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# Changes in physicochemical characteristics of guava fruits due to chitosan and calcium chloride treatments during storage

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#### Abstract

Effect of post-harvest treatments of chitosan and CaCl<sub>2</sub> alone and in combination on the physicochemical characteristics of guava (Hisar Surkha) fruits were studied. The fruits were treated with different concentrations of chitosan (0.5-3.0%) and CaCl<sub>2</sub> (1.0-3.0%) for 5 min and evaluated for various physico-chemical parameters. Best concentration for chitosan (1.5%) was selected on the basis of qualityrelated parameters. For the storage study of guava, fruit were then treated with the selected concentration of chitosan in combination with different concentrations of CaCl<sub>2</sub> (1.0-3.0%) for 5 min and then stored at room temperature (18°C). Pre-treatment of fruits with chitosan and CaCl<sub>2</sub> alone and in combination significantly delayed decline in physiological loss in weight, total soluble solids and more retention of firmness, acidity, ascorbic acid, sugars, phenols and total antioxidant activity during storage. The treatment of CaCl<sub>2</sub> (1.5%)+chitosan (1.5%) was most effective treatment in modulating physico-chemical changes in guava fruits and enhancing keeping quality of guava during storage.

Keywords: guava, storage, chitosan, calcium chloride, quality

#### 1. Introduction

Guava (Psidium guajava L.) is one of the important commercial fruits in India with annual production of 3.66 million tonnes (Saxena and Gandhi, 2014) [58]. The guava is rich in antioxidants like phenolics and carotene (Joseph and Priya, 2011)<sup>[36]</sup> and a source of minerals like iron, calcium, phosphorus as well as many vitamins like ascorbic acid, pantothenic acid, vitamin A and niacin (Embaby and Hassan, 2015) <sup>[18]</sup>. Guava is a highly perishable fruit having high moisture content and intense metabolic activities which continues post-harvest, therefore loses its texture and quality during storage (Kanwal et al. 2016) [37]. Marketable life is also significantly limited by the abrupt softening during post-harvest handling. Therefore, guava fruits are required to be managed appropriately through judicious use of post-harvest treatments (Golding *et al.* 2005)<sup>[20]</sup>. The exogenous application of chemicals such as chitosan, CaCl<sub>2</sub>, polyamines and gibberellins are being used to retard the physiological changes of the produce so as to increase the shelf-life. Chitosan is a high molecular weight cationic polysaccharide derived from a low acetyl form of chitin, mainly composed of glucosamine and N-acetylglucosamine with a  $\beta$ -1-4 glycosidic linkage (Hadwiger and McBride, 2006) <sup>[22]</sup>. Chitosan has great potentialities as a biodegradable, exhibits excellent biocompatibility, nontoxicity, antioxidant, antimicrobial activity (Zhelyazkov et al. 2014; Hussein et al. 2015) [74, 30] and also possesses film-forming and barrier properties (Elsabee and Abdou, 2013) [17], thus making it a potential raw material for coatings. It acts as an excellent semi-permeable barrier against oxygen, carbon dioxide and moisture, thereby reducing respiration and water loss and counteracting the dehydration and shrinkage of the fruit (Velickova et al. 2013; Petriccione et al. 2015b) [68, 54] hence retarding ripening and senescence. Calcium ions play an essential role in the structural maintenance of membranes and cell walls (Oms-Oliu et al. 2010) [52]. Calcium (Ca) delays the process of ripening particularly the softening and hence, increases the shelf-life by altering intercellular and extracellular processes (Shehata et al. 2009) <sup>[60]</sup>. However, no published studies about guava fruits treated with combination of chitosan with CaCl<sub>2</sub> in case of improving quality parameters have been found. The objective of this research was to investigate the effect of chitosan and CaCl<sub>2</sub> alone and in combination on the physico-chemical characteristics of guava during storage.

#### 2. Materials and Methods

#### 2.1 Plant material

Guava (*Psidium guajava* L.) fruits of variety Hisar Surkha (shelf-life 4-5 days) were selected for this study. The fruits were procured from the Horticulture Farm, CCS Haryana Agricultural

University, Hisar at mature green stage. To optimize concentration of chitosan treatments for increasing shelf-life of guava, fruit free of any visible defects and approximately of same size, were treated with different concentrations of chitosan *viz.* 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% for 5 min. The fruits were then taken out, extra solution wiped off, air dried and were analyzed for physico-chemical parameters and then stored at room temperature. Samples were taken at two day interval until complete decay. All the observations were taken in triplicates.

# **2.2 Analytical Methods**

The physiological loss in weight (PLW) of fruit was calculated on initial weight basis and expressed in percent. Flesh firmness was measured by hand held fruit pressure tester penetrometer. Firmness of three fruits per treatment was measured and it was expressed in Kg cm<sup>-2</sup>. Total soluble solids of juice was measured with the help of hand refractometer (0-32 °brix) and expressed as per cent soluble solids. The titratable acidity was estimated by titrating against 0.1 N NaOH using phenolphthalein as an indicator (Ranganna, 2003) <sup>[56]</sup>. Appearance of pink colour was observed. From the volume of alkali used, acidity was calculated and expressed as g citric acid /100 g fruit pulp.

# **2.3 Biochemical Parameters**

## 2.3.1 Ascorbic acid

Fruit tissue of one g tissue was macerated in 5 ml HClO<sub>4</sub> (0.8 N) and centrifuged at 10,000 x g for 25 min. The supernatant was used for estimation of ascorbic acid by the method of Mukherjee and Choudhuri (1983) <sup>[49]</sup> which was based on the reduction of 2,4- dinitrophenyl hydrazine. The absorbance was read at 530 nm and quantity of ascorbic acid (10-100  $\mu$ g).

## 2.3.2 Total and reducing sugars

Total and reducing sugars were extracted by refluxing dried fruit samples (500 mg) in 80% ethanol. The alcohol was evaporated from the supernatant by heating on water bath. The residue was dissolved in distilled water to a volume of 100 ml. This served as extract for total sugars and reducing sugars. Total sugars were estimated by the method of Yemm and Willis (1954) <sup>[71]</sup>. Color developed by anthrone reagent was measured at 625 nm against a reagent blank and concentration was calculated by preparing standard curve of glucose solution. Reducing sugars were estimated by the method of Nelson (1944) <sup>[51]</sup> as modified by Somogyi (1952) <sup>[66]</sup>. A stable blue colour developed using arsenomolybdate reagent was read at 520 nm. Concentration of reducing sugars was calculated from the standard curve of glucose (10-100 µg) prepared simultaneously.

## 2.3.3 Total phenols

The same extract prepared for estimation of total and reducing sugars was used for estimation of total phenols. Total phenolic content was estimated according to the Folin-Ciocalteau procedure (Swain and Hillis, 1959)<sup>[67]</sup>. The absorbance was measured at 725 nm after 1 h against a reagent blank. Standard curve was prepared using different concentration of tannic acid. Total phenol value was expressed as mg tannic acid equivalents (TAE)/ g dry weight (DW).

## 2.3.4 Antioxidant activity

Antioxidant activity was measured using stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical as per the method described by Shimada *et al.* (1992) <sup>[61]</sup>. Five hundred mg of fruit pulp was macerated in 10 ml methanol and centrifuged at 4,000 rpm for 15 min. The volume of supernatant was diluted with methanol and used for the estimation of antioxidant activity. The absorbance was read at 517 nm on spectrophotometer. Dye mixed with 0.5 ml methanol was used as blank and the per cent scavenging of DPPH was calculated using the following formula:

% Scavenging capacity of DPPH =  $[(Ao - A_1)/Ao] \times 100$  %

Where Ao = Absorbance of blank $A_1 = Absorbance of sample$ 

The antioxidant activity was also expressed in terms of Vit. C equivalents/g using (5 to  $30 \mu g$ ) ascorbic acid.

## Statistical analysis

Estimation of all the biochemical parameters was done in triplicates. The data were statistically analyzed in factorial CRD for calculating CD using software 'Statistical Package for Agriculture Scientists', OPSTAT (available online at www.hau.ernet.in).

## 3. Results and Discussion

The physiological loss in weight (PLW), major determinant of storage life and quality of fruits, increased progressively throughout the storage period (Table 1). Treatment of fruits with chitosan alone and in combination with CaCl<sub>2</sub> retarded the weight loss of guava fruits during storage and minimum weight loss was observed in the fruits treated with 1.5% chitosan in combination with 1.5% CaCl<sub>2</sub> (8.25%) followed by 1.5% chitosan with 2% CaCl<sub>2</sub> (8.97%) and 1.5% chitosan alone (9.17%). Loss of weight in fruit is mainly due to respiration and chitosan coating act as barriers, thereby restricts evaporation, water transfer thus delays dehydration and maintains tissue rigidity (Krishna and Rao, 2014) [39]. Calcium plays an effective role in membrane functionality and integrity maintenance by binding to the polar head group of the phospholipids. Hence the lower loss of phospholipids with reduced ion leakage could be responsible for the lower weight loss in calcium treated fruits (Lester and Grusak, 1999) <sup>[40]</sup>. The reduction in weight loss in the guava fruit treated with chitosan is similar with the result in litchi (Lin et al. 2011) [42] and banana (Hossain and Iqbal, 2016) [26] Apart from guava, chitosan has been effective in reducing weight loss in other fruits including strawberry (Hernandez- Munoz *et al.* 2008) <sup>[24]</sup>, papaya (Ali *et al.* 2011) <sup>[2]</sup>, mango (Chien *et al.* 2007) <sup>[9]</sup>, mushroom (Jiang *et al.* 2012) <sup>[34]</sup>, longan (Jiang and Li, 2001) <sup>[35]</sup> fruits. Dhillon and Kaur, 2013 <sup>[13]</sup> reported that guava treated with 6% CaCl<sub>2</sub> recorded lowest weight loss as compared to the control.

Fruit firmness is one of the most important quality parameter for determining shelf-life and the market value of fruit. Firmness of guava fruit decreased with the advancement of storage period from 9.37 to 1.07 Kg cm<sup>-2</sup> at 15 DOS in control fruits (Table 2). Fruit softening occurs due to deterioration in the cell structure, the cell wall composition and the intracellular materials (Vogler *et al.* 2015; Romanazzi *et al.* 2016) <sup>[69, 57]</sup>. Though all the treatments led to delay in loss of fruit firmness but treatments of chitosan alone and in combination with CaCl<sub>2</sub> helped in maintaining fruit firmness. The maximum retention (3.76 and 3.37 Kg cm<sup>-2</sup>) was obtained in fruit treated with chitosan in combination with 1.5% CaCl<sub>2</sub> and 1.5% chitosan alone at 15 DOS with a mean value of 6.99 and 6.81 Kg cm<sup>-2</sup>. The maintenance of fruit firmness in the fruits treated with chitosan could be due to their higher antifungal activity and covering of the cuticle and lenticels, thereby reducing infection, respiration and other ripening processes during storage (Ali et al. 2005)<sup>[1]</sup>. These results with chitosan treatment were agreed with those observed in strawberries, raspberries, tomato, peaches, mango, papaya, guava (El Ghaouth et al. 1991a, 1992; Li and Yu, 2001; Zhu et al. 2008; Ali et al. 2011; Hong et al. 2012) [14, 15, 41, 75, 2, 25]. Similarly, post-harvest treatment of calcium chloride has been reported to maintain firmness of peach during storage (Gupta et al. 2011; El-Badawy, 2012) [21, 16]. The retention of firmness in calcium treated fruits might be due to the calcium binding to free carboxyl groups of polygalacturonate polymer, stabilizing and strengthening the cell wall (Conway and Sams, 1983) <sup>[10]</sup>. The other factor involved in maintaining the structure of fruits by chitosan containing 1.5% CaCl<sub>2</sub> might be because of interaction of calcium with pectic acid in cell walls to form calcium pectate, a compound helpful for maintaining structure of the fruit (Hussain et al. 2012)<sup>[29]</sup>.

Data depicted in Table 3 revealed that total soluble solids (TSS) content increased with prolongation in storage duration from 8.30 to 12.23 °Brix at 9 DOS and declined thereafter to 11.23 °Brix and 10.48 °Brix respectively at 12 and 15 DOS respectively in control fruits. The initial increase in TSS content during storage might be due to hydrolysis of starch into sugars and subsequent declined due to the metabolism of sugars into organic acids during respiration. The increase in TSS content was delayed in the fruits treated with CaCl<sub>2</sub> and chitosan alone or in combination. Among the treatments, combination of chitosan (1.5%) with CaCl<sub>2</sub>(2%) was the most effective in maintaining the TSS content to 9.00 °Brix at 9 DOS stage and overall to 8.61 °Brix during total period of storage. The delay in the rise of TSS content could be due to the slowing down of respiration and metabolic activity (Hong et al. 2012) <sup>[25]</sup>. A suppressing respiration rate also slows down the synthesis and the use of metabolites, resulting in lower TSS, due to the slower hydrolysis of carbohydrates to sugars (Das et al. 2013) [12]. The present experimental results are in close conformity with the findings of Kittur et al. (2001) <sup>[38]</sup> and Liu et al. (2014) <sup>[43]</sup>, where a slow rise in TSS was recorded in mango, banana and plums treated with chitosan. The effect of calcium treatment on delaying the increase in TSS are in harmony with those reported by Montanaro et al. (2006) [48] in kiwifruit and Sohail et al. (2015) <sup>[65]</sup> in peach fruit.

The titratable acidity (TA) is an important character to determine quality and acceptability of fruits. In general, TA declined linearly with storage ranging from 0.56% to 0.21% in control fruits (Table 4). All the fruits treated with different concentrations of chitosan and CaCl<sub>2</sub> (except 2.0 and 2.5% CaCl<sub>2</sub>) and their combinations (except 1.5% chitosan and 3.0% CaCl<sub>2</sub>) showed higher values of titratable acidity at all the stages of storage as compared to the control. But, maximum acidity (0.49%) was retained in fruits treated with combination of 2% CaCl<sub>2</sub> with 1.5% chitosan while the lowest value (0.37%) was recorded in the control and 3% CaCl<sub>2</sub> treatment. The decline in titratable acids might be due to increased catabolism of organic acids into sugars (Ibrahim *et al.* 2014) <sup>[31]</sup>. The higher acidity in fruits treated with

chitosan and CaCl<sub>2</sub> could be attributed to reduction in metabolic activities, thereby preventing loss of organic acids. Similar results with chitosan have been reported in apricot (Ghasemnezhad *et al.* 2010) <sup>[19]</sup>, plum (Bal, 2013) <sup>[8]</sup>, pomegranate (Zahran *et al.* 2015) <sup>[72]</sup> and banana (Hossain and Iqbal, 2016) <sup>[26]</sup>. The present results with calcium chloride treatment are in agreement with those reported in strawberry (Amini and Habibi, 2015) and mango (Dhillon and Kaur, 2013) <sup>[13]</sup>.

Ascorbic acid is an essential attribute in judging fruit's antioxidant and reducing capacity. Total ascorbic acid content in control fruits increased within the early 9 days (from 134.0 to 149.0 mg/100 g FW) and then decreased thereafter to 143.0 mg/100 g FW and 140.0 mg/100 g FW respectively at 12 and 15 DOS stage in control fruits (Table 5). Though fruits treated with different concentrations of chitosan and CaCl2 had more ascorbic acid content as compared to control, 1% chitosan exhibited maximum (158.0 mg/100 g FW) ascorbic acid content. The best treatment for maintaining maximum ascorbic acid content (159.3 mg/100 g FW) was 2% CaCl<sub>2</sub> in combination with 1.5% chitosan. An initial increase in ascorbic acid could be due to availability of fruit sugar, a precursor of ascorbic acid synthesis but during later stages, oxidative destruction of ascorbic acid by oxidase might have contributed to decrease in ascorbic acid (Mapson, 1970; Singh et al. 2005) [46, 63]. The higher level of ascorbic acid in chitosan treated fruit might reflect the low oxygen permeability, slowing down the respiration rate, which delays the deteriorative oxidation reaction of ascorbic acid of fruit (Dang et al. 2010) [11]. The present results of chitosan treatment are in conformity with the findings in mango (Jain and Mukherjee, 2011)<sup>[33]</sup>, strawberries (Wang and Gao, 2013) <sup>[70]</sup> and kiwifruit (Huang et al. 2016) <sup>[27]</sup>. Similarly, postharvest application of calcium chloride in the present study retained ascorbic acid content during storage, might be attributed to the slow rate of oxidation in the respiration process (Hussain et al. 2011)<sup>[28]</sup>. Results are in agreement with those reported in jujube (Al-Obeed, 2012)<sup>[3]</sup> and guava (Shaaban Fatma, 2006)<sup>[59]</sup> fruits.

The total antioxidant activity is an indicator of the capacity of total antioxidants to counter oxidative stress. In the present investigations, the antioxidant activity of guava fruits decreased progressively throughout the storage period from 4.40 to 2.49 mg Vit C eq g<sup>-1</sup> FW (Table 6). Treatments of chitosan and CaCl<sub>2</sub> alone or in combination delayed the loss in antioxidant activity and loss was minimum in fruits treated with combination treatment of 1.5% chitosan with 1.5% CaCl<sub>2</sub> followed by 2% chitosan treatment thus retaining maximum antioxidant activity. Antioxidant mechanism of chiotsan could be due to chelation of metal ions found at enzyme active sites, rendering oxidation enzymes inactive (Badawy and Rabea, 2009)<sup>[6]</sup>. Our results with chitosan treatment are consistent with those reported in strawberry (Badawy, 2016)<sup>[7]</sup> and grapes (Shiri et al. 2013)<sup>[62]</sup>. A similar result of calcium chloride treatments on antioxidant activity has been reported in pomegranate fruits during storage (Mirdehghan and Ghotbi, 2014)<sup>[47]</sup>.

Phenolic compounds are very important constituents in the food because they retard oxidative degradation of lipids and nutritional value of food is improved (Pan *et al.* 2011) <sup>[53]</sup>. The results revealed a steady increase from 17.99 mg TAE g<sup>-1</sup> DW at 0 DOS to 24.76 mg TAE g<sup>-1</sup> DW at 9 DOS followed by decline to 23.38 and 21.74 mg TAE g<sup>-1</sup> DW at 12 and 15 days of storage in the untreated (control) fruits (Table 7). Phenylalanine ammonia lyase (PAL) activity is the initial

regulatory enzyme in the biosynthesis of phenolics and the same might have contributed to increase in phenolics content in guava fruits during initial days of storage. The increase in phenolics content is an indication of the activation of defense mechanism. The decreasing trend of phenolic compounds at the end of storage might be due to breakdown of cell structure in order to senescence phenomenon during storage (Macheix et al. 1990)<sup>[44]</sup>. Among the treatments, 1.5% chitosan+1.5% CaCl<sub>2</sub> treated fruits had maximum phenolic content with a mean value of 24.84 mg TAE g<sup>-1</sup> DW. Treatment with chitosan may form a protective barrier on the fruit surface and reduce the oxygen supply for enzymatic oxidation of phenolics (Zhang and Quantick, 1997)<sup>[73]</sup>. The present results with chitosan treatment are in agreement with those reported in tomato (Mustafa *et al.* 2014) <sup>[50]</sup>, apricot (Ghasemnezhad *et* al. 2010) <sup>[19]</sup>, grapes (Shiri et al. 2013) <sup>[62]</sup> and sponge gourd (Han et al. 2014)<sup>[23]</sup>. Similarly, post-harvest CaCl<sub>2</sub> treatment maintained the nutritional quality of pomegranate fruit with higher total phenols content (Ramezanin et al. 2010)<sup>[55]</sup>.

The sugars present in fruits impart sweetness, which influence the taste and flavour of the fruit. Increase in total sugars and reducing sugars upto 9 DOS followed by decline till the end of storage was observed (Table 8-9). All the treated fruits had lower value of total as well as reducing sugars at all the stages of storage as compared to the control, suggesting that CaCl<sub>2</sub> and chitosan caused inactivation of hydrolyzing enzymes responsible for conversion of starch into sugars. Among the treatments, 1.5% CaCl<sub>2</sub> with 1.5% chitosan had pronounced effect in keeping minimum levels of total and reducing sugars throughout the storage period. Results are in accordance with those reported by Amarjeet *et al.* (2016) <sup>[4]</sup> that 2% CaCl<sub>2</sub> was effective in delaying the hydrolysis of polysaccharides in guava (Mahajan *et al.* 2011) <sup>[45]</sup> and papaya (Singh *et al.* 2012) <sup>[64]</sup> fruit, thereby post-porning the production of sugars. Similar effect of calcium chloride has been observed by Ismail *et al.* (2010) <sup>[32]</sup> in guava. In the present research work, we presented a novel strategy of

post-harvest treatments of chitosan and  $CaCl_2$  alone and in combination on physico-chemical and quality changes in guava fruit. The fruits treated with 1.5% chitosan maintained the quality of guava fruits but the fruits treated with 1.5% chitosan in combination with 1.5%  $CaCl_2$  was the most effective in retaining quality of fruit as is evident from delayed decline in PLW, TSS and retention of higher fruit firmness, acidity, phenols, ascorbic acid and antioxidant activity during storage. Therefore, this novel strategy would be feasible for guava storage on a commercial scale.

Physiol	logical loss in	weight (	%)			
The sector of th			Days	of storage		
Treatment	3	6	9	12	15	Mean
Control	4.04	8.68	13.49	19.62	26.81	14.53
0.5% chitosan	3.92	8.20	12.85	18.86	25.78	13.92
1.0% chitosan	3.47	6.99	10.93	15.52	20.97	11.58
1.5% chitosan	2.37	4.91	8.05	12.77	17.73	9.17
2.0% chitosan	2.68	5.72	8.89	13.85	19.05	10.04
2.5% chitosan	3.50	7.54	11.07	16.75	22.60	12.29
3.0% chitosan	3.85	7.86	11.87	17.82	24.26	13.13
1.0% CaCl <sub>2</sub>	3.65	8.11	11.95	17.69	24.01	13.08
1.5% CaCl <sub>2</sub>	2.69	6.53	11.22	16.28	21.89	11.72
2.0% CaCl <sub>2</sub>	3.12	7.35	12.28	17.44	23.17	12.67
2.5% CaCl <sub>2</sub>	3.72	8.52	13.24	18.98	25.92	14.08
3.0% CaCl <sub>2</sub>	3.96	8.63	13.45	19.51	26.14	14.34
1.5% chitosan + 1.0% CaCl <sub>2</sub>	2.47	8.09	12.27	18.42	24.65	13.18
1.5% chitosan + 1.5% CaCl <sub>2</sub>	1.75	4.06	7.08	11.72	16.66	8.25
1.5% chitosan + 2.0% CaCl <sub>2</sub>	1.99	4.51	7.81	12.72	17.82	8.97
1.5% chitosan + 2.5% CaCl <sub>2</sub>	2.03	5.51	9.56	14.24	19.45	10.16
1.5% chitosan + 3.0% CaCl <sub>2</sub>	2.38	6.97	12.65	18.39	23.56	12.79
Mean	3.03	6.95	11.10	16.50	22.38	

Table 1: Effect of chitosan and calcium chloride treatments on physiological loss in weight in guava fruit during storage

CD (P ≤0.05)

a (Treatments): 0.053; b ( Days of storage): 0.029; Interaction ( a×b ) : 0.119

Table 2: Effect of chitosan and calcium chloride treatments on firmness in guava fruit during storage

	Firmness (Kg	cm <sup>-2</sup> )					
T			D	ays of st	orage		
Treatment	0	3	6	9	12	15	Mean
Control	9.37	8.28	7.01	5.53	3.56	1.07	5.80
0.5% chitosan	9.37	8.29	7.06	5.61	3.73	1.36	5.90
1.0% chitosan	9.37	8.35	7.23	5.87	4.16	2.08	6.18
1.5% chitosan	9.37	8.69	7.73	6.59	5.12	3.37	6.81
2.0% chitosan	9.37	8.56	7.48	6.13	4.44	2.40	6.40
2.5% chitosan	9.37	8.33	7.18	5.79	4.03	1.88	6.10
3.0% chitosan	9.37	8.32	7.15	5.72	3.89	1.55	6.00
1.0% CaCl <sub>2</sub>	9.37	8.32	7.19	5.78	3.99	1.81	6.08
1.5% CaCl <sub>2</sub>	9.37	8.65	7.67	6.50	5.01	3.22	6.74
2.0% CaCl <sub>2</sub>	9.37	8.54	7.44	6.06	4.34	2.25	6.33
2.5% CaCl <sub>2</sub>	9.37	8.31	7.14	5.71	3.86	1.57	5.99
3.0% CaCl <sub>2</sub>	9.37	8.29	7.04	5.57	3.64	1.32	5.87

1.5% chitosan + 1.0% CaCl <sub>2</sub>	9.37	8.41	7.09	5.41	3.34	1.15	5.80
1.5% chitosan + 1.5% CaCl <sub>2</sub>	9.37	8.73	7.84	6.79	5.45	3.76	6.99
1.5% chitosan + 2.0% CaCl <sub>2</sub>	9.37	8.61	7.60	6.37	4.83	2.88	6.61
1.5% chitosan + 2.5% CaCl <sub>2</sub>	9.37	8.49	7.33	5.89	4.03	2.05	6.19
1.5% chitosan + 3.0% CaCl <sub>2</sub>	9.37	8.44	7.21	5.62	3.63	1.58	5.98
Mean	9.37	8.45	7.32	5.94	4.18	2.08	

CD ( P ≤0.05)

a (Treatments): 0.114 ; b (Days of storage ): 0.067 ; Interaction ( a×b ) : 0.278

Table 3: Effect of chitosan and calcium chloride treatments on total soluble solids in guava fruit during storage

Το	tal soluble	e solids (	°Brix)				
T				Days of st	orage		
Treatment	0	3	6	9	12	15	Mean
Control	8.30	9.32	10.67	12.23	11.23	10.48	10.37
0.5% chitosan	8.30	9.30	10.33	11.58	10.61	9.62	9.96
1.0% chitosan	8.30	8.49	8.73	9.07	8.83	8.48	8.65
1.5% chitosan	8.30	8.56	8.90	9.38	9.00	8.60	8.79
2.0% chitosan	8.30	8.65	9.11	9.70	9.19	8.65	8.93
2.5% chitosan	8.30	8.88	9.60	10.52	9.77	9.57	9.44
3.0% chitosan	8.30	8.76	9.34	10.07	9.46	8.82	9.13
1.0% CaCl <sub>2</sub>	8.30	8.51	8.77	9.14	8.87	8.49	8.68
1.5% CaCl <sub>2</sub>	8.30	8.58	8.94	9.44	9.03	8.59	8.81
2.0% CaCl <sub>2</sub>	8.30	8.67	9.16	9.80	9.26	8.68	8.98
2.5% CaCl <sub>2</sub>	8.30	8.91	9.68	10.62	9.84	9.60	9.49
3.0% CaCl <sub>2</sub>	8.30	9.30	10.63	12.18	11.18	10.39	10.33
1.5% chitosan + $1.0%$ CaCl <sub>2</sub>	8.30	8.68	9.17	9.81	9.33	8.84	9.02
1.5% chitosan + 1.5% CaCl <sub>2</sub>	8.30	8.55	8.85	9.27	8.90	8.52	8.73
1.5% chitosan + 2.0% CaCl <sub>2</sub>	8.30	8.47	8.68	9.00	8.78	8.45	8.61
1.5% chitosan + 2.5% CaCl <sub>2</sub>	8.30	8.78	9.39	10.17	9.60	9.02	9.21
1.5% chitosan + 3.0% CaCl <sub>2</sub>	8.30	8.93	9.69	10.63	9.92	9.74	9.54
Mean	8.30	8.78	9.39	10.15	9.58	9.09	

# CD ( P ≤0.05)

a (Treatments): 0.033 ; b ( Days of storage ): 0.019 ; Interaction (  $a \times b$  ) : 0.080

Table 4: Effect of chitosan and calcium chloride treatments on titratable acidity in guava fruit during storage

Т	itratable acid	ity (%)					
Trissetin			D	ays of st	orage		
Treatment	0	3	6	9	12	15	Mean
Control	0.56	0.47	0.39	0.32	0.26	0.21	0.37
0.5% chitosan	0.56	0.49	0.44	0.40	0.37	0.36	0.44
1.0% chitosan	0.56	0.50	0.46	0.44	0.43	0.43	0.47
1.5% chitosan	0.56	0.51	0.47	0.44	0.42	0.41	0.47
2.0% chitosan	0.56	0.50	0.45	0.41	0.38	0.36	0.44
2.5% chitosan	0.56	0.49	0.43	0.38	0.35	0.33	0.42
3.0% chitosan	0.56	0.48	0.40	0.34	0.30	0.27	0.39
1.0% CaCl <sub>2</sub>	0.56	0.48	0.42	0.36	0.32	0.29	0.41
1.5% CaCl <sub>2</sub>	0.56	0.49	0.44	0.39	0.35	0.33	0.43
2.0% CaCl <sub>2</sub>	0.56	0.50	0.45	0.40	0.37	0.35	0.44
2.5% CaCl <sub>2</sub>	0.56	0.47	0.39	0.32	0.27	0.22	0.37
3.0% CaCl <sub>2</sub>	0.56	0.47	0.40	0.33	0.29	0.25	0.38
1.5% chitosan + 1.0% CaCl <sub>2</sub>	0.56	0.49	0.43	0.37	0.33	0.30	0.41
1.5% chitosan + 1.5% CaCl <sub>2</sub>	0.56	0.50	0.44	0.39	0.35	0.32	0.43
1.5% chitosan + 2.0% CaCl <sub>2</sub>	0.56	0.52	0.49	0.47	0.46	0.45	0.49
1.5% chitosan + 2.5% CaCl <sub>2</sub>	0.56	0.49	0.43	0.38	0.34	0.32	0.42
1.5% chitosan + 3.0% CaCl <sub>2</sub>	0.56	0.48	0.40	0.33	0.28	0.25	0.38
Mean	0.56	0.49	0.43	0.38	0.35	0.32	

CD ( P ≤0.05)

a (Treatments): 0.024 ; b ( Days of storage ): 0.014 Interaction ( a×b ) : 0.059

Table 5: Effect of chitosan and calcium chloride treatments on ascorbic acid content in guava fruit during storage

Ascort	ic acid con	tent (mg/1	<b>00 g FW</b> )								
Treatment		Days of storage									
Treatment	0	3	6	9	12	15	Mean				
Control	134.0	136.0	141.0	149.0	143.0	140.0	140.5				
0.5% chitosan	134.0	138.0	145.0	154.0	147.0	142.0	143.3				
1.0% chitosan	134.0	148.0	165.0	176.0	164.0	161.0	158.0				
1.5% chitosan	134.0	145.0	158.0	174.0	161.0	151.0	153.8				
2.0% chitosan	134.0	143.0	155.0	170.0	159.0	152.0	152.2				

2.5% chitosan	134.0	139.0	147.0	158.0	149.0	143.0	145.0
3.0% chitosan	134.0	141.0	152.0	166.0	154.0	146.0	148.8
1.0% CaCl <sub>2</sub>	134.0	144.0	158.0	175.0	158.0	152.0	153.5
1.5% CaCl <sub>2</sub>	134.0	142.0	152.0	164.0	152.0	143.0	147.8
2.0% CaCl <sub>2</sub>	134.0	141.0	150.0	161.0	151.0	144.0	146.8
2.5% CaCl <sub>2</sub>	134.0	139.0	146.0	154.0	146.0	140.0	143.2
3.0% CaCl <sub>2</sub>	134.0	138.0	143.0	149.0	142.0	138.0	140.7
1.5% chitosan + 1.0% CaCl <sub>2</sub>	134.0	144.0	154.0	167.0	158.0	152.0	151.5
1.5% chitosan + $1.5%$ CaCl <sub>2</sub>	134.0	144.0	156.0	171.0	161.0	154.0	153.3
1.5% chitosan + $2.0%$ CaCl <sub>2</sub>	134.0	146.0	162.0	182.0	167.0	165.0	159.3
1.5% chitosan + 2.5% CaCl <sub>2</sub>	134.0	142.0	151.0	163.0	156.0	151.0	149.5
1.5% chitosan + $3.0%$ CaCl <sub>2</sub>	134.0	140.0	148.0	158.0	152.0	149.0	146.8
Mean	134.0	141.8	151.9	164.2	154.1	148.4	

CD ( P ≤0.05)

a (Treatments): 3.580 ; b (Days of storage): 2.127 ; Interaction (a×b ) : 8.768

Table 6: Effect of chitosan and calcium chloride treatments on total antioxidant activity in guava fruit during storage

Total antioxida	nt activity (1	ng Vit C	eq g <sup>-1</sup> F	<b>W</b> )			
Truceton ent			D	ays of st	orage		
Treatment	0	3	6	9	12	15	Mean
Control	4.40	4.06	3.48	2.88	2.24	1.59	3.11
0.5% chitosan	4.40	4.08	3.55	2.99	2.42	1.82	3.21
1.0% chitosan	4.40	4.16	3.80	3.38	2.94	2.47	3.53
1.5% chitosan	4.40	4.21	3.99	3.76	3.48	3.14	3.83
2.0% chitosan	4.40	4.25	4.06	3.81	3.52	3.19	3.87
2.5% chitosan	4.40	4.11	3.62	3.10	2.57	1.98	3.30
3.0% chitosan	4.40	4.13	3.71	3.22	2.73	2.20	3.40
1.0% CaCl <sub>2</sub>	4.40	4.14	3.83	3.50	3.11	2.71	3.62
1.5% CaCl <sub>2</sub>	4.40	4.17	3.90	3.61	3.25	2.85	3.70
2.0% CaCl <sub>2</sub>	4.40	4.24	3.73	3.38	2.95	2.53	3.54
2.5% CaCl <sub>2</sub>	4.40	4.12	3.61	3.26	2.82	2.35	3.43
3.0% CaCl <sub>2</sub>	4.40	4.08	3.54	3.03	2.59	2.14	3.30
1.5% chitosan + $1.0%$ CaCl <sub>2</sub>	4.40	4.23	4.03	3.83	3.57	3.28	3.89
1.5% chitosan + 1.5% CaCl <sub>2</sub>	4.40	4.27	4.10	3.87	3.60	3.30	3.92
1.5% chitosan + $2.0%$ CaCl <sub>2</sub>	4.40	4.18	3.82	3.43	3.02	2.61	3.58
1.5% chitosan + 2.5% CaCl <sub>2</sub>	4.40	4.15	3.76	3.29	2.83	2.36	3.47
1.5% chitosan + $3.0%$ CaCl <sub>2</sub>	4.40	4.11	3.65	3.12	2.53	1.86	3.28
Mean	4.40	4.16	3.78	3.38	2.95	2.49	

CD ( P ≤0.05)

a (Treatments): 0.050; b (Days of storage): 0.030 ; Interaction (  $a \times b$  ) : 0.123

Table 7: Effect of chitosan and calcium chloride treatments on total phenols in guava fruit during storage

Το	tal phenols (	ng TAE g	g <sup>-1</sup> DW)				
Tractor			Da	ys of stor	age		
Treatment	0	3	6	9	12	15	Mean
Control	17.99	20.30	22.55	24.76	23.38	21.74	21.79
0.5% chitosan	17.99	20.84	22.98	25.08	23.96	22.57	22.24
1.0% chitosan	17.99	22.77	24.60	26.15	25.54	24.76	23.64
1.5% chitosan	17.99	24.11	25.67	26.90	26.41	25.82	24.49
2.0% chitosan	17.99	23.68	25.29	26.64	26.06	25.38	24.17
2.5% chitosan	17.99	21.27	23.37	25.30	24.52	23.54	22.66
3.0% chitosan	17.99	22.02	24.00	25.78	24.83	23.65	23.04
1.0% CaCl <sub>2</sub>	17.99	22.93	24.76	26.31	25.48	24.73	23.70
1.5% CaCl <sub>2</sub>	17.99	23.31	25.13	26.53	26.08	25.46	24.08
2.0% CaCl <sub>2</sub>	17.99	22.29	24.22	25.94	25.26	24.31	23.33
2.5% CaCl <sub>2</sub>	17.99	21.16	23.31	25.35	24.66	23.97	22.74
3.0% CaCl <sub>2</sub>	17.99	20.89	23.15	25.19	24.23	23.63	22.51
1.5% chitosan + 1.0% CaCl <sub>2</sub>	17.99	24.22	25.72	26.90	25.86	24.35	24.17
1.5% chitosan + 1.5% CaCl <sub>2</sub>	17.99	24.81	26.05	27.17	26.97	26.08	24.84
1.5% chitosan + 2.0% CaCl <sub>2</sub>	17.99	23.47	25.13	26.47	25.98	25.11	24.03
1.5% chitosan + 2.5% CaCl <sub>2</sub>	17.99	23.09	24.81	26.31	24.05	23.13	23.23
1.5% chitosan + 3.0% CaCl <sub>2</sub>	17.99	23.85	24.34	26.72	25.78	24.82	23.92
Mean	17.99	22.65	24.42	26.09	25.24	24.30	

CD ( P ≤0.05)

a (Treatments): 0.657 ; b ( Days of storage ): 0.390 ; Interaction ( a×b ) : NS

	Total su	gars (%)					
Treestereet			Da	ys of stor	age		
Treatment	0	3	6	9	12	15	Mean
Control	10.62	11.60	13.28	15.27	14.04	13.03	12.97
0.5% chitosan	10.62	11.55	13.12	15.03	13.87	12.91	12.85
1.0% chitosan	10.62	11.38	12.50	14.02	13.15	12.53	12.37
1.5% chitosan	10.62	11.07	12.04	13.22	12.61	12.18	11.96
2.0% chitosan	10.62	11.23	12.32	13.63	12.85	12.28	12.16
2.5% chitosan	10.62	11.43	12.71	14.38	13.43	12.65	12.54
3.0% chitosan	10.62	11.51	12.92	14.71	13.68	12.81	12.71
1.0% CaCl <sub>2</sub>	10.62	11.28	12.46	13.84	13.08	12.54	12.30
1.5% CaCl <sub>2</sub>	10.62	11.45	12.67	14.29	13.43	12.81	12.55
2.0% CaCl <sub>2</sub>	10.62	11.51	12.91	14.69	13.75	12.97	12.74
2.5% CaCl <sub>2</sub>	10.62	11.54	13.05	14.93	13.94	13.08	12.86
3.0% CaCl <sub>2</sub>	10.62	11.59	13.23	15.19	14.02	13.08	12.96
1.5% chitosan + 1.0% CaCl <sub>2</sub>	10.62	11.41	12.57	14.14	13.31	12.73	12.46
1.5% chitosan + 1.5% CaCl <sub>2</sub>	10.62	11.26	12.38	13.74	13.02	12.50	12.25
1.5% chitosan + 2.0% CaCl <sub>2</sub>	10.62	11.48	12.84	14.57	13.65	12.92	12.68
1.5% chitosan + 2.5% CaCl <sub>2</sub>	10.62	11.53	13.00	14.86	13.89	13.07	12.83
1.5% chitosan + 3.0% CaCl <sub>2</sub>	10.62	11.57	13.19	15.12	13.99	13.07	12.93
Mean	10.62	11.43	12.78	14.45	13.51	12.77	

Table 8: Effect of chitosan and calcium chloride treatments on total sugars in guava fruit during storage

CD ( P ≤0.05)

a (Treatments): 0.529; b ( Days of storage ): 0.315; Interaction (  $a \times b$  ) : NS

Table 9: Effect of chitosan and calcium chloride treatments on reducing sugars in guava fruit during storage

R	educing suga	rs (%)					
There is a second			D	ays of st	orage		
Treatment	0	3	6	9	12	15	Mean
Control	5.98	6.76	7.89	8.74	5.44	4.39	6.53
0.5% chitosan	5.98	6.70	7.83	8.61	5.42	3.75	6.38
1.0% chitosan	5.98	6.47	7.19	8.05	5.25	3.61	6.09
1.5% chitosan	5.98	6.35	7.02	7.80	4.89	3.52	5.93
2.0% chitosan	5.98	6.41	7.16	8.01	5.02	3.59	6.03
2.5% chitosan	5.98	6.49	7.33	8.21	5.31	3.66	6.16
3.0% chitosan	5.98	6.52	7.59	8.34	5.38	3.71	6.25
1.0% CaCl <sub>2</sub>	5.98	6.49	7.22	7.96	5.07	4.03	6.13
1.5% CaCl <sub>2</sub>	5.98	6.52	7.35	8.13	5.14	4.12	6.21
2.0% CaCl <sub>2</sub>	5.98	6.57	7.45	8.26	5.35	4.16	6.30
2.5% CaCl <sub>2</sub>	5.98	6.62	7.74	8.61	5.37	4.29	6.44
3.0% CaCl <sub>2</sub>	5.98	6.69	7.84	8.72	5.41	4.35	6.50
1.5% chitosan + 1.0% CaCl <sub>2</sub>	5.98	6.46	7.25	8.07	5.13	4.08	6.16
1.5% chitosan + 1.5% CaCl <sub>2</sub>	5.98	6.45	7.16	7.94	5.04	4.01	6.10
1.5% chitosan + 2.0% CaCl <sub>2</sub>	5.98	6.52	7.41	8.30	5.32	4.14	6.28
1.5% chitosan + 2.5% CaCl <sub>2</sub>	5.98	6.56	7.67	8.59	5.39	4.28	6.41
1.5% chitosan + 3.0% CaCl <sub>2</sub>	5.98	6.62	7.81	8.74	5.43	4.33	6.49
Mean	5.98	6.54	7.47	8.30	5.26	4.00	

CD ( P ≤0.05)

a (Treatments): NS ; b ( Days of storage ): 0.514 ; Interaction ( a×b ) : NS

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