

Physico-chemical and Enzymatic Changes in Cold Stored ‘Dusehri’ Mango Fruits in Response to Beeswax and Aloe Vera Gel Coatings

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Received November 21, 2020; Revised December 22, 2020; Accepted January 02, 2021

Abstract Mango is harvested in summer months and exhibits short postharvest life due to rapid biochemical changes. These ripening changes occur even at low temperature storage, though at slower rates. Hence, the effect of *Aloe vera* gel (AVG) and beeswax coatings on physico-chemical parameters and enzymatic activities in mango cv. Dusehri were evaluated for 28 days under low temperature (9-11 °C, 90-95 % RH). Fruits were coated with AVG (50 and 100 %) and beeswax (1.5 and 3.0 %) while control fruits were left uncoated. As compared to control, both AVG and beeswax coatings significantly delayed the pulp colour development in fruits throughout the storage. These coatings also reduced weight loss and maintained titratable acidity in mango fruits. At 21st day of storage, beeswax 3.0 % coated fruits retained 54.06 % higher fruit firmness and 43.18 % lower weight loss than the untreated fruits. Both edible coatings efficiently retarded the polygalacturonase (PG) and cellulase activities as compared to control fruits. In coated fruits, the enzymatic activities increased up to 21 days of storage, while the control fruits exhibited a decline in enzymatic activities after 14 days. The beeswax coatings were more effective in increasing the storage life of mango fruits under low temperature conditions.

Keywords: cellulase, fruit firmness, mango, polygalacturonase, titratable acidity

Cite This Article: Abubakar Mshora, PPS Gill, SK Jawandha, Aeshna Sinha, and Nirmaljit Kaur, “Physico-chemical and Enzymatic Changes in Cold Stored ‘Dusehri’ Mango Fruits in Response to Beeswax and *Aloe Vera* Gel Coatings.” *Journal of Food and Nutrition Research*, vol. 9, no. 1 (2021): 1-9. doi: 10.12691/jfnr-9-1-1.

1. Introduction

Mango, one of the most important tropical fruits, is known for attractive flavour, aroma and high nutritional value. In north-western India, its plantations are concentrated in the sub-mountainous strip adjacent to the *Shivalik* foothills between 32°17' N' and 75°42' E to 30°73' N and 76°78' E. The fruits of Dusehri mango, a predominant cultivar in this region, are harvested during the first fortnight of July. During this time high humidity and temperature prevails in the region leading to short shelf-life of fruits. Due to intense metabolic activity after harvest, the fruit undergoes various physiological and biochemical changes resulting in increased respiration rate, ethylene production, depolymerisation of cell wall resulting into textural losses and deterioration of fruit quality [1]. Cellulase, polygalacturonase and pectin methylesterase are the major enzymes that cause fruit softening [2].

So far different technologies have been identified to enhance the postharvest life of fruits. However, lately the growing health awareness among consumers in nutrition

and food safety, the use of edible coatings as an environment friendly technology for preserving postharvest life of mango has gained high importance [3]. Edible coatings such as *Aloe vera* gel (AVG) and beeswax forms a physical barrier on the fruit surface and regulate the selective permeability to gases such as oxygen, carbon dioxide and water vapour, thus delaying the natural physiological ripening process. AVG reduces the microorganism activity, delay the oxidative stress in fruits and decelerates the respiration rate and ripening process [4]. Studies demonstrated that AVG coatings maintained quality attributes and prolonged the storage life in apricot [5], delayed browning incidence in litchi [6], and enhanced the antioxidant activity in raspberry [7]. Similarly, beeswax is considered as important hydrophobic lipid based edible wax forming a protective layer against moisture losses and also imparts glossiness to the fruits [8]. Beeswax coatings were effective in maintaining various physico-chemical properties of fruits such as nectarines [9] and plums [10]. Elementary information is available on effect of beeswax [11] and AVG [12] on physico-chemical characteristics of mango fruits under low temperature storage conditions. However in these studies, the activities of cell wall degrading

enzymes that play an important role in softening and ripening of fruits by sequential disassembly of pectic substances and hemicellulose were not assessed [13]. The efficacy of these coatings on quality attributes and activities of cell wall degrading enzymes in commercial mango *cv.* Dusehri is yet to be ascertained. Hence, the present work was designed to elucidate the potential of AVG and beeswax coatings in maintaining the fruit texture and quality attributes in mango under low temperature storage.

2. Material and Methods

2.1. Experimental Setup

Mature green, uniform and healthy fruits of mango *cv.* Dusehri (specific gravity: 0.98 ± 0.01 ; firmness: 94.5 ± 2.5 N) were harvested from the orchard of Department of Fruit Science, Punjab Agricultural University, Ludhiana (30.89° N, 75.80° E), India. The fruit were sorted, washed with 100 ppm chlorinated water and dried in shade. Beeswax (1.5 and 3.0%) and AVG (50 and 100 %) coatings were applied on the fruit surface using a soft brush and the control fruit left uncoated. Each treatment comprised of 80 fruits in 4 replications with 20 fruits for replicate. The fruit were packed in corrugated fibre board boxes (5 % ventilation) with newspaper lining and stored at $9-11^\circ\text{C}$, 90-95 % RH [14]. Fruit were taken on the day of harvest and at 7, 14, 21 and 28 days of cold storage for analytical determination and fruit quality.

2.2. Preparation of Coating Formulations

Beeswax emulsion was prepared following the method by Efendi and Hermawati [15]. For the emulsion preparation, 1.5 g and 3.0 g of beeswax (obtained from Apiary of the Institute) was placed in a container and melted at 70°C , heated continuously to attain a temperature of $80-90^\circ\text{C}$. The molten wax was added with 2 mL of oleic acid and 6 mL of TEA (Triethanolamine) and the final volume was made to 100 mL. This formulation gives a very fine emulsion as the oleic acid dissolves in the wax phase and TEA dissolves in the water phase.

Aloe vera gel was prepared according to method of Adetunji et al. [16]. Fresh leaves of *Aloe vera* were harvested and washed with 100 ppm chlorine solution. Further, the *Aloe vera* gel matrix was separated from the outer cortex of leaves and the colourless hydroperenchyma was mixed in a blender and filtered to remove the fibres. This gel matrix was pasteurized at 70°C for 45 min. The gel was cooled and pH was set at 4.0.

2.3. Physico-chemical Analysis

2.3.1. Determination of Pulp Colour

The pulp colour of fruit was recorded at the colour coordinates of L^* , a^* and b^* in *Commission International de l'Eclairage* (CIE) units using a ColorFlex spectrophotometer (Hunter Lab ColorFlex, Hunter Associates Inc., Reston, VA, USA) with the head of

15 mm diameter to fit fruit surface. L^* value represents intensity of lightness (0= black to 100= white). The chromaticity dimension a^* indicates redness and b^* measures yellowness. For recording colour clarity, chroma values were derived from the formula $C^* = (a^{*2} + b^{*2})^{1/2}$ and range from 0 i.e least intense to 60 the most intense. While, hue angles were derived from $h^\circ = \tan^{-1}(b^*/a^*)$ and the values range from 0 to 360 (0= red, 90= yellow and 180= green).

2.3.2. β -carotene Estimation

Carotenoids were estimated in the form of β -carotene from the pulp of mango fruits using method of Ranganna [17]. Fruit pulp of 5g was taken and dried for 3 h in a hot air oven at $40-45^\circ\text{C}$. The dried sample was pulverized with 5 g of sodium sulphate in a pestle mortar. Further extraction was done with 3.0 % acetone and the final volume was made to 25 mL with petroleum ether. The colour intensity of samples was read at 452 nm in a spectrophotometer (Spectronic 20D⁺ Thermo Fischer Scientific, USA) against petroleum ether used as blank.

2.3.3. Sensory Quality

Sensory quality of mango fruits was in terms of visual appearance, texture, taste and aroma of the mango fruits. Judges six in number were selected based on their interest to taste the fruit, availability and who had previous experience of profiling mango fruit. Fruits from each replicate were randomly selected, cut into six slices and were equally distributed. Sensory evaluation was done on the basis of observations recorded from the panellists based on 9 point hedonic scale where, 9 = extremely like, 8= like very much, 7 = like moderately, 6= like slightly, 5 = acceptable (limit of marketability), 4= dislike, 3= dislike moderately (limit of unacceptability), 2= dislike very much, 1=extremely poor (off odour).

2.3.4. Weight Loss

Weight loss of fruit was determined considering the initial fresh weight and the weight of fruits at subsequent interval. The weight loss was expressed as percentage loss and calculated using the following formula:

$$\text{Weight loss \%} = \frac{\text{initial weight} - \text{Final weight}}{\text{Initial weight}} * 100$$

2.3.5. Fruit Firmness

Fruit firmness was measured by the destructive method using stand mount penetrometer (Model FT-327, USA). Selected fruits were punctured to 1cm depth using 8mm plunger at two opposite points on the fruit's equator after peeling and the values were expressed in Newton (N).

2.3.6. Soluble Solids Content and Titratable Acidity

SSC was measured using hand held digital refractometer (ATAGO, PAL -1, Japan). Pulp tissue of fruit meshed and juice obtained was filtered through cheese cloth. Two drops of filtrate was placed on the prism of refractometer the reading was recorded and expressed in $^\circ\text{Brix}$. TA was determined taking 2 mL of strained juice, adding phenolphthalein indicator and titrating this solution against 0.1 N NaOH till the colour changed to pink. TA

was expressed as a percentage of maleic acid per 100 g fresh weight.

2.4. Polygalacturonase and Cellulase Activities

2.4.1. Enzyme Extraction for Polygalacturonase and Cellulase Enzymes

Tissue for polygalacturonase and cellulase activity was extracted using the method of Malik and Singh [18] with slight modification. Fruit pulp of 0.1 g was ground in mortar with 5 mL of 0.1M sodium acetate buffer (pH 5.2) and the homogenate was centrifuged at 12000 rpm for 15 min at 4 °C. The clear supernatant was used for the enzyme estimation.

2.4.2. PG Assay

PG activity was determined by assaying the reducing end of galacturonic acid as standard. The reaction mixture comprised 1.0 mL of 0.1 M sodium acetate buffer (pH 5.2), 1.0 mL of 0.5% pectic acid and 1.0 mL of enzyme extract. This reaction mixture was incubated at 37 °C for 1 h and afterwards added with 1.0 mL of DNS reagent. The contents were boiled for 10 min and then cooled. Thereafter, the absorbance of the enzyme was recorded on spectrophotometer at 560 nm Spectronic 20D⁺ Thermo Fischer Scientific, USA).

2.4.3. Cellulase Assay

Cellulase activity was determined in terms of μg D-glucose acid g^{-1} FW min^{-1} . The reaction mixture consisted 1.0 mL of 0.1 M sodium acetate buffer (pH 5.2), 1.0 mL of 0.5 % carboxymethyl cellulose and 1.0 mL of enzyme extract. This reaction mixture was then incubated at 55°C for 1 h. After adding 1.0 mL of DNS reagent, contents were boiled for 10 min and then cooled at room temperature. The absorbance of the enzyme was recorded on a spectrophotometer at 560 nm Spectronic 20D⁺ Thermo Fischer Scientific, USA).

2.5. Statistical Analysis

The data for various parameters were analyzed by two-way analysis (coating x storage period) of variance in Completely Randomized Design (Factorial) and the significant difference between different treatments was analyzed by HSD Tukey's test at $P \leq 0.05$ level of

significance using statistical package SAS 9.3 (The SAS system for Windows, Version 9.3, SAS Institute, Cary, NC). Further, data were subjected to Pearson's correlation analysis and regression analysis to assess the nature and extent of the relationship between the colour attributes.

3. Results

3.1. Pulp Colour

Edible coatings maintained the higher brightness (L^*) of mango pulp than those of control fruits during low temperature storage (Table 1). The minimum decrease in pulp L^* value was observed with beeswax 3.0 % treatment (84.40 to 62.51), followed by its lower concentration at 1.5 % (84.40 to 61.73), whereas, the control fruits registered maximum decline in L^* value (84.40 to 60.21) up to 28 days of cold storage. Similar results were observed in AVG treatments where a slow decline in L^* value was observed in fruit coated with 100 % AVG than the uncoated fruits.

Compared to control, the edible coatings slowed down the pulp colour development, estimated in terms of a^* and b^* values (Table 1). The fruits treated with beeswax 3.0 % recorded minimum increase in a^* (69.02 %) and b^* values (39.12 %) of pulp than the uncoated fruits which registered a maximum increase in a^* (70.49 %) and b^* (41.56 %) values during the whole study. Among AVG coatings, 100 % concentration effectively delayed the increase in pulp a^* (70.12 %) and b^* (40.78 %) values in comparison to control fruits.

Irrespective of coating treatments, the pulp chroma increased with progression in storage interval (Table 1). Beeswax at 3.0 % significantly delayed the changes in pulp C^* values, while a rapid increase in C^* was observed in uncoated fruits. At the end of the storage, minimum C^* value (67.6) was obtained with beeswax 3.0 % treatment, while the highest C^* value (70.5) was observed in uncoated fruits. While fruits coated with 100 % and 50 % AVG recorded significantly lower pulp C^* values by 3.4 % and 1.3 %, respectively as compared to uncoated mangoes. A significant decline in hue angle was observed in control fruits than that of beeswax coated fruits (Table 1). Beeswax at 3.0 % recorded minimum decrease (from 77.6 to 66.6) in pulp h° and it was at par with 100 % AVG coated fruits.

Table 1. Effect of AVG and Beeswax Coating on Pulp Colour Parameters in Mango Fruits during Storage at 9-11 °C, 90-95% RH

Parameter	Coatings	Storage Intervals (Days)				
		0	7	14	21	28
L^*	AVG 50 %	84.40±0.70 ^a	73.00±1.08 ^{cd}	64.50±1.02 ^{feh}	61.20±0.82 ^{jk}	59.30±0.70 ^k
	AVG 100 %		74.40±0.88 ^c	66.90±1.08 ^{ef}	62.10±0.75 ^{hij}	60.90±0.63 ^{jk}
	Bee wax 1.5%		77.40±0.52 ^b	67.80±0.82 ^e	64.80±0.82 ^{fg}	61.70±1.1 ^{ijk}
	Bee wax 3.0%		79.70±0.96 ^b	71.50±1.72 ^d	66.70±0.78 ^{ef}	62.50±0.96 ^{ghij}
	Control		73.50±0.85 ^{cd}	64.00±1.22 ^{ghi}	60.30±0.84 ^{jk}	60.20±0.99 ^{jk}
	Control		84.40±0.70 ^a	73.00±1.08 ^{cd}	64.50±1.02 ^{feh}	61.20±0.82 ^{jk}
a^*	AVG 50 %	8.33±0.27 ^l	13.80±0.68 ^{ijk}	18.30±0.87 ^{fg}	25.80±0.17 ^{bc}	29.50±0.89 ^a
	AVG 100 %		13.00±0.07 ^{ijk}	17.60±0.08 ^{fg}	24.10±0.07 ^{cd}	27.80±0.10 ^{ab}
	Bee wax 1.5%		12.30±0.04 ^{jk}	16.80±0.04 ^{gh}	23.40±0.04 ^{cd}	27.30±0.04 ^{ab}
	Bee wax 3.0%		11.10±0.09 ^k	15.70±0.08 ^{ghi}	22.10±0.07 ^{de}	26.90±0.07 ^{ab}
	Control		14.60±1.91 ^{hij}	19.60±1.55 ^{ef}	26.00±2.08 ^{bc}	28.20±3.29 ^{ab}
	Control		8.33±0.27 ^l	13.80±0.68 ^{ijk}	18.30±0.87 ^{fg}	25.80±0.17 ^{bc}

Parameter	Coatings	Storage Intervals (Days)				
		0	7	14	21	28
<i>b</i> *	AVG 50 %	37.80±0.36 ^l	47.10±0.84 ^j	55.80±0.89 ^f	62.50±0.04 ^{bc}	64.90±0.83 ^a
	AVG 100 %		46.20±0.40 ^j	53.20±0.69 ^e	62.40±0.04 ^{bc}	63.80±0.64 ^{ab}
	Bee wax 1.5%		45.70±0.63 ^j	54.10±0.04 ^e	60.10±0.07 ^d	63.10±0.80 ^{bc}
	Bee wax 3.0%		42.40±0.92 ^k	50.60±0.04 ^b	58.70±0.04 ^{de}	62.10±0.04 ^c
	Control		48.80±0.88 ⁱ	57.70±0.10 ^e	62.80±0.99 ^{bc}	64.7±0.14 ^a
<i>C</i> *	AVG 50 %	38.72±01.13 ^l	49.11±0.58 ^{ik}	58.72±0.41 ^e	67.62±0.68 ^{cd}	71.28±0.84 ^a
	AVG 100 %		47.98±0.60 ^k	56.05±0.50 ^b	66.81±0.43 ^d	69.67±0.66 ^{abc}
	Bee wax 1.5%		47.36±0.94 ^k	56.67±0.10 ^{gh}	64.45±0.99 ^e	68.77±1.21 ^{bcd}
	Bee wax 3.0%		47.36±0.94 ^k	52.94±0.74 ⁱ	62.72±1.41 ^{ef}	67.68±0.80 ^{cd}
	Control		50.95±0.84 ^{ij}	60.97±0.95 ^f	67.77±0.71 ^{cd}	70.59±1.11 ^{ab}
<i>h</i> ^o	AVG 50 %	77.60±0.27 ^a	73.70±0.49 ^{de}	71.80±0.60 ^{gh}	67.60±0.17 ^j	65.60±0.49 ^m
	AVG 100 %		74.30±0.55 ^{cd}	71.70±0.35 ^h	69.00±0.40 ⁱ	66.40±0.34 ^{lm}
	Bee wax 1.5%		74.90±0.44 ^{bc}	72.70±0.41 ^{fg}	68.70±0.13 ⁱ	66.60±0.29 ^{kl}
	Bee wax 3.0%		75.40±0.32 ^b	72.80±0.50 ^{ef}	69.300.43± ^j	66.60±0.18 ^{kl}
	Control		73.30±0.48 ^{ef}	71.20±0.47 ^h	67.80±0.37 ^{jk}	66.40±0.54 ^{lm}

Mean followed by the same letter is not significantly different within the columns according to an LSD test ($P < 0.05$). $n=4$ replicates, \pm SE.

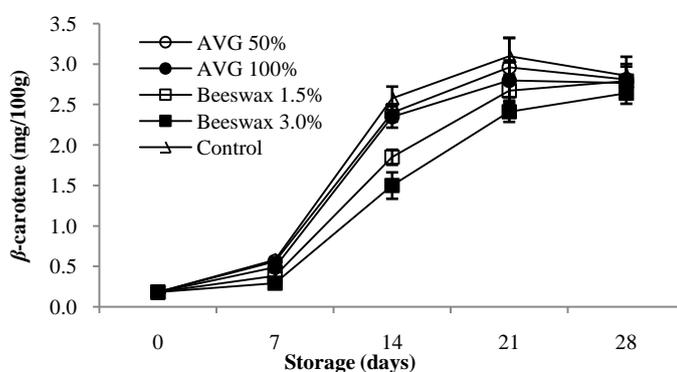


Figure 1. Effect of AVG and beeswax coatings on β -carotene content of mango fruits during low temperature storage. Vertical bars represent \pm standard error of mean of 4 replicates

3.2. β -carotene

There was progressive increase in β -carotene content in both coated as well as uncoated mangoes during cold storage (Figure 1). However, the maximum increase in β -carotene content was recorded in uncoated fruits than edible coating treatments. Fruits treated with AVG 100 % registered 7.6 % lower β -carotene pigment than the uncoated fruits. Throughout the storage period the maximum delay in β -carotene development was observed in 3.0 % beeswax coated mangoes which exhibited a minimum (0.18 to 2.64 mg/100g) variation in β -carotene level. The control fruits exhibited a maximum increase (0.18 to 2.86 mg/100g) in β -carotene up to 28 days of low temperature storage. Overall the increase in β -carotene

content in control fruits was 32.8 % higher as compared to fruits coated with 3.0 % beeswax treatment.

3.3. Sensory Quality

Sensory quality in mango varied significantly with the progression of storage period (Figure 2). Untreated and AVG 50 % coated mangoes recorded maximum sensory score (8.29 and 8.07, respectively) on 14th day of storage while the rest of coatings (AVG 100 % and beeswax 1.5 & 3.0 %) registered maximum sensory scores on 21st day followed by a decline. The rate of sensory quality decline in uncoated fruits was higher due to rapid loss in taste, texture and aroma. At the end of storage, the maximum sensory score was observed in fruits coated with beeswax 1.5 %.

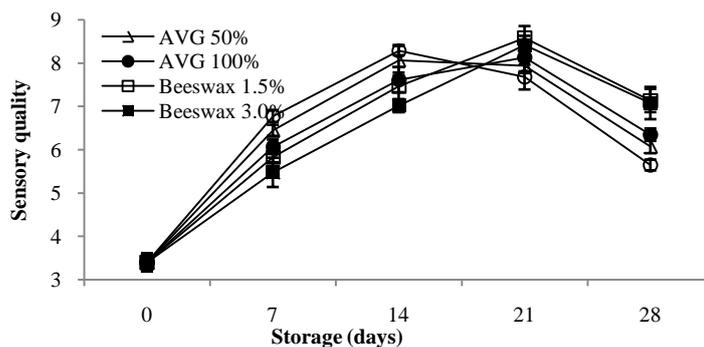


Figure 2. Effect of AVG and beeswax coatings on sensory quality of mango fruits during low temperature storage. Vertical bars represent \pm standard error of mean of 4 replicates

3.4. Weight Loss

During low temperature storage, a gradual loss in weight was observed in all the coated as well as uncoated fruits (Figure 3). The weight loss in control fruit was significantly higher at initial storage period than that of AVG and beeswax coated mangoes. On 21 days of storage, beeswax 3.0 % coated mangoes recorded only 5.0 % weight loss which is less than critical weight loss while in controls this loss was 8.8 %. At the end of the storage period, control fruits recorded maximum weight loss (11.50 %).

3.5. Fruit Firmness

The fruit firmness declined in all the treatments during the storage period (Figure 4). At initial sampling, rate of fruit softening was rapid in uncoated fruits than those in wax coated fruits. From harvest to 21 days of storage, the 100 % AVG coated and control fruits recorded 77.58 % and 81.94 % decline in fruit firmness. However at the end of storage, beeswax 3.0 % coated fruits retained maximum firmness (7.88 N) followed by 1.5% beeswax coating

(6.45N), while uncoated fruit recorded minimum firmness (4.17 N).

3.6. Soluble Solids Content and Titratable Acidity

The SSC in coated as well as uncoated fruits increased progressively during cold storage (Figure 5A). However, the rate of increase in SSC was higher in uncoated fruits as compared to fruits coated with AVG or beeswax. From harvest to the end of the storage, the maximum rate of increase in SSC was registered in uncoated fruits (57.02 %) followed by 50% AVG (55.47 %) coatings treatment. While the minimum increase in SSC values was recorded in beeswax 3.0 % and 1.5 % (50.05 % and 51.17 %, respectively) coatings.

A significant decline in titratable acidity was observed with in all the fruits irrespective of the coating applied (Figure 5B). The rate of decline in acidity was sharp up to 21 days of storage followed by a gradual decline. At the end of storage, maximum titratable acidity was recorded in beeswax 3.0% (0.34%) coated fruits and minimum in uncoated fruits (0.27%).

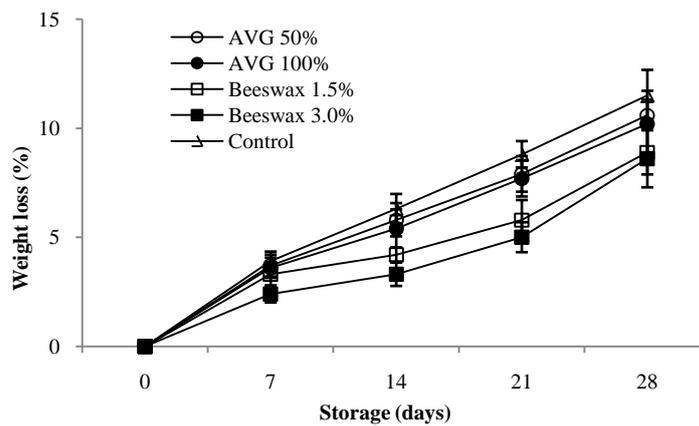


Figure 3. Effect of AVG and beeswax coatings on weight loss of mango fruits during low temperature storage. Vertical bars represent \pm standard error of mean of 4 replicates

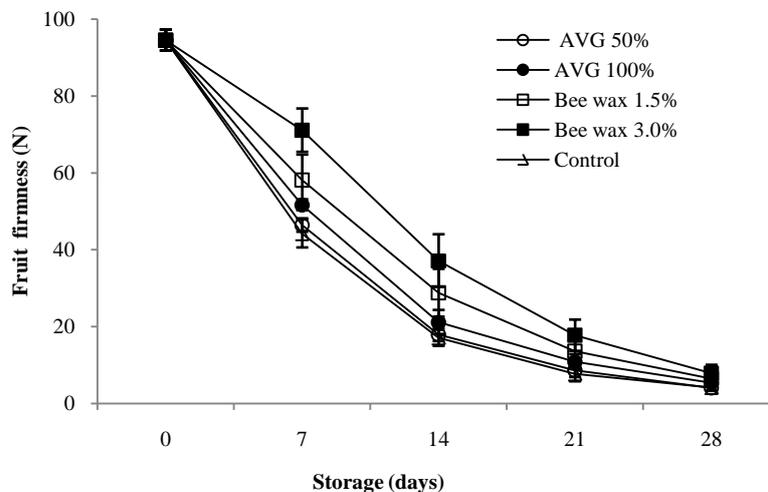


Figure 4. Effect of AVG and beeswax coatings on fruit firmness of mango fruits during low temperature storage. Vertical bars represent \pm standard error of mean of 4 replicates

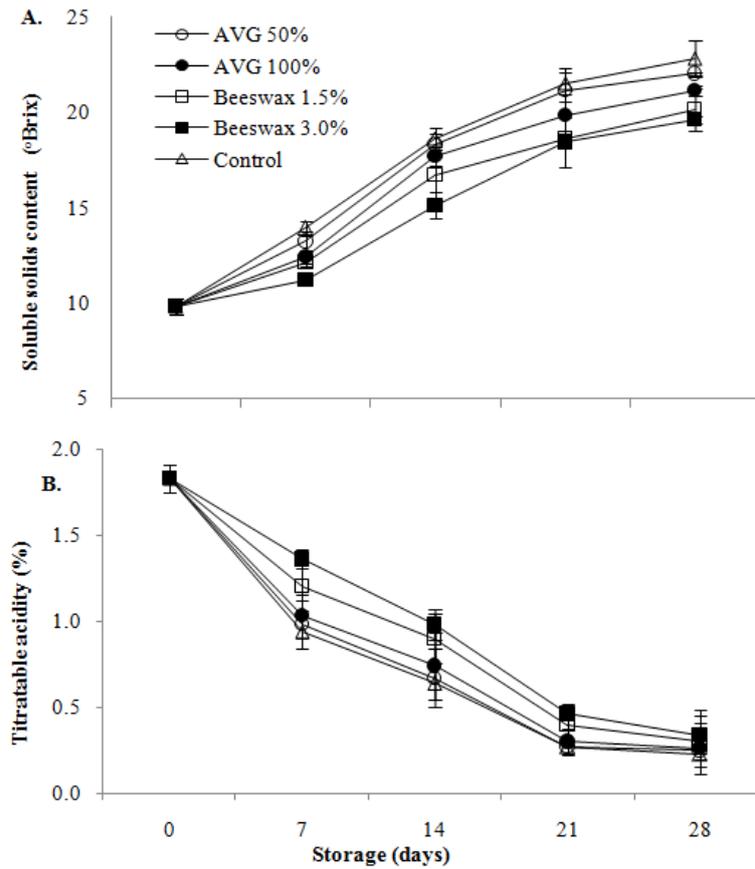


Figure 5. Effect of AVG and beeswax coatings on (A.) soluble solids contents, and (B.) titratable acidity of mango fruits during low temperature storage. Vertical bars represent \pm standard error of mean of 4 replicates

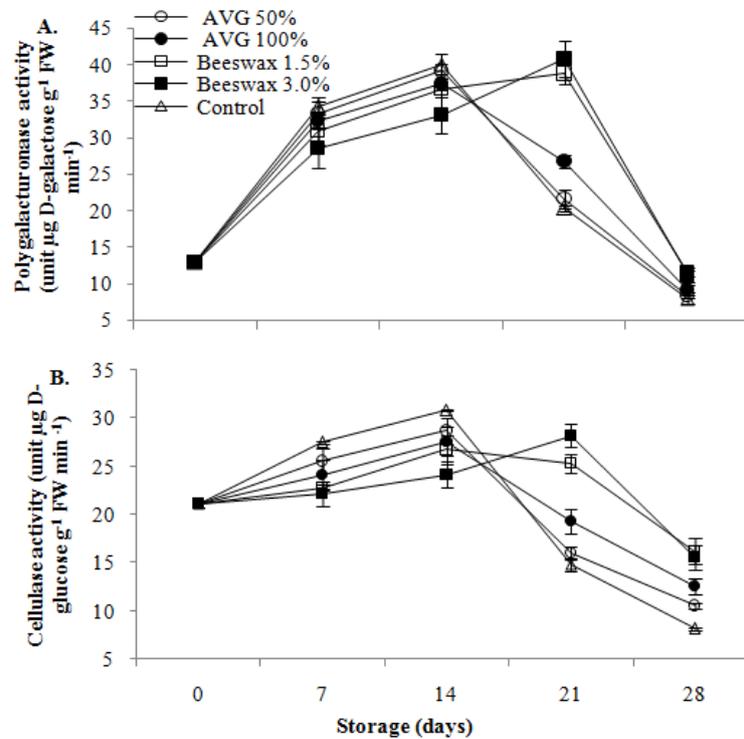


Figure 6. Effect of AVG and beeswax coatings on (A.) Polygalactouronase, and (B.) Cellulase enzyme activities of mango fruits during low temperature storage. Vertical bars represent \pm Standard error of mean of 4 replicates

3.7. PG and Cellulase Enzyme Activity

In the present study, both PG and cellulase enzyme activities showed similar trend depending upon the storage

interval (Figure 6A and 6B). The PG activity in uncoated samples increased at a faster pace as compared to wax coated samples. At the time of fruit harvest, the PG activity in mango was $12.9 \mu\text{g D-galacturonic acid g}^{-1} \text{FW min}^{-1}$ and it

reached to maximum ($40.0 \mu\text{g D-galacturonic acid g}^{-1} \text{FW min}^{-1}$) level after 14 days of the cold storage in control fruits, thereafter, it declined rapidly. However, both AVG and beeswax coatings retarded the increase in PG activity in fruits during low temperature storage. In beeswax coatings, the increase in activities of PG enzyme was recorded till 21 days of the storage period, followed by a decline, but at a slower pace. At the end of the storage, the maximum ($11.4 \mu\text{g D-galacturonic acid g}^{-1} \text{FW min}^{-1}$) PG activity was observed in 1.5 % beeswax coated fruits and it was at par with 3.0 % beeswax coatings, while minimum PG ($8.0 \mu\text{g D-galacturonic acid g}^{-1} \text{FW min}^{-1}$) activity was measured in uncoated samples. The results indicated that beeswax (3.0 % and 1.5 %) coatings were capable of retaining 30.5 % and 28.5 % higher enzymatic activity as compared to uncoated fruits after 28 days of cold storage period.

Similarly, the cellulase enzyme activity in mango increased linearly up to 21 days in beeswax coated fruits. Both beeswax and AVG coatings significantly ($p < 0.05$) restricted the cellulase activities in fruits. Maximum cellulase activity ($30.8 \mu\text{g D-glucose g}^{-1} \text{FW min}^{-1}$) in uncoated fruit was recorded at earlier sampling stage i.e. 14 days as compared to beeswax coated fruits (1.5 % and 3.0 %), which measured maximum (25.3 and $28.1 \mu\text{g D-glucose g}^{-1} \text{FW min}^{-1}$) enzyme activity after 21 days. At the end of storage period, beeswax coatings (1.5 % and 3.0 %) retained 47.8 % and 49.8 % higher enzymatic activities respectively, as compared to uncoated fruits.

4. Discussion

Effect of beeswax and AVG coating on quality and enzymatic changes in Dusehri mango was assessed during low temperature storage. Results exhibited that the fruits coated with beeswax significantly slowed down the degeneration of yellow colour of pulp and retained a lighter yellow pulp colour as compared to AVG coatings and control fruits. The decrease in hue angle in beeswax coated fruits was comparatively less which may be attributed to the slow pulp colour changes as compared to other treatments, while higher chroma values were recorded in beeswax coated fruits. This is evidence that beeswax coatings delayed the climacteric peak in fruits. Colour change in fruit is mainly due to the degradation of chlorophyll caused by various enzymatic activities as well as accumulation of carotenoids in response to the climacteric rise in respiration rate and ethylene production [19]. Similar findings were reported by Moalemiyan et al. [20] who observed delay in colour developed in mango fruits by edible coatings. The wax coated fruits also recorded lower chroma and hue values that indicate the delay in senescence process. Carotenoids are the most crucial pigments that define the qualitative characteristic of mango fruit and increase with the progress of the fruit ripening. β -carotene increased in all the waxed as well as non-waxed fruits, but their increase was recorded at a slower pace in 3.0 % beeswax coated fruits. This delay in the ripening process in beeswax coated fruits may be due to the modification of internal atmospheric conditions which suppress the enzymatic activities and reduces the chlorophyll degradation and carotenoid biosynthesis. The

similar inhibitory effect of wax treatments on carotenoid synthesis was reported in mango [21].

Beeswax coatings effectively maintained the mango sensory attributes at acceptable levels up to 21 days as compared to uncoated fruits where the visual quality, taste and aroma started to decline after 14 days of cold storage. Maintenance of sensory quality attributes in coated fruits might be due to the significant delay in ripening and senescence process which further prevents the fruits from rapid postharvest deterioration. The observations are in line with the findings of Anjum et al. [22] in guava fruits.

Weight loss in fruits is mainly due to surface transpiration and is a major cause of shrivelling in fruits. Fruits coated with 3.0 % beeswax recorded minimum weight loss as compared to AVG and uncoated fruits as beeswaxes have formed an effective barrier on the fruit surface, thus limiting the water loss and protecting fruits against dehydration losses. Results are in agreement to previous studies where lower weight loss was observed in beeswax coated mango fruits [3].

Fruit texture is an important characteristic of fresh horticultural produce. During storage, quality in mango is characterized by textural softening. The present study showed a decline in fruit firmness throughout the 28 days of low temperature storage. This loss in textural quality in fruits is mainly due to the change in the composition of the cell wall. Middle lamella mainly comprises of pectin substances which maintain the cohesiveness of the fruit and structural integrity in the cell wall. But with the progression of ripening, these pectic polymers get depolymerized and induces solubilisation of pectic polysaccharides in response of the activation of various cell wall degrading enzymes such as cellulase, polygalacturonase, pectin methylesterase and pectate lyase [2]. The control fruit became highly soft after 21 days of storage and lost consumer acceptability while beeswax 3.0 % treated fruits retained acceptable firmness of 17.34 N. These results can be correlated with the findings of Thai et al. [23] in mango where maximum firmness was retained in the wax coated fruits. The coatings create a modified atmospheric condition around the fruit surface, thus reducing any alteration in pectic substances and cell wall degrading enzymatic activity [24].

A progressive increase in SSC was observed in all the coated as well as uncoated fruits in the current study which may be attributed to the hydrolysis of starch and complex carbohydrate into simple sugars. Slow increase of SSC in 3.0% beeswax coatings is due to the formation of semi-permeable film around the fruit which modifies the internal atmosphere of fruit by reducing the oxygen level [25] and thus lowering the respiration rate which ultimately reduces the consumption of metabolites resulting into low SSC and slower breakdown of carbohydrates into sugars [26]. Similar results were reported in mango [3] fruits where higher SSC were recorded in uncoated fruits as compared to coated fruits.

The results in this study showed that the titratable acidity declined throughout the storage period in coated and uncoated fruits which may be due to utilization of organic acids during respiration for enzymatic reactions [26]. Due to a lower rate of respiration in fruits coated with AVG and beeswax the lesser organic acid were utilized, contributing to higher titratable acidity at the end

of storage period. In our study, the highest titratable acidity was recorded in 3.0% beeswax coated fruits throughout the storage period indicating preservation of organic acids due to reduced respiration rate. Results are in line with the previous findings in guava fruits [22] where the decline in titratable acidity in coated fruits was effectively inhibited in comparison to the control fruits.

Polygalactouronase, PME, cellulase are the major cell wall degrading enzymes responsible for the breakdown of pectic substances, celluloses and hemicellulose in the middle lamella resulting into reduction of cohesive forces binding cells together, weakening of cell wall and fruit softening [27]. The enzymatic activity of polygalacturonase catalyzes the depolymerisation and hydrolytic cleavage of de-esterified polygalacturonoid chain [28]. Beeswax coatings significantly delayed the enzymatic activity in mango which implies the slowdown in the degradation process of insoluble proto-pectins into the soluble pectic acid and pectins. In our studies the PG activities were lower in 3.0% beeswax coated fruits than the AVG coatings and control. In uncoated fruits, PG activity increased approximately 2 folds up to 14 days of storage period than 3.0 % beeswax coated fruits where this increase was only 1.4 fold. A similar finding was reported in wax coated 'Manila' mango where PG activity was delayed during storage [29].

The cellulase enzymes cleave the β -1, 4 glucosidic bonds of cellulose in the cell wall [30] and its activity is directly correlated with fruit softening. Both AVG and beeswax coatings inhibited the increase in enzymatic activities, while 3.0 % beeswax coated fruits maintained highest enzymatic activities at the end of storage as

compared to uncoated fruits which signify that waxed fruits are capable of alleviating the degradation of cell wall components and accumulated higher substrate levels.

The results from linear regression between different colour attributes of mango pulp are shown in Figure 7. High significant positive correlation was observed between colorimetric parameter a^* value & β -carotene ($R^2 = 0.811$) and b^* value & β -carotene ($R^2 = 0.890$). The increase in a^* and b^* values of the pulp can be directly correlated with an increase in β -carotene levels. Carotenoid pigments impart yellow, red or orange colour to the fruit and with an increase in β -carotene level, there is an increase in yellowness or redness of the fruit pulp colour. Mango pulp colour is not only important from consumers perception of quality but also an important recognition for nutritional content i.e. vitamin A.

5. Conclusion

Among two coatings, beeswax was more effective in maintaining the fruit texture and quality under low temperature storage. It retarded the activities of PG and cellulase enzymes in mango thus demonstrating its protective role in the maintenance of cell wall integrity. Furthermore, a high significant positive correlation was obtained between the colorimetric parameter a^* and b^* values & β -carotene. Overall, beeswax 3.0 % coating extended the storage life of mango *cv.* Dusehri during cold storage up to one week (upto 21 days) as compared to untreated fruits.

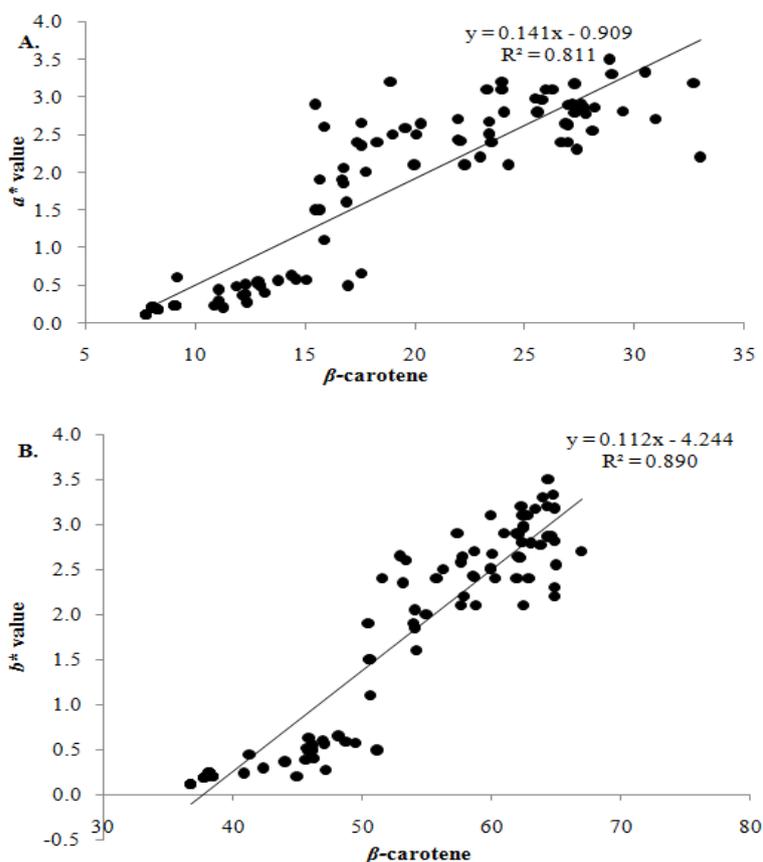


Figure 7. Linear regression relationship between different colour attributes and β -carotene of mango fruits in relation to different edible coating treatments. Significant at $P < 0.01$.

Acknowledgments

The authors are thankful to Punjab Agricultural University, Ludhiana, India for providing the necessary research facilities.

Conflicts of Interest

The author declares no conflict of interest.

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