

Original Research Article

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Bioactive Compounds and Quality Attributes of Edible Coated Ready-to-Eat Arils of Pomegranate cv. Bhagwa Packed in Clamshells during Storage

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The present research study focuses on organic edible coatings such as chitosan (1%), aloe vera gel (100%), honey (10%) as an substitute for chemical based coatings showed promising results in extending shelf life of ready-to-eat arils of pomegranate cv. Bhagwa packed in clamshells. Chitosan (1%) treated pomegranate arils packed in clamshells and stored at cold temperature of $4\pm1^{\circ}\text{C}$ was found to be promising to maintain several quality parameters such as total anthocyanins ($25.38 \text{ mg } 100 \text{ g}^{-1}$), β -carotene content ($25.56 \mu \text{g } 100\text{g}^{-1}$) and sensory characters like colour, taste and flavour of arils besides keeping the microbial load at minimum level at twenty days of storage.

Introduction

Pomegranate (*Punica granatum* L.) which is regarded as the ‘Fruit of paradise’ and ‘Elixir of life’ is one of the choicest table fruit cultivated in India on commercial scale. The edible part of the fruit comprises juicy arils which range from 50 to 70% of the fruit. Pomegranate arils are juicy and rich in anthocyanins and other phenolic compounds. Scientific evidence has linked increasing consumption of pomegranate arils to improved human health as a result of active phenolic

compounds which have potent pharmacological activities (Vinda-Martos *et al.*, 2010). Further, pomegranate arils are an excellent dietary source rich in organic acids, anthocyanins, vitamin-C, fatty acids and mineral elements (Fawole and Opara, 2012). Production of pomegranate arils in ‘ready-to-eat’ form would be a convenient and desirable alternative to the consumption of fresh fruits and may increase pomegranate demand by consumers. However, maintaining the nutritional quality of pomegranate arils is a major challenge because arils easily deteriorate in texture, colour together with an

increasing in microbial and fungal spoilage.

Minimally processed pomegranate arils have greatly reduced postharvest life compared with whole fruits. It is therefore important to try and improve the preservation and quality of minimally processed arils in order to extend their shelf life. Recent advances in post-harvest treatments include, the use of organic edible coatings comprising polysaccharides (cellulose and its derivatives, starch and its derivatives, resins) chitosan, aloe vera gel, honey etc. are of great significance in reducing the post- harvest losses.

The present study was conducted to determine if edible coatings and storage temperatures could be an alternate way to preserve the minimally processed pomegranate arils, with reduced microbial population and extend the shelf life.

Materials and Methods

Well developed Bhagwa fruits at optimum stage of maturity, free from pest and disease attack were harvested from the field and brought to the laboratory. The arils from fruits were extracted manually after splitting the fruits with the help of sterilized knife. The entire process of aril extraction and packing was done under hygienic conditions. Edible coatings viz., Chitosan (1%), Aloe vera gel (100%) and Honey (10%) were used for treating the arils. The treated arils packed in clamshells were kept at $4\pm1^{\circ}\text{C}$, $7\pm1^{\circ}\text{C}$ and room temperature ($26\text{-}29^{\circ}\text{C}$).

Preparation of edible coatings

Chitosan (1%): Chitosan with 90% deacetylation and a molecular weight of 360 kDa was prepared at 1% (w/v) concentration in an aqueous solution of acetic acid (0.5% v/v). The solution was warmed to 45°C and stirred for complete dissolution of chitosan,

adjusting its pH to 5.2 with NaOH. After cooling at 20°C , the arils were dipped in the chitosan solution for 60 seconds to generate a uniform film.

Aloe vera (100%): Matured leaves from Aloe vera plant were harvested and washed with a mild chlorine solution of 25%. Aloe vera gel matrix was then separated from the outer cortex of leave and this colourless hydro parenchyma was ground in a blender. The resulting mixture was filtered to remove the fibres. The gel matrix was pasteurized at 70°C for 45min. For stabilization, the gel was cooled immediately to an ambient temperature.

Honey (10%): Honey solution @ 10g was dissolved in one liter of warm water to get honey (10%) solution.

Estimation of quality parameters

Total anthocyanins (mg 100g^{-1}): The procedure outlined by Harborne (1973) was used for analyzing anthocyanin content in pomegranate arils. One gram of pomegranate arils was macerated in one millilitre of methanol containing one per cent hydrochloric acid. The content was kept overnight at 0°C temperature in a deep freezer. The absorbance of red colour solution was recorded at 530 nm on spectrophotometer. Anthocyanin content was expressed as absorption units at 530 nm per gram fresh arils.

The total anthocyanin content of arils was determined by using the following formula

$$\text{Total optical density/mg} = \frac{\text{Optical density} \times \text{Volume made up} \times 100}{\text{Weight of the sample}}$$

$$\text{Total anthocyanins (mg)} = \frac{\text{Total optical density}/100 \text{ g}}{98.2}$$

β -carotene content ($\mu \text{ 100g}^{-1}$): β -carotene

content of pomegranate arils was estimated by using the methodology of Srivastava and Kumar (2002). β -carotene was extracted from the sample by crushing one gram of sample with 10 ml acetone and adding crystals of anhydrous sodium sulphate. The supernatant was decanted and collected in a beaker. The process was repeated twice. 10 ml of petroleum ether was added and mixed thoroughly. The content was transferred in to a separating funnel and two layers were separated out on standing solution. Lower layer was discarded and upper layer was collected and volume was made up to 20 ml with petroleum ether. The optical density was recorded at 452 nm using petroleum ether as blank.

The β -carotene was estimated by using the formula

$$\beta - \text{carotene } (\mu \text{g } 100\text{g}^{-1}) = \frac{\text{Optical Density} \times 13.9 \times 10^4 \times 100}{\text{Weight of sample (g)} \times 560 \times 1000}$$

Sensory evaluation

The stored arils of pomegranate were examined for their sensory qualities by assessing the colour, flavour, texture and overall acceptability. Sensory evaluation was carried out by a panel of 5 judges and the rating was done with score on 9 point Hedonic scale (Amerine *et al.*, 1965). Organoleptic evaluation of pomegranate arils was carried out by Gil *et al.*, (1996). The overall rating was obtained by averaging the score of evaluation. Fruits with sensory score of 5.5 and above were rated as acceptable.

Results and Discussion

Total anthocyanins ($\text{mg } 100 \text{ g}^{-1}$)

In general, most of the pomegranate cultivars are predominant of cyanidin 3,5-diglucoside, while pelargonidin 3-glucoside anthocyanin was present in the lowest amount. Changes in

the total anthocyanins were evaluated for all treatments across storage period. The results showed that with progressing storage duration, total anthocyanins declined throughout the storage period in all the treatments (Table.1). However, arils in control treatment (non-chitosan-coated arils) that were stored at 4°C, 7 °C and room temperature (26-29°C) showed an increase in total anthocyanins during initial days of storage but at the end of storage period, a rapid and considerable reduction was observed that reached the minimum value. In addition, a minor increase compared with that of control was recorded in coated pomegranates with 1% chitosan stored at 4°C, 7 °C and room temperature (26-29°C) during storage days and maintained better during 20 days of storage except, on 4th day, minimum total anthocyanin ($\text{mg } 100\text{g}^{-1}$) content (30.95) was recorded in C₁ (1% chitosan) coated arils was lower compared to un-coated arils in C₄ (control) (33.86). Reduction of anthocyanin content recorded in this study was similar to those reported earlier by Ayhanand and Estruck (2009), Salama *et al.*, (2012) and Caleb *et al.*, (2013) in pomegranate and strawberries.

The maximum total anthocyanins ($\text{mg } 100\text{g}^{-1}$) (28.56, 27.73, 26.11) was observed in C₁ (1% chitosan) and the minimum values (27.52, 25.84 and 24.03) were obtained in C₄ (control) on 8th, 12th and 16th day of storage, respectively. One per cent chitosan treatments showed better anthocyanin-keeping properties compared to control. This might be due to the barrier effect of the chitosan coating which imposes in its endogenous CO₂ and O₂ levels as reported by Zhang and Quantick (1998) in strawberries and raspberries. Chitosan application has also been demonstrated to have beneficial effects in maintaining anthocyanin content in several fruits such as longan fruit (Jiang and Li, 2001) and peeled litchi fruit (Donga *et al.*, 2004).

The interaction effects between edible

coatings and storage temperatures revealed that significant differences were observed on all the days of storage except, on 4th and 12th day of storage. C₁T₁ (1% chitosan and 4±1°C) was found superior with regard to retention of total anthocyanins (mg 100 g⁻¹) (29.72), (26.78) and (25.38) on 8th, 16th and 20th day of storage, respectively.

Beta-carotene (μ 100g⁻¹)

The influence of different edible coatings and storage temperatures β -carotene content (μ 100g⁻¹) of arils of pomegranate cv. Bhagwa was studied and the data is presented in Table.2. The β -carotene content (μ 100g⁻¹) of arils decreased with increase in storage period. The highest β -carotene content in pomegranate arils (29.68, 28.36, 28.03, 26.35 and 24.94) was recorded in C₁ (1% chitosan) on 4th, 8th, 12th, 16th and 20th day of storage, respectively.

The lowest β -carotene content (μ 100g⁻¹) was recorded in C₄ (control) (27.93, 27.91, 26.57, and 24.01). With regard to storage temperatures, T₁ (4±1°C) recorded high β -carotene content (μ 100g⁻¹) during storage period. Whereas, it was low at T₃ (room temperature) on 4th (26.42 μ 100g⁻¹) and 8th (25.06 μ 100g⁻¹) day of storage and the spoilage of arils observed after 8th day of storage.

The interaction between edible coatings and storage temperatures had significant effect on β -carotene content (μ 100g⁻¹) of arils. The highest β -carotene content (μ 100g⁻¹) was recorded in C₁T₁ (1% chitosan and 4±1°C) (31.26, 30.26, 28.65, 26.82 and 25.56). The lowest β -carotene content (μ 100g⁻¹) (24.92) was recorded in C₄T₃ (control and room temperature) on 4th day of storage and spoilage of arils observed after 4th day of storage.

Organoleptic evaluation

Colour of pomegranate arils

The colour of arils of pomegranate cv. Bhagwa was significantly influenced by edible coatings and storage temperatures Figure 1. It is evident from the data that a minor increase in aril colour was observed in un-coated arils compared with that of coated arils during initial days and steady decrease thereafter was noticed during storage period. Correlation between colour parameters and anthocyanin levels was reported in several fruits Jiang *et al.*, 2005 in Litchi, Goncalves *et al.*, 2007 in Cherries, Sepulveda *et al.*, 2010 in pomegranate.

On 4th day, minimum aril colour (8.21) was recorded in C₁ (1% chitosan) coated arils compared to un-coated arils in C₄ (control) (8.59). Similar findings were also reported by Ayhanand and Estruck (2009) and Salama *et al.*, (2012) in pomegranate, and also in strawberries and raspberries, where delayed colour change due to chitosan coating was observed by Han *et al.*, (2004).

Maximum aril colour (8.03, 7.37, 6.78 and 6.26) observed on 8th, 12th, 16th and 20th day of storage with C₁ (1% chitosan) is due to low enzymatic activity and polyphenol oxidase (PPO) activity. The preservation of colour and retardation of browning have been improved by the use of films or coatings as stated by Olivas *et al.*, 2009. Minimum aril colour (7.49, 6.00 and 5.78) was observed in C₄ (control) on 8th, 12th and 16th day of storage. The Interaction effects between edible coatings and storage temperatures revealed that significant differences were observed on all the days of storage except, on 4th day of storage (Fig. 2).

Table.1 Effect of different edible coatings and storage temperatures on total anthocyanin content ($\text{mg } 100 \text{ g}^{-1}$) of arils of pomegranate cv. Bhagwa

		Total anthocyanin content ($\text{mg } 100 \text{ g}^{-1}$)																								
		Storage period (days)																								
	0	4					8					12					16					20				
		C ₁	C ₂	C ₃	C ₄	Mean	C ₁	C ₂	C ₃	C ₄	Mean	C ₁	C ₂	C ₃	C ₄	Mean	C ₁	C ₂	C ₃	C ₄	Mean	C ₁	C ₂	C ₃	C ₄	Mean
T ₁	29.31	30.06	30.18	30.13	30.77	30.28	29.72	29.60	29.67	28.03	29.25	28.18	28.11	28.16	26.33	27.69	26.78	26.71	26.75	24.03	26.07	25.38	25.27	25.31	*	25.32
T ₂	29.31	30.76	30.82	30.78	31.27	30.90	28.88	28.78	28.82	27.01	28.37	27.28	27.16	27.21	25.36	26.75	25.44	25.38	25.40	*	25.41	24.14	24.06	24.11	*	24.10
T ₃	29.31	32.02	32.15	32.10	33.86	32.53	27.12	27.07	27.09	*	27.09	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Mean	29.31	30.95	31.05	31.03	31.97		28.57	28.48	28.53	27.52		27.73	27.66	27.68	25.84		26.11	26.04	26.07	24.03		24.76	24.66	24.71	*	
Statistics		SE.m±		CD @ P=0.05			SE.m±		CD @ P=0.05			SE.m±		CD @ P=0.05			SE.m±		CD @ P=0.05			SE.m±		CD @ P=0.05		
C		0.28		0.83			0.25		0.74			0.21		0.62			0.18		0.52			0.14		0.42		
T		0.24		0.72			0.22		0.66			0.18		0.54			0.16		0.45			0.12		0.36		
C×T		0.49		NS			0.44		1.29			0.37		NS			0.31		0.91			0.25		0.72		

C₁ : Chitosan (1%)
 C₂ : Aloe vera gel (100%)
 C₃ : Honey (10%)
 C₄ : Control (Un-treated)

T₁ : 4±1°C
 T₂ : 7±1°C
 T₃ : Room temperature (26-29°C)

* Decayed arils

Table.2 Effect of different edible coatings and storage temperatures on beta-carotene content ($\mu 100 \text{ g}^{-1}$) of arils of pomegranate cv. Bhagwa

		Beta-carotene content ($\mu 100 \text{ g}^{-1}$)																								
		Storage period (days)																								
	0	4					8					12					16					20				
		C ₁	C ₂	C ₃	C ₄	Mean	C ₁	C ₂	C ₃	C ₄	Mean	C ₁	C ₂	C ₃	C ₄	Mean	C ₁	C ₂	C ₃	C ₄	Mean	C ₁	C ₂	C ₃	C ₄	Mean
T ₁	32.07	31.26	31.23	31.25	30.02	30.94	30.26	30.20	30.24	28.38	29.77	28.65	28.52	28.61	27.11	28.22	26.82	26.78	26.80	24.01	26.10	25.56	25.50	25.53	*	25.53
T ₂	32.07	30.82	30.79	30.80	28.86	30.31	29.72	29.68	29.70	27.45	29.13	27.42	27.37	27.40	26.04	27.06	25.65	25.60	25.62	*	25.62	24.32	24.28	24.30	*	24.30
T ₃	32.07	26.96	26.90	26.91	24.92	26.42	25.11	25.01	25.08	*	25.06	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Mean	32.07	29.68	29.64	29.65	27.93		28.36	28.30	28.34	27.91		28.03	27.94	28.00	26.57		26.35	26.19	26.21	24.01		24.94	24.84	24.91	*	
Statistics		SE.m±		CD @ P=0.05			SE.m±		CD @ P=0.05			SE.m±		CD @ P=0.05			SE.m±		CD @ P=0.05			SE.m±		CD @ P=0.05		
C		0.27		0.79			0.26		0.74			0.22		0.63			0.18		0.53			0.17		0.48		
T		0.23		0.68			0.22		0.64			0.19		0.55			0.16		0.46			0.14		0.42		
C×T		0.47		NS			0.44		1.29			0.38		NS			0.31		0.91			0.29		0.84		

C₁ : Chitosan (1%)
 C₂ : Aloe vera gel (100%)
 C₃ : Honey (10%)
 C₄ : Control (Un-treated)

T₁ : 4±1°C
 T₂ : 7±1°C
 T₃ : Room temperature (26-29°C)

* Decayed arils

Table.3 Effect of different edible coatings and storage temperatures on flavour of arils of pomegranate cv. Bhagwa

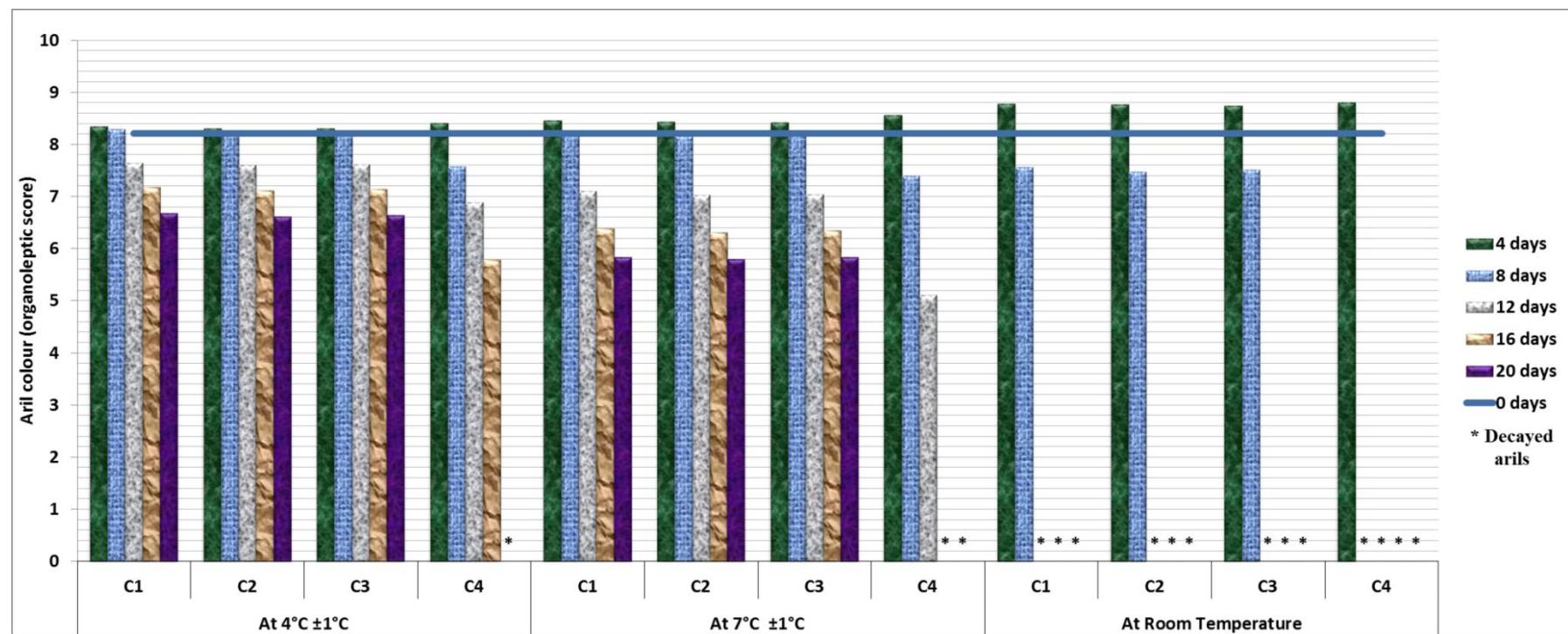
		Flavour of arils (organoleptic score)																								
		Storage period (days)																								
	0	4					8					12					16									
		C ₁	C ₂	C ₃	C ₄	Mean	C ₁	C ₂	C ₃	C ₄	Mean	C ₁	C ₂	C ₃	C ₄	Mean	C ₁	C ₂	C ₃	C ₄	Mean					
T ₁	8.90	8.86	8.83	8.84	8.01	8.63	8.68	8.61	8.67	7.31	8.32	7.78	7.72	7.76	7.01	7.57	7.38	7.32	7.36	5.61	6.91	6.64	6.57	6.61	*	6.60
T ₂	8.90	8.37	8.27	8.34	7.66	8.16	8.19	8.16	8.17	6.55	7.77	7.06	7.01	7.03	5.32	6.60	6.56	6.51	6.53	*	6.53	5.62	5.56	5.58	*	5.59
T ₃	8.90	6.11	6.06	6.08	5.33	5.90	5.43	5.36	5.39	*	5.93	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Mean	8.90	7.78	7.72	7.75	7.00		7.43	7.38	7.41	6.93		7.42	7.36	7.39	6.16		6.97	6.91	6.94	5.61		6.13	6.06	6.09	*	
Statistics		SE.m±		CD @ P=0.05			SE.m±		CD @ P=0.05			SE.m±		CD @ P=0.05			SE.m±		CD @ P=0.05			SE.m±		CD @ P=0.05		
C		0.07		0.21			0.07		0.19			0.05		0.16			0.05		0.13			0.03		0.10		
T		0.06		0.18			0.06		0.17			0.05		0.14			0.04		0.12			0.03		0.09		
CxT		0.12		NS			0.11		0.33			0.09		0.28			0.08		0.23			0.06		0.18		

C₁ : Chitosan (1%)
 C₂ : Aloe vera gel (100%)
 C₃ : Honey (10%)
 C₄ : Control (Un-treated)

T₁ : 4±1⁰C
 T₂ : 7±1⁰C
 T₃ : Room temperature (26-29⁰C)

* Decayed arils

Fig.1 Effect of different edible coatings and storage temperatures on aril colour of pomegranate cv. Bhagwa



Among the storage temperatures, T_1 ($4\pm1^{\circ}\text{C}$) was found significantly superior with regard to retention of aril colour (8.34, 8.10, 7.44, 6.80 and 6.64) on 4th, 8th, 12th, 16th and 20th day of storage. Maximum aril colour score recorded in T_3 (room temperature) on 4th (8.77) might be due to high enzymatic activity, and minimum at 8th (7.51) day of storage and spoilage was observed after 8 days of storage. The colour change observed in the present study might be due to lower enzymatic activity which is most likely related to arils stored at lower temperatures.

Taste of pomegranate arils

There was a reducing trend in organoleptic score of taste of arils during the period of storage and this might be due to fluctuations in acids, pH and sugar/acid ratio as reported by Malundo *et al.*, (1997) in mango. The small variation in taste scores of treated pomegranate arils was for chitosan coating, which maintained taste and retained the quality until 20 days of storage. Munoz *et al.*, (2006) stated that, the influence of the chitosan on strawberries stored at 20°C for 4 days showed better maintenance of eating quality. The best organoleptic score for aril taste was recorded in C_1 (1% chitosan) and the least score was recorded in C_4 (control). The low temperature maintained the aril quality which influenced the aril taste Figure 3. Jiang and Li, (2001) reported that chitosan treated longan fruit had good eating quality even after 30 days of storage at 2°C. The interaction effect between coatings and temperatures revealed that the organoleptic score for taste of arils was maximum in C_1T_1 (1% chitosan and $4\pm1^{\circ}\text{C}$) and the minimum organoleptic score for this trait was recorded in C_4T_3 (control and room temperature).

Flavour of pomegranate arils

The organoleptic score for flavor of arils of pomegranate cv. Bhagwa as influenced by

edible coatings and storage temperatures are presented in Table.3. There was a decreasing trend in flavor score of arils throughout the storage period. The best flavor score for arils was recorded in C_1 (1% chitosan) during 20 days of storage. The maximum off flavor with least flavor score was recorded in C_4 (control). These results tally with Munoz *et al.*, (2006) and Sayak *et al.*, (2014) who reported influence of chitosan for better maintenance of eating quality in pineapple and strawberries. The best flavor score of arils was recorded in T_1 ($4\pm1^{\circ}\text{C}$). With regard to interaction effects, significant differences were observed between edible coatings and storage temperatures for all the storage days except, 4th day, which was found non-significant. The best flavor score of arils was recorded in C_1T_1 (1% chitosan and $4\pm1^{\circ}\text{C}$) during 20 days of storage. Similar findings were also reported by Doreyappa and Huddar (2001) in mango.

Based on the results obtained from the study, it is concluded that chitosan (1%) edible coating to arils of pomegranate cv. Bhagwa proved to be good in maintaining the quality of arils during storage period of twenty days. Integrating chitosan (1%) treated arils with cold storage temperature of $4\pm1^{\circ}\text{C}$, was found to be promising in maintaining several quality parameters such as total anthocyanins, β -carotene ($\mu 100\text{g}^{-1}$), and organoleptic scores.

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