concentration level, phenolic, antioxidant and anthocyanin content due to the packaging material with minimum processed arils in sensory analysis.

Result: When the pomegranate Arils packed in breathable film indicated high retention of phenols antioxidants, anthocyanins, ascorbic acid and total than arils packed in LDPE or PP. Breathable film shown better quality attributes for minimally processed arils over an 18-day storage period as compared to other packaging materials.

Key words: Bioactive, Minimal treatment, Packaging, Pomegranate, Storage period.

INTRODUCTION

Pomegranate fruits have been reported to have significant atherosclerotic properties, antimutagen, anti-inflammatory, antioxidant capacity, anti-hypertension properties (O'Grady *et al*., 2014). Moreover, the pomegranate is an excellent source for bioactive substances , organic acids, micro- and macronutrients, vitamin C and fatty acids (Opara *et al*., 2009). In comparison to whole fruits, ready-to-eat and minimally processed pomegranate arils are an attractive product that increases the chances of production (Artés *et al*., 2000; O'Grady *et al*., 2014). Packaging has significant role to play in preserving the microbial and nutritional characteristics of fresh and fresh cut perishable commodities (Opara and Mditshwa, 2013). Packaging mainly helps to protect perishable commodities from microorganisms, food borne illness and attracting due to remarkable feature of disease management to provide structural support for easy storage and transport (O'Grady *et al*., 2014). Packaging outlines a novel approach to increase the sustainability and minimizes food borne diseases (Riudavets *et al*., 2009). Various packaging and storage applications have been investigated for the pomegranate arils, such as rigid polystyrene vessels, bi-axially oriented polypropylene films, polypropylene trays, heat-sealed trays with polypropylene bags perforated polyethelene bags (Ayhan and Eştürk, 2009; Ergun and Ergun, 2009; O'Grady *et al*., 2014).

The MAP introduces a new approach for increasing shelf life by dynamically changing the composition of the gases contained inside a package. The shelf life of fresh products depends on numerous characteristics such as storage temperature, respiration rate, gas permeability of film, headspace composition and weight of product and their interaction will influence during storage period (Ergun and Ergun, 2009; Mahajan *et al*., 2007; O'Grady *et al*., 2014; Oliveira *et al*., 2012).

Pomegranate production has increased globally as a result of growing customer demand and for the international markets supply various post harvest handling procedure and refrigerated supply chain management is necessary (Caleb *et al*., 2012). The main aim of our study is to find out the various packaging materials effect on the pomegranate arils (Breathable film, PP and LDPE) bioactive components like antioxidant activity, ascorbic acid, anthocyanins and physicochemical properties during refrigerated storage.

MATERIALS AND METHODS

The pomegranate (*Punica granatum* L.) fruit cvs. 'Bhagwa' was purchased from fruit market and the fruits has been immediately stored at 5°C. The aril was removed from the whole pomegranate fruit manually and hundred (100) grams of arils were packed in three various types of material like

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Breathable film (PP), low density polyethylene (LDPE) and polypropylene (PP).

Ascorbic acid

1% metaphosphoric acid and 2, 6-dichlorophenolindophenol dye is used to find total ascorbic acid content using spectrophotometer (Mditshwa *et al*., 2013). 1:9 ratio of juice and 1% metaphosphoric acid has been vortexed and sonicated into cold water for 180 seconds to extract ascorbic acid content from PJ sample. To obtain a clear, homogenous solution, the mixture was centrifuged at 4°C for 10 minutes at 10,000 rpm (Mditshwa *et al*., 2013; O'Grady *et al*., 2014). In a glass test tube, 0.0025% 2, 6 dichlorophenolindophenol dyes was added (9 ml) to the PJ sample (1 ml). At 515 nm the standard curve and extracts of sample DCP-MPA was measured after dark incubation (30 minutes) (O'Grady *et al*., 2014). Pomegranate juice ascorbic acid value was calculated as (mg L^{-1}) (O'Grady *et al*., 2014).

Total phenol content

Folin-Ciocalteu method was used to obtain the phenol content of pomegranate arils (Ramasamy *et al*., 2018). 300 µl of pomegranate juice was mixed with 1.5 ml of a Folin-Ciocalteu reagent (10-fold diluted) and 1.2 ml of sodium carbonate at 7.5% concentration was mixed with diluted at a methanol: water ratio (6:4) of 1:100. Total phenolics were measured using spectrophotometry at 750 nm after the solution could remain at room temperature for up to 90 minutes. Gallic acid was used for the standard. The findings were expressed in GAE mg ml^{-1} (Mditshwa *et al*., 2013).

Anthocyanins content

Total anthocyanins in pomegranate juice was measured using a differential pH technique with two buffer systems; pH 1.0 potassium chloride buffer (0.025 M) with pH 4.5 sodium acetate buffer (0.4 M) (Fazaeli *et al*., 2013). The samples were diluted with a potassium chloride buffer until they were absorbed at a wavelength of 510 nm across the linear range of the spectrophotometer (Cecil 2010 UV-visible). The sample was then diluted with a sodium acetate buffer using this dilution factor. After 15 minutes of incubation, the wavelength of each sample was measured four times, diluted in two separate buffers, with two different wavelengths of 510 nm and 700 nm. The anthocyanin content of PJ was expressed in milligrams of 3-glucoside cyanidin/100 mL.The amount of anthocyanins in pomegranate juice was estimated to be equivalent to cyanidin 3-glucoside cyanidine (molecular weight of 449.2 and molecular extinction coefficient of 26,900) per 100 ml of crude juice (mg of C3gE/100 ml) (Fazaeli *et al*., 2013).

Antioxidant activity

Moon and Terao described the DPPH technique for finding the antioxidant role in pomegranate juice (1998). The combination of the sample has formed by mixing 1 ml of DPPH, Tris-Hcl buffer 0.9 ml, along with 0.1 ml of pomegranate juice .After a vigorous shake, the mixture was set aside for 30 minutes. An UV-Visible spectrophotometer was used to determine the solution's absorbance at 517 nm. The activity of antioxidants may be calculated using the equation below.

$$\text{Antioxidant activity (\%)} = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100$$

Sensory evaluation

Pomegranate arils which is obtained from cultivars was packed in three various packaging material which is further processed for sensory evaluation with the below rating from 01 to 09 (Dislike extremely to like extremely). For commercial reasons, a score of 6 or above was regarded acceptable. Color, flavor, texture, juiciness and general acceptability were the criteria used to evaluate the product.

Statistical analysis

The influence of a number of factors on all dependent variables was investigated using statistical analysis. Statistical analysis was done with tabulated data for Windows® software, as per Snedecor and Cochran's usual technique (1994). The researchers used variance analysis (ANOVA) to see whether there was any significant influence on packaging materials, physiological weight reduction, bioactive component content and color value.

RESULTS AND DISCUSSION

Ascorbic acid content changes

Table 1 represents Ascorbic acid content changes of minimally treated arils during storage.

During storage, the ascorbic acid content of minimally treated arils decreased. There were significant positive between packaging materials and ascorbic acid content with regard to storage concerned.

This finding confirms that irrespective of the packing method, it is evident that the ascorbic acid level decreased over the storage time. Our results confirm during the storage period, Breathable film showed significant ascorbic acid retention and ascorbic acid concentration level declined due to oxidative enzymes activity. It was found that decline in ascorbic acid level during storage could be ascribed to oxidative enzymes. In this study, ascorbic acid level declining trend was observed in the most of the packages. The enzyme ascorbic acid oxidase is required for the oxidative breakdown of vit C in presence of molecular oxygen (Singh *et al*., 2011).

According to (Arendse *et al*., 2014), decreasing the ascorbic acid content due to prolonged storage and degradation in the MAP packages due to delayed biosynthesis. The significant influence of packaging applications on biochemical compounds of Pomegranate corroborates previous study reported by (Ayhan and Eştürk, 2009). On the other hand Coultate (2007) found that enzyme phenolase is sensitive towards ascorbic acid, variation in temperature, pH, oxygen and light. Along with water loss

and cell wall destruction, other factors contributing to the declining trend of ascorbic acid include relative humidity and the packing environment (Bhatia *et al*., 2013).

Changes of phenol content

A significant change (P<0.05) was noticed in the total phenol concentration due to the packaging materials, storage days and this interaction was observed in Table 1.

Breathable packaging film showed that highest retention of phenol content compared to PP and LDPE materials during storage periods. The total phenol content gradually increased until the 12th day, following which a decline pattern was observed over storage periods. This is in good agreement with (Espı and Toma, 2014), the increasing phenolic content throughout storage periods may be attributable to the increased in cold storage due to activity of enzymes in phenolic production. As reported by (Fan, 2005) the evidence we found that during minimal processing, increased phenolic compounds that cause physical damage to plant tissue can increase PAL activity and stress response to wounding (synthesis of stress-relieving phytochemical). The phenolic compounds degradation induced by enzymatic activity during storage may be responsible for the decrease in total phenolic content of pomegranate fruit (Fawole and Opara, 2013). The significant effect of packaging applications on phenolic concentrations confirms previous study reported by Bhatia *et al*. (2013).

Anthocyanin content changes

A significant difference in the anthocyanin content of the pomegranate aril (p<0.05) was found in Table 1. Due to the packaging materials, storage days and their interaction, significant difference was observed in anthocyanin content. It was noticed that, due to prolonged storage periods anthocyanin content was gradually decreased. Breathable film packed arils showed highest retention of anthocyanin content followed by PP and LDPE. In the processing and storage of pomegranate arils, anthocyanins were unstable and susceptible to degradation. Anthocyanin stability was affected by temperature, storage conditions, the use of postharvest methods, anthocyanin chemical composition, metals, sugar, pH and exposure to light (Maghoumi *et al*., 2013). The hydrolysis of protective 3-glucoside linkages, which causes the formation of unstable anthocyanins, may be responsible for the reduction in anthocyanin levels. The amount of anthocyanin found in colorful perishable commodities is affected by the pH and the structure of the anthocyanin. During storage, it was found that the anthocyanin content of hardly processed pomegranate arils decreased (Artés *et al*., 2002).

According to (Zhang *et al*., 2003) there is a correlation between phenolic content and antioxidants, suggesting that polyphenol content influences antioxidant activity. Most likely, higher anthocyanin pigment retention and phenolic compound synthesis were responsible for the increased antioxidant activity.

Table 1: Packaging material and storage effects on complete phenols, antioxidant activity, ascorbic acid content and anthocyanins content in the low processed pomegranate fruit (5±2°C and 85±5% RH).

Biochemical parameters	Various types of materials for packing	Storage days						
		0	3	6	9	12	15	18
Totalanthocyanins (mg/100 g)	Breathable film	24.18±0.30	22.94±0.23	21.58±0.28	20.62±0.19^{cd}	19.64±0.12^{ef}	18.78±0.03^{fg}	18.02±0.24
	PP	24.18±0.20	21.3±0.02	20.15±0.33	19.5±0.32	17.33±0.35	15.46±0.14	15.2±0.35
	LDPE	24.18±0.49	21.16±0.22	20.9±0.17	17.49±0.37	15.02±0.12	13.82±0.12	13.04±0.31
Ascorbic acid (mg/100 g)	Breathable film	8.28±0.06	8.23±0.11	7.85±0.10	6.97±0.12	6.53±0.19	5.98±0.37	5.76±0.14
	PP	8.28±0.03	7.47±0.11	6.53±0.05	6.20±0.05	5.53±0.02	5.20±0.02	5.08±0.12
	LDPE	8.28±0.12	7.12±0.05	6.21±0.12	6.11±0.08	5.29±0.11	4.96±0.05	4.78±0.08
Antioxidant activity (%)	Breathable film	56.16±1.34	56.83±1.42	57.00±0.83	57.38±0.53	57.01±0.10	55.28±1.35	54.08±0.19
	PP	56.16±0.93	56.81±1.21	57.23±0.71	58.12±1.28	57.34±0.10	54.04±0.74	52.24±0.62
	LDPE	56.16±0.551	56.76±0.06	57.22±0.53	58.13±0.30	57.46±0.89	54.00±0.08	51.02±0.91
Total phenols (µg gallic acid equiv. g)	Breathable film	156.92±0.34	160.37±3.56	163.26±2.46	166.72±1.47	164.34±0.85	162.15±1.26	161.08±0.92
	PP	156.92±2.l57	158.38±0.41	163.02±2.46	165.76±0.60	162.3±1.60	160.15±0.16	157.08±3.51
	LDPE	156.92±0.15	158.14±1.48	163.47±1.10	165.73±3.27	162.3±4.05	160.15±0.50	157.08±3.43

Antioxidant activity

The interaction between different packaging materials and the selected storage temperature on the antioxidant activity is given in Table 1.

There was considerable variation in the antioxidant activity of pomegranate arils across the three packing materials investigated, with values ranging from 54.08±0.19 to 53.02±0.91. The breathable film showed the highest retention of anti-oxidant activity and the lowest in the LDPE packaging film. Significant differences of antioxidant activity found to be in pomegranate arils ($P<0.05$) were revealed among the effect of interaction along with storage period and packaged materials.

Antioxidant activity and total phenolic concentration showed positive correlation and it was observed by (Zhang *et al*., 2003), indicating that polyphenol content impacts antioxidant activity. Phenolic compounds synthesis and anthocyanin pigment retention (high) was probably due to the increase in antioxidant activity of arils. Antioxidant activity decreased as total flavonoids, total polyphenols and ascorbic acid were decreased. Fawole and Opara (2013) resulted that O_2-promoted oxidation of the constitutive phenolic components may be responsible for a decrease in the antioxidant activity of arils as storage time increases.

Sensory evaluation

Sensory scores greater than 6 out of 9 indicate that product attributes such as aril juiciness, sweetness, color and texture are acceptable. Sensory evaluations conducted during storage revealed that packed pomegranate in Polypropylene bags scored higher on color, crispness, sweetness, juiciness and whole acceptance than arils packed in Low density poly ethylene or Poly propylene bags (Data not shown). The Breathable film bags have scored well because of the decreased water loss, improved color preservation and organoleptic quality. The overall acceptance of pomegranate in low density poly ethylene bags was less commercially viable only till 9 days. On the 18th day of storage, pomegranate fruit packed in Breathable film and Polypropylene exceeded commercial acceptance bound. In the current study minimum 10 to maximum 14 days of the primary shelf life of the packed pomegranate fruit was determined.

CONCLUSION

The best effect on the shelf life, antioxidant properties phytochemical retention of the fruit (CV 'Bhagwa') and these attributes were related with increased for 18 days in breathable film. This study examines need of the appropriate packaging materials for handling minimally processed fresh product, with a focus on the pomegranate cultivar 'Bhagwa'.

conflict of interest

The authors declare no conflict of interest.

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