Indian Journal of Agricultural Sciences **89** (12): 2003–8, December 2019/Article
https://doi.org/10.56093/ijas.v89i12.96262

Shelf-life and quality of guava (*Psidium guajava*) affected by chitosan based coating

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Received: 10 April 2017; Accepted: 27 April 2019

ABSTRACT

The present study was carried out during 2013–14 to evaluate the effect of 1% chitosan + 2% calcium gluconate as texture enhancer + 0.2% tocopherol as antioxidant + 100 ppm kinetin as antisenescent + 1.5% tulsi as antimicrobial agent on shelf-life of guava fruits under room temperature (RT) (33±3ºC, 80±5% RH) and low temperature (LT) (i.e. 10±1ºC, 55±5% RH) storage conditions. This coating significantly reduced the weight loss, rate of ripening, decay loss, respiration rate, ethylene evolution and accumulation of total sugars in guava fruits both at RT and LT storage. Coated fruits retained firmness (9.3 kg/cm^2, 12.2 kg/cm^2), total soluble solids (10.7%, 10.0%), acidity (0.474%, 0.487%), ascorbic acid (124.8 mg/100g, 107.8 mg/100g), phenols (3.57 mg/g, 3.36 mg/g), pectin (0.71%, 0.69%), total chlorophyll (1.18 mg/100mm^2, 1.15mg/100mm^2) and total carotenoids (0.22 mg/100mm^2, 0.208 mg/100mm^2) significantly better than uncoated fruits both at RT and LT storage, respectively. This chitosan based functional edible coating enhanced the shelf-life of guava up to 8 and 20 days at RT and LT conditions, respectively as against 4 and 12 days for uncoated fruits at the respective storage conditions.

Key words: Chitosan, Edible coating, Guava, Shelf-life, Quality

Guava (*Psidium guajava* L.) is an important fruit crop of sub-tropical and tropical regions of the world. It is a highly perishable fruit known for its delightful flavour, nutritional status and moderate price in market. It is one of the choicest fruits having a rich source of vitamin C along with appreciable amount of minerals such as phosphorus, calcium, iron as well as vitamins like niacin, pantothenic acid, thiamin, riboflavin and vitamin A (Rana *et al.* 2015). But, guava due to its high respiration rate has a very short shelf-life (Miranda-Castro 2016), which in turn makes transportation and storage difficult (Jain *et al.* 2003). Storage below 10°C may cause severe chilling injury symptoms in the form of skin surface pitting and flesh browning, and susceptibility to chilling injury limits the potential for its commercialization (Wang *et al.* 2009).

Sardar (Lucknow-49) is the most popular variety of guava for commercial cultivation which is having high total soluble solids and vitamin C but has medium keeping quality (Singh 2012). Edible coatings can extend the shelf life and improve the quality of fruits and vegetables by creating a modified atmosphere inside the fruit due to their barrier properties to gases and moisture (Diab *et al.*2001). Chitosan has been proven as one of the best edible coatings for different types of fruits because of its film-forming properties, antimicrobial actions, and lack of toxicity, biodegradability and biochemical properties (Shiekh *et al.* 2013). Keeping the above information in mind, the present investigation was planned to develop a functional edible coating having chitosan as the main component for extending the shelf-life of guava during storage.

MATERIALS AND METHODS

Uniform and healthy medium sized disease free fruits of guava cv. Lucknow-49 (Sardar) harvested at firm but mature stage were procured from Horticultural Farm, CCS HAU, Haryana during 2013–14. Chitosan and α-tocopherol were procured from Sigma-Aldrich Chemicals Pvt Ltd, New Delhi (India), calcium gluconate and kinetin from Titan Biotech Ltd, Bhiwadi (India) and tulsi leaves for preparing tulsi extract were procured locally from Hisar.

Chitosan coating was prepared by dissolving chitosan (1.0%) in 1% acetic acid followed by magnetic stirring at room temperature for 10 min. Then the glycerol (1:2::glycerol:chitosan) as plasticizer was added and all the functional components calcium gluconate (2%), tocopherol (0.2%), kinetin (100 ppm) and tulsi extract (1.5%) were mixed with magnetic stirring for 5 min. Mixture of edible coating was applied to guava fruits by dipping for 2 min and subsequent dripping followed by air drying. Control

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(uncoated) and coated fruits were packaged in corrugated fibreboard (CFB) boxes with newspaper lining and stored at room temperature (RT) (33±3ºC, 80±5% RH) and at low temperature (LT) (i.e. 10±1ºC, 55±5% RH). Six replicates of eight fruits per pack (~700 g) were taken for each treatment. The observations were recorded during storage on every 2nd day under RT and on every 4th day under LT by recording percent weight loss.

The fruits turned dark yellow were regarded as ripened, whereas, decay loss was assessed as total rotten fruits in terms of percentage on number basis. Firmness for guava fruits was measured by hand held fruit pressure tester (Model FT 327) using cylindrical plunger of 8 mm diameter. Respiration rate and ethylene evolution was determined as per head space analysis procedure adopted by Banks *et al.* (1994).

Total soluble solids were determined by using Abbe's hand refractometer of 0–32% range at room temperature and expressed as % total soluble solids of fruit. Acidity and ascorbic acid were determined as per method described by AOAC (1990). For estimation of pigments, four discs of 78.57 sq mm area from peel were placed in 10 ml dimethyl sulphoxide (DMSO) and incubated at 60±1ºC for 4 h. The optical density of the liquid extract was then measured at 652 nm for total chlorophyll and 440 nm for carotenoids using method of Barnes *et al.* (1992).Total sugars were estimated by the method of Hulme and Narain (1931).Total pectin as calcium pectate was estimated by the method described by Ranganna (2009).The total phenols were estimated by the method of Amorium *et al.* (1977) using Folin Denin's reagent.The data obtained in the present investigation were subjected to statistical analysis of variance (ANOVA) technique using 2 factorial completely randomized designs (CRD), except for PLW, where simple CRD was used.

RESULTS AND DISCUSSION

Physiological loss in weight (PLW): PLW of uncoated guava fruits during storage at RT increased up to 12.5% on 4th day as compared to 12.2% on 8th day in coated fruits. Similarly, PLW of uncoated guava fruits stored at LT increased up to 15.9% on 16th day as compared to 10.5% on 20th day in coated fruits. Thus, coated fruits significantly reduced the PLW as compared to uncoated ones (Table 1). Chitosan coatings act as moisture barrier, thereby restricting water transfer and protecting fruit skin from mechanical injuries, as well as sealing small wounds and thus reducing dehydration and weight loss in fruits during storage (Ribeiro *et al.* 2007). Incorporation of calcium gluconate, tocopherol and kinetin in coating maintained the integrity of cells, reduced the respiration and other oxidative metabolic reactions responsible for senescence and thus, reduced the weight loss by preventing loss of moisture. The favourable effects of chitosan coating (1% and 2%) in reducing the PLW have also been reported in guava cv. Allahabad Safeda (Krishna and Rao 2014).

Ripening and decay loss: The control fruits attained 100% ripening by 4th day of storage at RT and 45.8% ripening by 16th day of storage at LT, while the fruits coated with functional edible coating did not show any ripening throughout the storage period of 8 days at RT and 20 days at LT. The slowing down of ripening process by coating resulted in better preservation of color, aroma and firmness. Similarly, Ferreira *et al.* (2011) also reported that green peel color of guava fruits was maintained due to delayed ripening during the storage, when treated with antimicrobial coatings with chitosan. There was 100% decay loss on 4th day of storage at RT and on 20th day of storage at LT, whereas, the fruits coated with functional edible coating did not show any decay loss by 6th day of storage at RT and by

Table 1 Effect of functional edible coating on physiological parameters of guava during storage

Physiological parameter	Treatment	Period of storage (days)												
		Room temperature (33±1°C)						Low temperature (10±1°C)						
		0	2	4	6	8	Mean	0	4	8	12	16	20	Mean
PLW (%)	Control		5.2	12.5	(12.5)*	(12.5)*	10.7		3.7	6.4	10.9	15.9	(15.9)*	10.6
	Coated		3.1	8.3	10.5	12.2	8.5		1.5	3.7	5.3	8.2	10.5	5.8
	Mean		4.1	10.4	11.5	12.4			2.6	5.1	8.1	12.0	13.2	
	CD at 5%	T = 0.32; S = 0.41; T × S = 0.63						T = 0.51; S =0.81; T × S = 1.15						
Ripening (%)	Control	0	45.8	100	(100)*	(100)*		0	0	0	0	45.8	(45.8)*	
	Coated	0	0	0	0	0		0	0	0	0	0	0	
Decay loss (%)	Control	0	0	12.5	(100)*	(100)*		0	0	0	0	62.5	(100)*	
	Coated	0	0	0	0	12.5		0	0	0	0	0	0	
Firmness (N)	Control	13‡	9.4	6.8	(6.8)*	(6.8)*	8.6	13‡	12.4	7.2	6.2	4.4	(4.4)*	7.9
	Coated	13‡	13‡	12.4	12.2	9.3	12.0	13‡	13.0	13.0	12.5	12.3	12.2	12.7
	Mean	13‡	11.2	9.6	9.5	8.1		13‡	12.7	10.1	9.3	8.4	8.3	
	CD at 5%	T = 0.31; S = 0.49; T × S = 0.70						T= 0.12; S = 0.21; T × S = 0.29						

T, Treatment; S, Storage; * Treatment was terminated due to spoilage of the fruits, Values in the parenthesis are assumed values equivalent to the values at the last day before termination of the treatment.The values have been taken for the purpose of ANOVA only;‡ values exceeded instrumental limit of 13 (kg/cm^2), Firmness at 0 day = 13‡ (kg/cm^2).

20th day of storage at LT (Table 1). The antimicrobial activity of chitosan could be related to the ability of this biopolymer to cause severe cellular damage to the mold and interfere in the secretion of poly-galacturonases (Hernandez-Munoz *et al*. 2008). Moreover, antimicrobial component present in tulsi leaf extract may also have inhibited growth of spoilage causing microbes and thus reduced decay loss.

Firmness: The flesh firmness of fruits at 0-day of storage was recorded >13 kg/cm^2 (more than the instrumental limit of 13 kg/cm^2) which decreased progressively with storage period both at RT and LT conditions. Guava fruits coated with functional edible coating retained firmness significantly better than control during storage at both conditions (Table 1). Similar delayed loss in fruit firmness has also been reported in red guava coated with cashew gum and carboxymethylcellulose based edible coatings (Forato *et al.* 2015).

Respiration rate and ethylene evolution: Guava being climacteric fruit showed a climacteric rise in respiration rate and ethylene production during storage at RT and LT conditions. Coated guava fruits not only showed a delay but also reduction in the climacteric rise in respiration rate and ethylene evolution as compared to control at RT and LT conditions (Fig 1). The coating by providing a semi-permeable barrier against gas movement, may have modified internal atmosphere of the fruit, and thereby resulted in reduced rates of respiration and ethylene production (Ali *et al.* 2013).

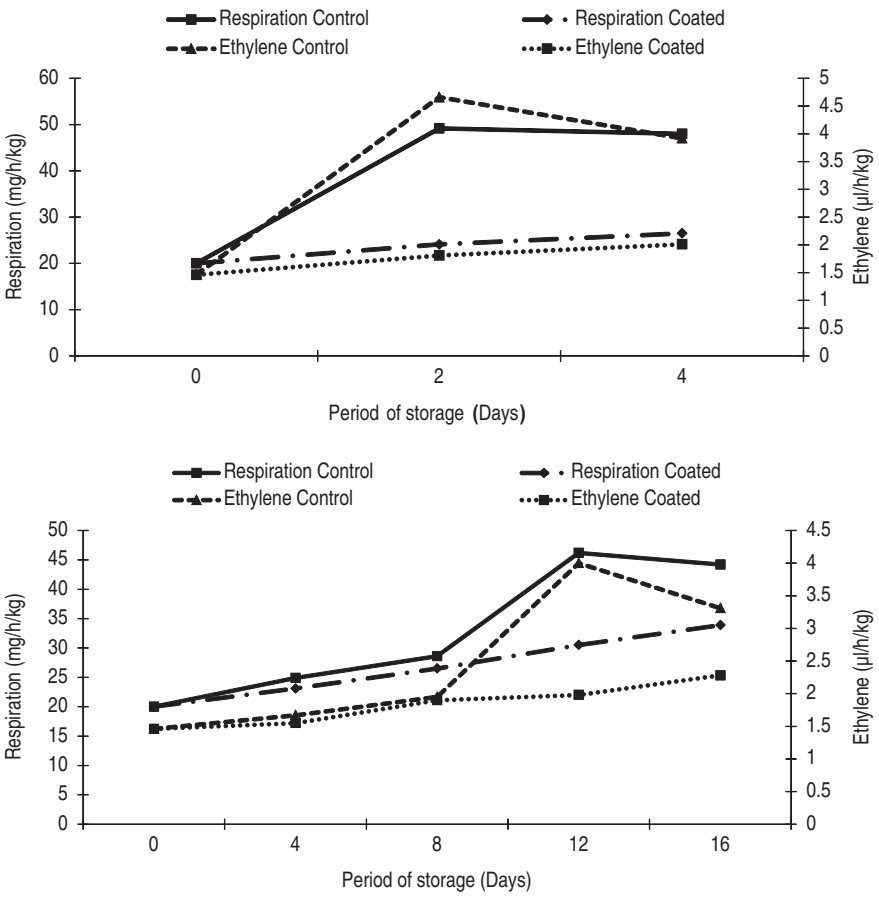

Fig 1 Effect of functional edible coatings on respiration rate (mg CO_2/h/kg) and ethylene evolution (µl C_2H_4/h/kg) of guava during storage at RT (A) and at LT (B).

Total soluble solids and total sugars: Coated fruits showed lower increase in TSS and total sugars as compared to uncoated fruits both at RT and LT storage conditions (Table 2). Decrease in TSS and total sugars could be attributed due to delay in ripening process, which could be due to less conversion of starch to sugars. Similar reduction in TSS in mango with application of coating containing pectin and chitosan has been reported by Medeiros *et al.* (2012).

Acidity and ascorbic acid: Acidity of guava fruits decreased with increase in storage period, both at RT and LT conditions, the decrease being slower in coated fruits (Table 2). Ascorbic acid content of guava fruits increased initially and thereafter showed a decreasing trend during further storage period at RT and LT conditions (Table 2). The increase in ascorbic acid during initial stage of fruit ripening might be due to its synthesis while the decrease inascorbic acid content as the ripening advances could be due to its utilization as a terminal oxidase in competition with cytochrome oxidase in the electron transport system. The higher reduction in ascorbic acid during storage may be due to high respiration rate of uncoated fruits, as is also evident in the present investigation.

Chlorophyll and carotenoids: With advancement of storage period under both RT and LT storage conditions, a progressive decrease in chlorophyll and an increase in carotenoids were observed in guava fruits. During the ripening process, the chlorophyll degrades exposing the carotenoids which are the main pigment responsible for most of the yellowish tinge (Forato *et al.* 2015). In the present investigation, presence of higher chlorophyll and lower carotenoid contents in chitosan coated fruits under both RT and LT storage conditions indicated reduced/ delayed ripening during storage (Table 2). The findings are in conformity with Ali *et al.* (2013) in tomato fruits coated with gum Arabic based edible coating.

Pectin and total phenols: The coated guava fruits showed higher retention of pectin as compared to control and thereby, also retention of better firmness (Table 2). Similar results have been reported by Zhou *et al.* (2011) in the edible film coated pear.Total phenolic content was recorded more in coated fruits as compared to control during storage both at RT and LT conditions (Table 2). The coating forms a protective barrier on the surface of the fruit and may

Table 2 Effect of functional edible coating on biochemical parameters of guava during storage

Biochemical parameter	Treatment	Period of storage (days)												
		Room temperature (33±1°C)						Low temperature (10±1°C)						
		0	2	4	6	8	Mean	0	4	8	12	16	20	Mean
TSS (%)	Control	9.2	10.5	10.8	(10.8)*	(10.8)*	10.4	9.2	9.7	9.8	10.5	10.7	(10.7)*	10.0
	Coated	9.2	9.8	9.7	10.2	10.7	9.9	9.2	9.5	9.7	10.3	10.1	10 .0	9.8
	Mean	9.2	10.2	10.3	10.5	10.8		9.2	9.6	9.75	10.4	10.4	10 .4	
	CD at 5%	T =0.40; S = 0.60; T × S = NS						T = 0.20; S =0.60; T × S = NS						
Acidity (%)	Control	0.734	0.670	0.436	(0.436)*	(0.436)*	0.542	0.734	0.675	0.593	0.534	0.478	(0.478)*	0.582
	Coated	0.734	0.701	0.658	0.542	0.474	0.622	0.734	0.701	0.657	0.593	0.538	0.487	0.618
	Mean	0.734	0.686	0.547	0.489	0.455		0.734	0.688	0.625	0.564	0.508	0.483	
	CD at 5%	T = 0.009; S = 0.015; T × S = 0.021						T = 0.007; S = 0.011; T × S = 0.016						
Ascorbic acid (mg/100g)	Control	131.0	150.4	128.7	(128.7)*	(128.7)*	133.5	131.0	142.6	134.1	121.7	110.1	(110.1)*	124.9
	Coated	131.0	143.4	152.7	140.3	124.8	138.5	131.0	141.9	139.5	133.3	120.2	107.8	128.9
	Mean	131.0	146.9	140.7	134.5	126.7		131.0	142.3	136.8	127.5	115.1	108.9	
	CD at 5%	T = 3.96; S = 6.26; T × S = 8.86						T= 3.16; S = 5.48; T × S = NS						
Chlorophyll (mg/100mm^2)	Control	1.44	1.19	0.79	(0.79)*	(0.79)*	1.00	1.44	1.38	1.29	1.08	0.99	(0.99)*	1.20
	Coated	1.44	1.39	1.30	1.24	1.18	1.31	1.44	1.41	1.33	1.27	1.21	1.15	1.30
	Mean	1.44	1.29	1.05	1.02	0.98		1.44	1.39	1.31	1.17	1.10	1.07	
	CD at 5%	T = 0.015; S = 0.024; T × S = 0.033						T = 0.017; S = 0.030; T × S = 0.042						
Carotenoids (mg/100 mm^2)	Control	0.165	0.211	0.328	(0.328)*	(0.328)*	0.272	0.165	0.189	0.218	0.250	0.274	0.274	0.228
	Coated	0.165	0.182	0.195	0.208	0.22	0.194	0.165	0.169	0.18	0.191	0.206	0.208	0.187
	Mean	0.165	0.197	0.261	0.268	0.274		0.165	0.179	0.199	0.22	0.24	0.241	
	CD at 5%	T = 0.002; S = 0.003; T × S = 0.005						T = 0.002; S =0.003; T × S = 0.004						
Pectin (%)	Control	1.07	0.87	0.54	(0.54)*	(0.54)*	0.71	1.07	0.95	0.85	0.70	0.59	(0.59)*	0.79
	Coated	1.07	0.94	0.85	0.80	0.71	0.87	1.07	0.98	0.90	0.87	0.77	0.69	0.88
	Mean	1.07	0.90	0.69	0.67	0.62		1.07	0.97	0.88	0.78	0.68	0.64	

Contd.

Table 2 (*Concluded*)

Biochemical parameter	Treatment	Period of storage (days)											
		Room temperature (33±1°C)						Low temperature (10±1°C)					
		0	2	4	6	8	Mean	0	4	8	12	16	20	Mean
	CD at 5%	T = 0.03; S = 0.04; T × S = 0.06						T = 0.02; S = 0.03; T × S = 0.04						
Total phenols (mg/g)	Control	4.21	3.73	2.77	(2.77)*	(2.77)*	3.25	4.21	3.97	3.81	3.41	3.01	(3.01)*	3.57
	Coated	4.21	4.05	3.89	3.79	3.57	3.90	4.21	4.16	4.03	3.89	3.65	3.36	3.88
	Mean	4.21	3.89	3.33	3.28	3.17		4.21	4.07	3.92	3.65	3.33	3.19	
	CD at 5%	T = 0.125; S = 0.197; T × S = 0.279						T = 0.082; S = 0.142; T × S = 0.201						
Total sugars (%)	Control	5.0	5.9	6.8	(6.8)*	(6.8)*	6.2	5.0	5.8	6.1	6.5	6.7	(6.7)*	6.1
	Coated	5.0	5.5	5.9	6.2	6.6	5.8	5.0	5.2	5.8	6.2	6.3	6.5	5.8
	Mean	5.0	5.7	6.3	6.5	6.7		5.0	5.5	6.0	6.4	6.5	6.6	
	CD at 5%	T = 0.26; S = 0.41; T × S = NS						T = 0.20; S = 0.35; T × S = NS						

T, Treatment; S, Storage; * Treatment was terminated due to spoilage of the fruits, Values in the parenthesis are assumed values equivalent to the values at the last day before termination of the treatment. The values have been taken for the purpose of ANOVA only; NS, Non-significant.

have reduced the supply of oxygen for enzymatic oxidation of phenolic, resulting in better retention of total phenols as compared to control. In similar studies, chitosan based edible coating has also been reported to reduce the loss of total phenolic in apricot fruits (Ghasemnezhad *et al.* 2010) .

Thus coating Sardar guava fruits with edible coating composed of 1% chitosan + 2% calcium gluconate + 0.2% tocopherol + 100 ppm kinetin + 1.5% tulsi extract extended their shelf life from 4 days to 8 days at RT and from 12 days to 20 days at LT storage conditions slowing down the weight loss, ripening, decay and respiration rate of fruits with better retention of organoleptic and nutritional quality.

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to the Centre of Food Science and Technology, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India for providing the necessary facilities to undertake the present studies.

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