

Influence of chitosan coating and storage temperatures on postharvest quality of guava

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ABSTRACT

The effect of chitosan coating on postharvest quality of guava cv. Allahabad Safeda fruits stored at room temperature, 12° and 8°C was investigated. The fruits were either treated with chitosan (1 and 2%), acetic acid 1% or untreated and various quality attributes were studied at the end of storage period. Among the different treatments and temperatures, the chitosan 1% treated fruits stored at 12°C had shown higher firmness, TSS, titratable acidity and maintained greenness with a slow increase in yellow colour by the end of storage life. Also, the total antioxidant capacity, total phenols and total flavonoids of these fruits were well maintained by the time they are full ripe. Chitosan 1% and storage temperature of 12°C can be used for extending the storage life of guava upto 21 days with least deterioration in postharvest quality.

Key words: Antioxidants, acetic acid, edible coating, post-harvest.

Chitosan is one among various per-treatments extensively used nowadays for postharvest treatment of fruits. Chitosan has a chemical structure close to that of cellulose, has long been known to protect perishable produce from deterioration by reducing transpiration, respiration and maintaining the textural quality. Chitosan (poly β -(1-4)N-acetyld-glucosamine), a deacetylated form of chitin, is a natural compound obtained from crustacean shells (crabs, shrimp and cray fishes) either by chemical or microbiological processes and can be produced by some fungi too. India has a vast cost line (7,517 km) owing to its high capability of harvesting crustaceans from the sea, producing a large quantity of crustacean shell waste. Chitosan has been successfully tried and recommended for enhancing the shelf life of several fruits such as litchi, mango and guava.

Although, there has been some research into the use of chitosan as a preservative coating in some fruits, but very few published information on the use of chitosan coatings and different storage temperatures on postharvest quality of guava is available. In the present investigation, an attempt has been made to know the interaction effect of chitosan coating and storage temperatures on physio-chemical characteristics of guava after harvest.

Physiologically mature green fruits of guava cv. Allahabad Safeda were harvested manually from nearby orchards of IIHR, Bengaluru, during early hours (8.00-9.00 am). The fruits were transported to the laboratory in plastic crates, where they were sorted out to remove immature, misshaped, bruised, diseased and insect-infested fruits if any. These fruits were graded as floaters (≤ 1) and sinkers (>1) based on their specific gravity among which floaters (mature) were taken for the experiment. The fruits were then washed, air-dried and treated with chitosan. Acetic acid (1%) was used to dissolve and prepare 1% (C_{a}) and 2% (C₂) chitosan solutions. The solution was stirred for sufficient time using mechanical stirrer for complete dissolution of chitosan. Fruits were dipped in these chitosan solutions for 2 min., drained and surface dried. Acetic acid (1%) (C₁) was also taken as one of the treatment since the same was used in dissolving and preparing the chitosan solutions and un-treated as control (C_0). These fruits were then packed in non-ventilated CFB boxes, each with 20 fruits and stored at room temperature (T₁) (28-32°C and 32-41% RH), 12°C (T₂) and 8°C (T₃).

Fruit firmness, as the force required to puncture the fruit, was measured using an Instron-Universal testing machine (Model 4201, USA) and expressed as kg/cm². Quality components like total soluble solids (TSS) and titratable acidity were estimated according to standard AOAC methods (Ranganna, 8). Total antioxidants were estimated using FRAP (Ferric Reducing Antioxidant Potential) method as described by Benzie and Strain (3). Total phenols were estimated according to the procedure given by Singleton and Rossi (9). Total flavonoids in the methanol extract were determined as per Chun *et al.*

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(4). The surface colour of the fruit was measured with colour difference meter (Model: Color Reader, CR-10, Konica Minolta, Japan) in terms of (*L*, *a*, *b*) values. The experiment consisted of 12 treatments, *i.e.*, three storage conditions (including room temperature) and four pre-treatments (including control) under each storage condition. These treatments were replicated thrice. The observations recorded under each parameter at the end of storage life (7th at RT, 21st at 12°C and 35th at 8°C) were statistically analysed using factorial completely randomised design.

It is quite apparent from the table 1 that at the final stage of ripening, the higher firmness was retained at room temperature (RT) followed by 12°C irrespective of the pre-treatment given. Among the pre-treatments, chitosan 2% (5.39 kg/cm²) treated fruits retained significantly higher firmness followed by chitosan (1%) treated fruits than acetic acid (1%) treated fruits and control. The interaction effect revealed that T₁C₃ has the highest firmness followed by T₂C₂ and the least firmness was observed in T₂C₁. Softening of guava fruit was remarkably delayed with chitosan (1 and 2%) treatment during storage at all the temperatures (Table 1). The increase in pectin solubilisation and disruption of the xyloglucan-cellulose micro fibril networks of guava fruit moderated by an increase in the activities of exo-polygalacturonase (PG), pectin methylesterase, $\beta(1\rightarrow 4)$ -glucanase and β -galactosidase has been proposed to be associated with the rapid softening of fruit (Ali et al., 2). The maintenance of firmness in the fruits treated with 1 and 2% chitosan coatings could be due to the covering of the cuticle and lenticels and their higher antifungal activity thereby reducing infection, respiration and other ripening processes during storage. The retardation of fruit softening in response to chitosan treatment has been reported in many fruits such as papaya (Al Eryani et al., 1).

It was evident from the table 1 that, irrespective of the pre-treatments, at the fully ripe stage the titratable acidity was significantly retained at 12°C followed by 8°C. Among the pre-treatments, chitosan (2%) treated fruits retained significantly higher titratable acidity (0.62%), which is on par with chitosan (1%) treated fruits than acetic acid (1%) treated fruits and control. The interaction effect revealed that T₂C₂ (0.79%) shown higher titratable acidity which is on par with T₂C₃ and lowest was observed in remaining all other interaction effects which are on par with each other. The decrease in acidity during storage may be attributed to an increase in malic enzyme and pyruvate decarboxylation reaction during the climacteric period in apples. The fruits treated with Chitosan maintained higher acidity during storage probably due to delay in the ripening process (Table 1). Al Eryani et al. (1) observed lower acidity loss during storage in papaya.

A perusal of data in Table 1, showed that with respect to storage temperatures guava fruits stored at 8°C had shown highest TSS (12.85°Brix) followed by the fruits stored at 12°C which was on par with RT. Among the pre-treatments, C₂ treated fruits had the highest amount of TSS followed by C₃ treated fruits. The interaction of the two factors showed T₃C₃ and T_3C_2 had a higher content of TSS, followed by T_3C_0 , T_3C_1 and T_3C_1 while lowest TSS content was found in T_1C_0 (10.13°Brix) and T_1C_1 . The fruit treated with chitosan registered maximum TSS content, while the lowest average TSS was recorded by control and acetic acid 1% treated fruits. The increase in TSS/ sugars during storage/ripening may be possibly due to hydrolysis of starch into sugars and on complete hydrolysis of starch, no further increase occurs and subsequently a decline in these parameters is predictable as they along with other organic acids are primary substrate for respiration. Chitosan delayed

Treatment	Firmness At harvest (34.94 kg/ cm²)				Titratable acidity At harvest (1.25%)				TSS At harvest (11.10°B)			
	T ₁	T ₂	T ₃	Mean	T ₁	T ₂	T ₃	Mean	T ₁	T ₂	T ₃	Mean
C ₀	3.56	2.41	2.41	2.79	0.50	0.62	0.48	0.53	10.13	11.12	12.37	11.21
C ₁	3.81	3.19	2.26	3.09	0.48	0.55	0.49	0.51	10.63	11.47	12.23	11.44
C ₂	5.97	6.71	2.67	5.12	0.50	0.79	0.55	0.61	12.27	11.47	13.33	12.36
C ₃	8.50	5.02	2.65	5.39	0.50	0.73	0.62	0.62	10.97	11.27	13.47	11.90
Mean	5.46	4.33	2.50		0.50	0.67	0.54		11.00	11.33	12.85	
	Т	С	T×C		Т	С	T×C		Т	С	T×C	
CD at 1%	0.03	0.03	0.06		0.03	0.04	0.07		0.46	0.53	0.92	

Table 1. Effect of Chitosan coatings on firmness, titratable acidity and TSS in guava cv. Allahabad Safeda fruits at the end of storage.

 $C_0 = Control, C_1 = Acetic acid (1\%), C_2 = Chitosan (1\%), C_3 = Chitosan (2\%), T_1 = Room temperature, T_2 = 12°C, T_3 = 8°C.$

metabolic activity of fruits during storage due to reduced respiration rate with consequent delay in ripening as shown in Table 1. Keqian *et al.* (6) reported the delayed metabolic activity and respiration rate in the chitosan treated guava fruits.

The interaction effect showed that T₂C₃, which was on par with T₂C₂ is the best treatment combination to achieve higher total anti-oxidant capacity and the least total anti-oxidant capacity (177.41 mg ascorbic acid eqv. /100 g) was found under the treatment combination T_3C_2 . The changes in the total antioxidant capacity of the guava fruits are shown in Table 2. The total antioxidant capacity of the guava fruits increased at ripe stage compared to harvest. However, the results obtained were contradictory to Neeraj et al. (7) who reported the decline of antioxidants in fruits during their ripening. Among the storage temperatures, guava fruits stored at 12°C had shown higher amounts of total antioxidant capacity followed by those stored at RT and lowest at 8°C. The reduced antioxidant activity at 8°C might be due to more utilization of the antioxidants to neutralize the free radicals produced by the low-temperature stress (chilling injury). Among the pre-treatments highest anti-oxidant capacity was noticed in chitosan (2%) treated fruits at the full ripe stage, while the acetic acid (1%) treated fruits had the lowest antioxidant capacity. The chitosan treated guava fruits stored at 12°C had shown significantly higher total antioxidant capacity than other treatments, which shows the loss in antioxidants at RT was mainly due to increased respiration rate in this temperature compared to 12°C.

The interaction studies reveal that T_2C_3 (650.93 mg gallic acid eqv./100 g) had significantly higher total phenols followed by T_1C_3 , while lowest total phenols were recorded in T_3C_2 , which is on par with

 T_3C_3 . Total phenols were high in fruits stored at RT, followed by 12°C, whereas the fruits stored at 8°C have shown reduced total phenol content (Table 2). Similar observations were recorded in guava fruits (Hussain *et al.*, 5) stored at 10 or 20°C for 3 weeks and found that total phenols decreased significantly as storage period and temperature increased. Among pre-treatments chitosan treated fruits had highest total phenols content followed by control fruits whereas acetic acid (1%) treated fruits had the lowest phenol content. This might be due to, the reduction of ripening rate and respiration, which lead to maintained phenols in the post-storage ripening period.

Flavonoids are one of the major compounds contributing to the total antioxidant capacity of the fruits and vegetables. In nature, very large quantities of flavonoids are present in the form of catechins. The interaction of storage temperatures with pretreatments showed T_1C_0 (395.09 mg catechin eqv. /100 g) as the best treatment followed by T_2C_2 , which is on par with T_1C_2 , while the poor performing treatment was T_3C_3 . In this experiment, there were high total flavonoids at full ripe stage compared to the day of harvest and the total flavonoids were highest at 12°C followed by RT and lowest at 8°C (Table 2). Among the pre-treatments the highest flavanoid content in control might be due to a lesser number of days taken by them to reach a full ripe stage, which is followed by chitosan (1%) and chitosan (2%). The low flavonoids were found in acetic acid treated fruits.

At full ripe stage, irrespective of the storage period and pre-treatment given (Table 3), the L-values were significantly high at 12°C (68.86) followed by RT and were significantly low at 8°C (61.22). Among the three pre-treatments, control treated fruits had significantly higher L-values than acetic acid (1%)

Treatment	(mg as	scorbic a	dant cap cid eqv./ st (122.90	100 g)		gallic aci	phenols d eqv. / st (690.8	•	Total flavonoids (mg catechin eqv. / 100 g) At harvest (98.54)			
	T ₁	T ₂	T ₃	Mean	T ₁	T ₂	T ₃	Mean	T ₁	T ₂	T ₃	Mean
C ₀	235.51	233.16	197.12	221.93	625.48	607.55	452.14	561.72	395.09	311.17	234.88	313.71
C ₁	186.75	241.95	192.18	206.96	604.01	594.41	455.09	551.17	197.13	301.74	142.48	213.78
C ₂	232.56	274.91	177.41	228.30	618.00	625.36	443.50	562.29	322.14	330.86	208.76	287.25
C ₃	245.25	279.34	234.78	253.12	634.87	650.93	445.62	577.14	258.86	295.54	183.18	245.86
Mean	225.02	257.34	200.37		620.59	619.56	449.09		293.31	309.83	192.33	
	Т	С	T×C		Т	С	T×C		т	С	T×C	
CD at 1%	5.11	5.98	10.25		4.39	5.07	8.79		5.11	5.90	10.22	

Table 2. Effect of Chitosan coating on total antioxidant capacity, total phenols and total flavonoids in guava cv. Allahabad Safeda fruits at the end of storage.

 C_0 = Control, C_1 = Acetic acid (1%), C_2 = Chitosan (1%), C_3 = Chitosan (2%), T_1 = Room temperature, T_2 = 12°C, T_3 – 8°C.

L At harvest (51.22)								b			
				At harvest (-14.48)				At harvest (35.58)			
T ₁	Τ ₂	T ₃	Mean	T ₁	T_2	Τ ₃	Mean	T ₁	Τ ₂	T ₃	Mean
66.73	72.12	65.14	67.99	5.16	5.23	3.80	4.73	44.84	44.13	41.87	43.61
65.89	66.37	66.26	66.17	6.65	8.96	1.43	5.68	42.93	42.34	41.88	42.38
64.54	68.21	58.85	63.87	0.45	3.55	6.62	3.54	42.90	46.94	39.70	43.18
59.43	68.73	54.63	60.93	-5.06	4.26	2.52	0.57	39.63	46.32	35.95	40.63
64.15	68.86	61.22		1.80	5.50	3.59		42.58	44.93	39.85	
Т	С	T×C		Т	С	T×C		Т	С	T×C	
0.09	0.10	0.18		0.08	0.10	0.17		0.06	0.07	0.12	
	T ₁ 66.73 65.89 64.54 59.43 64.15 T	$\begin{array}{c ccc} \hline T_1 & T_2 \\ \hline 66.73 & 72.12 \\ \hline 65.89 & 66.37 \\ \hline 64.54 & 68.21 \\ \hline 59.43 & 68.73 \\ \hline 64.15 & 68.86 \\ \hline T & C \\ \end{array}$	$\begin{array}{c ccccc} T_1 & T_2 & T_3 \\ \hline 66.73 & 72.12 & 65.14 \\ \hline 65.89 & 66.37 & 66.26 \\ \hline 64.54 & 68.21 & 58.85 \\ \hline 59.43 & 68.73 & 54.63 \\ \hline 64.15 & 68.86 & 61.22 \\ \hline T & C & T \times C \\ \end{array}$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	At harvest (51.22) At harvest T ₁ T ₂ T ₃ Mean T ₁ T ₂ 66.73 72.12 65.14 67.99 5.16 5.23 65.89 66.37 66.26 66.17 6.65 8.96 64.54 68.21 58.85 63.87 0.45 3.55 59.43 68.73 54.63 60.93 -5.06 4.26 64.15 68.86 61.22 1.80 5.50 T C T×C T C	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$\begin{tabular}{ c c c c c c c } \hline At harvest (51.22) & At harvest (-14.48) \\ \hline T_1 & T_2 & T_3 & Mean & \hline T_1 & T_2 & T_3 & Mean \\ \hline 66.73 & 72.12 & 65.14 & 67.99 & 5.16 & 5.23 & 3.80 & 4.73 \\ \hline 65.89 & 66.37 & 66.26 & 66.17 & 6.65 & 8.96 & 1.43 & 5.68 \\ \hline 64.54 & 68.21 & 58.85 & 63.87 & 0.45 & 3.55 & 6.62 & 3.54 \\ \hline 59.43 & 68.73 & 54.63 & 60.93 & -5.06 & 4.26 & 2.52 & 0.57 \\ \hline 64.15 & 68.86 & 61.22 & 1.80 & 5.50 & 3.59 \\ \hline T & C & T \times C & T & C & T \times C \\ \hline \end{tabular}$	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $

Table 3. Effect of Chitosan coating on surface colour (*L*, *a* and *b* values) in guava cv. Allahabad Safeda fruits at the end of storage.

C₀ = Control, C₁ = Acetic acid (1%), C₂ = Chitosan (1%), C₃ = Chitosan (2%), T₁ = Room temperature, T₂ = 12°C, T₃ = 8°C.

treated fruits followed by chitosan (1%) and chitosan (2%). The interaction effect between the temperature and the pre-treatments indicate that T_2C_0 and T_2C_3 were the best possible combinations followed by T_2C_2 while T_3C_3 was the poorest performing combination.

It is evident from the data in table 3 that, with respect to storage temperatures alone, RT (1.80) had significantly lower a-value compared to 12°C and 8°C at thefull ripe stage. Among the pre-treatments given chitosan (2%) treated fruits had the lowest a-value followed by chitosan (1%), control and acetic acid (1%) treated fruits. The interaction studies reveal that T₁C₂ had significantly lower a-value than all other treatment combinations followed by T₁C₂. The data presented in the table 3 shows that, at full ripe stage irrespective of the pre-treatment, highest b-value was observed for fruits stored at 12°C followed by RT, while control, chitosan (1%) treated fruits had attained higher b-value compared to acetic acid 1% and chitosan (2%) treated fruits. The effect of interaction between storage temperature and pretreatments showed that $T_2 \tilde{C}_2$ was the best treatment combination followed by $T_2 \hat{C_3}$ and $T_3 \hat{C_3}$ was the poorly performed combination.

In our present study, chitosan (1 and 2%) treatments significantly delayed the green colour loss in guava fruits (Table 3). The occurrence of yellow colour on fruits was further delayed with a reduction in storage temperature of chitosan treated fruits. However, a slow but continuous increase in yellowness value of fruit was observed in chitosan (1 and 2%) treated fruits during later days of storage at 12° and 8°C. Similar results were reported by Yueming *et al.* (10) in chitosan (2%) at RT did not turn yellow at all which may be due to the high CO₂ accumulation in tissue of the fruit, which completely retarded the yellow colour development. There

were green mosaic patches on the fruits, attributed to the CO_2 injury. Among all the treatments and temperatures, chitosan (1%) and 12°C was found more appropriate in retention of fruit quality at the end of storage period, *i.e.*, upto 21 days. Even though 8°C extended the storage life for more than 30 days, it showed chilling injury after the fruits were shifted to room temperature condition. chitosan (2%) is also not recommended as the higher concentration lead to uneven ripening of fruits at room temperature.

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