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Changes in cell wall components and its hydrolyzing enzymes of guava fruits due to chitosan and calcium chloride treatments during storage at room and low temperature

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Abstract
Effect of post-harvest treatments of chitosan and $CaCl_2$ alone and in combination on the cell wall components and its hydrolyzing enzymes of guava (Hisar Surkha) fruits were studied. Guava fruits of variety Hisar Surkha treated with chitosan (1.5%), $CaCl_2$ (1.5%) and chitosan (1.5%) + $CaCl_2$ (1.5%), respectively were stored at room temperature (26.3ºC) and in the refrigerator (5 ± 2^0C).Cell wall components viz. hemicellulose, cellulose and pectin decreased progressively throughout the storage at room temperature and low temperature. The decrease in cell wall components were in co-ordination with enhanced activities of cell wall degrading enzymes viz. PME, PG and cellulase. However, low temperature was effective in slowing down the activities of PG and cellulase as compared to room temperature storage suggesting lower degradation of cell wall components. The treatment of $CaCl_2$ (1.5%)+chitosan (1.5%) was most effective treatment in enhancing keeping quality of guava during storage. Hence fruits given these treatments and stored at low temperature retained their quality for longer duration of time.

Keywords: Guava, storage, chitosan, calcium chloride, cell wall components and its hydrolyzing enzymes, room temperature and low temperature

Introduction
Guava is a climacteric fruit (Kanwal *et al*. 2016) [16]. It ripens rapidly after harvest and has a short shelf-life. It 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 (Mitra *et al*. 2012) [19]. This perishability makes this fruit prone to bruising, microbial decay, mechanical injuries and senescence and post-harvest losses are observed. 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 followed by storage at appropriate temperature (Golding *et al*. 2005) [11]. Fruit softening is associated with the solubilisation and degradation of cell wall contents particularly pectins and accompanied with their depolymerization during ripening. This phenomenon leads to changes in cell wall integrity (Brummell, 2006) [6]. These modifications are mediated by members of large gene families such as pectin methylesterases, polygalacturonases and cellulases (Wolf *et al*. 2009) [27]. Storage under low temperatures has been considered as an efficient method to maintain quality of fruits due to their effects on reducing respiration rate, transpiration, ethylene production, senescence and disease incidence. On the other hand, enzymatic reactions occur slowly at low temperatures, extending shelf-life of perishables (Bron *et al*. 2005) [5]. Chitosan has great potentialities as a biodegradable, exhibits excellent biocompatibility, non-toxicity, antioxidant, antimicrobial activity (Hussein *et al*. 2015) [13] and also possesses film-forming and barrier properties (Elsabee and Abdou, 2013) [9], thus making it a potential raw material for coatings. Chitosan can be used as promising edible and biologically safe preservative coating for maintaining sensory and nutritional quality of fruits (Jongsri *et al*. 2016) [15]. Pre-harvest and post-harvest treatments with calcium salts have been effective in controlling ripening, reducing post-harvest decay, controlling the development of many physiological disorders, reducing the

incidence of fungal pathogens and maintaining fruit firmness thus improving their nutritional value. Calcium chloride maintained visual quality by keeping the integrity of the cell wall and retarding flesh softening of fruits resulting in a longer shelf-life of fruits.

2. Material 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. Guava fruits of variety Hisar Surkha treated with chitosan (1.5%), $CaCl_2$ (1.5%) and chitosan (1.5%) + $CaCl_2$ (1.5%), respectively were stored at room temperature (26.3 ºC) and in the refrigerator (5±2^{0}C). The samples were analyzed for cell wall components and their hydrolytic enzymes at three days interval until complete decay. All the observations were taken in triplicates.

2.2 Cell wall components

2.2.1 Cellulose and hemicellulose

Cellulose and hemicellulose were estimated by the method of Van Soest (1967) [26] modified by (Pradhan and Bhatia 1986).

ADF extraction reagent

Cetrimide	5 g
Sulphuric acid (conc.)	28 ml
Volume	1L

To estimate cellulose, acid detergent fibres (ADF) were determined by refluxing 1 g sample with 100 ml ADF extraction reagent for 1 h. The solution was filtered, washed, dried overnight at 100 ºC and weighed.

$$\text{ADF (\%)} = \frac{\text{Residue weight}}{\text{Sample weight}} \text{ x } 100$$

Residue left after the extraction of ADF was taken in a sintered crucible and washed twice by stirring with 72% H_2SO_4. Acid-filled crucibles were kept for 3 h and after that acid was removed. Contents were made acid free by washing with hot water. Crucible was dried overnight at 100 ^{0}C and weighed. The loss in weight from original amount of acid detergent fibre corresponded to cellulose content which was expressed as per cent.

$$\text{Cellulose (\%)} = \frac{\text{Weight of residue after } H_2SO_4 \text{ treatment}}{\text{Weight of sample}} \text{ x } 100$$

2.2.2 Hemicellulose

To determine hemicellulose, neutral detergent fibres (NDF) were first estimated.

NDF extraction reagent

Sodium lauryl sulphate	10.00 g
EDTA	18.61 g
Borax (sodium borate decahydrate)	6.81 g
Disodium hydrogen phosphate (anhydrous)	4.56 g
Ethylene glycol monoethyl ether	10 ml
Volume	1L

NDF was determined by refluxing 1.0 g sample with 100 ml NDF extraction reagent for 1 h. The solution was filtered through sintered glass crucibles and the residue was washed first with hot distilled water and then with acetone, dried overnight at 100 °C and weighed. The residue represented NDF content and was expressed as per cent.

$$\text{NDF (\%)} = \frac{\text{Residue weight}}{\text{Sample weight}} \text{ x } 100$$

Hemicellulose content in the sample was calculated by the difference between NDF and ADF.

2.2.3 Pectin

Pectin was extracted by the method of Ahmed and Labavitch (1977) [1].

Extraction

To 1 g guava fruit, 25 ml 72% H_2SO_4 was added. The mixture was stirred for 30 min and made to 100 ml with distilled water. It was then filtered through Whatman No. 1 filter paper and the filtrate was used for pectin estimation by estimating uronic acid content by the method of Blumekrantz and Asboe-Hansen (1972) [4].

Estimation

Reagents

Metahydroxy diphenyl solution: 150 mg
Volume= 100 ml with 0.5 N NaOH

Borax solution

Sodium tetraborate	476 mg
Conc. H_2SO_4	100 ml

It was dissolved by keeping it in hot water bath for 10 min.

procedure

Suitable aliquot (0.2 ml) was mixed with 2 ml borax solution and kept for 5 min. After shaking vigorously, it was incubated in boiling water bath for 10 min and cooled. To each tube, 20 µl metahydroxy diphenyl solution was added and the contents were shaken vigorously till pink colour developed. The volume was made to 5 ml with conc. H_2SO_4 and then absorbance read at 520 nm. The amount of uronic acid was calculated from a calibration curve prepared by using D-galacturonic acid (10-50 µg) as standard.

2.3 Cell wall hydrolyzing enzymes

2.3.1 Pectin methylesterase (EC 3.1.1.11)

Pectin methylesterase (PME) was extracted and assayed by the method of Hagerman and Austin (1986) [12].

Extraction

Fresh fruit tissue (10 g) was homogenized in a pre-chilled pestle and mortar with 50 ml chilled 0.1 M Tris-HCl buffer (pH 7.5) containing 10% NaCl. Homogenate was centrifuged at 10,000 x g for 30 min. The supernatant represented the enzyme extract.

Assay

The reaction mixture contained 50 µl enzyme extract, 2.5 ml 0.5% (*w/v*) apple pectin in buffer (2 mM Tris-HCl, pH 7.5) and 0.4 ml bromothymol blue (0.01% *w/v*) in the same buffer. The absorbance at 620 nm was measured for 30 min. The difference in absorbance between 0 and 30 min was the measure of PME activity. Calculation of the activity was carried out against a standard curve of galacturonic acid (50 to 500 µg) prepared

under the same assay conditions and the enzyme activity expressed as mg galacturonic acid (carboxy group equivalent) released for 30 min g^{-1} FW. One enzyme unit was expressed as the amount of enzyme required to release 1 mg galacturonic acid/30 min.

2.3.2 Polygalacturonase (EC 3.2.1.15)

Extraction

Polygalacturonase (PG) was extracted according to the method of Singh and Singh (1993) [24]. Fruit sample (1.0 g) was extracted in 0.1 M sodium acetate buffer (pH 5.2) containing 0.02 M sodium metabisulphite and 10% (*w/v*) sodium chloride in a pre-chilled pestle and mortar. The homogenate was centrifuged at 10,000 x g for 30 min at 4ºC. Supernatant obtained was dialyzed against 0.01 M sodium acetate buffer (pH 5.2) for 4 h by changing buffer every hour.

Assay

The enzyme was assayed according to the method of Ahmed and Labavitch (1980). The assay mixture (1.0 ml) contained 0.2 ml enzyme extract, 0.2 ml chilled sodium acetate buffer (0.1 M, pH 5.2), 0.5 ml polygalacturonic acid (0.3% *w/v*) and 50 µl containing 125 µg each of chloramphenicol and cycloheximide. The mixture was incubated at 37 ºC for 20 h. Reaction was terminated by heating the tubes in a boiling water bath for 10 min and reducing sugars were estimated by the method described under the Section 3.4.1(B) using galacturonic acid as standard (20-100 µg). One enzyme unit was defined as the amount of enzyme required to release 1 mg galacturonic acid/20 h at 37 ºC.

2.3.3 Cellulase (EC 3.2.1.4)

Extraction and assay system were the same as for polygalacturonase except that 0.5% (*w/v*) sodium salt of carboxymethyl cellulose was used as substrate instead of polygalacturonic acid. The reaction was started by the addition of 0.5 ml of substrate solution and was terminated by heating the tubes in a boiling water bath for 10 min. reducing sugars were estimated by the method described under the Section 3.4.1(B) using glucose (20-100 µg) as the standard. One enzyme unit was expressed as the amount of enzyme required to release 1 mg glucose/20 h at 37 ºC.

2.4 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

Guava fruits of variety Hisar Surkha treated with chitosan (1.5%), $CaCl_2$ (1.5%) and chitosan (1.5%) + $CaCl_2$ (1.5%), respectively were stored at room temperature (26.3 ºC) and in the refrigerator (5±2 ºC). The samples were analyzed for cell wall components and their hydrolytic enzymes. The fruits stored at room temperature could be sampled upto 9 days while those at low temperature were sampled upto 24 DOS stage. During storage of guava fruits, cell wall components *viz*. cellulose, hemicelluloses and pectin were sequentially modified as levels of the wall materials decreased continuously throughout storage at both conditions. Low temperature storage was found to be effective in slowing down the degradation of cell wall components thereby delaying softening of fruits (Table 1-3). Results presented in Table 1 indicate that cellulose content decreased throughout the storage period from 23.37 to 11.17% (DW basis) at room temperature and 23.37 to 13.34% at low temperature in control fruits. Though application of both chitosan and $CaCl_2$ could slow down the rate of degradation of cell wall components significantly in both the conditions but low temperature storage was more effective in delaying loss of cell wall components at all the stages of storage. It was observed that fruits stored at low temperature for 21 days had similar cellulose content as in those of 6 DOS at room temperature. However, combined treatment of chitosan+$CaCl_2$ was observed to be most effective, thereby retaining cellulose content during storage from 16.98 to 20.72% at room temperature and 17.89 to 19.12% at low temperature. Low temperature could be an added advantage for much higher storage life of both treated and untreated fruits during storage. Similar effects of low temperature on cell wall components has been reported in guava (Deepthi *et al*. 2016) [8] and pear (Zhou *et al*. 2011) [28]. Concomitant with our results, the cell all components were found to be significantly maintained by pre-treatment of $CaCl_2$ in strawberry (Lara *et al*. 2004) [17] and blueberries (Angeletti *et al*. 2010) [2] fruits during storage. Like cellulose, hemicellulose content also decreased linearly and significantly throughout storage at both the temperatures. It decreased from 10.08% (DW basis) at 0 DOS to 3.55% at room temperature upto 9 DOS and to 4.51% at 24 DOS at low temperature (Table 2). Low temperature storage was found to be effective in inhibiting loss of hemicellulose content thereby delaying softening of guava fruits. Pre-treatment of fruits with chitosan with or without $CaCl_2$ could significantly slow down the decrease in hemicellulose content during storage at both temperatures. The treatment of chitosan alone and in combination with $CaCl_2$ had almost similar effect on retention on hemicellulose content attaining a mean value of 7.84% and 7.86% at room temperature. The maximum retention of 8.38% was observed in guava fruits given combined treatment of chitosan and $CaCl_2$ and stored at low temperature. Critical perusal of the data in Table 3 indicate that pectin content decreased continuously at both the temperatures. Fruits stored at room temperature had significantly lower pectin content as compared to those stored at low temperature in treated as well as in control fruits. Pre-treatment of fruits with chitosan, $CaCl_2$ and combination of chitosan with $CaCl_2$ delayed the loss of pectin and maximum effect was observed in chitosan+$CaCl_2$ treated fruits followed by chitosan 1.5% treatment. Though it resulted in retention of pectin content from 8.99 to 9.82% at room temperature and 9.55 to 10.41% at low temperature, the effect was found to be non-significant. Firming and resistance to softening as a result of calcium application have been attributed to the stabilization of membranes and the formation of Ca-pectates, which increased the rigidity of the middle lamella and cell walls, inhibited the degradation of pectins by polygalacturonase, thereby maintaining higher cell turgor pressure (Jayachandran *et al*. 2005) [14]. Due to possible role of calcium in protecting the pectin backbone from the enzymes, calcium was found to maintain the freshness of various types of cut fruits e.g. mango (Souza *et al*. 2006).

Modifications in cell wall during storage occurs due to coordination and interdependence of cell wall hydrolytic enzymes including PME, PG and cellulase. Pectin methylesterase is an important enzyme which plays a critical role in softening of fruit tissues. During fruit storage, it is responsible for de-esterification of the highly methyl-esterified polygalacturonans in the cell wall (Payasi *et al*. 2009) [20]. It is also responsible for the hydrolyzation of pectins to demethylated

pectins which are more easily solubilized by PG and results in pectin degradation (Zhou *et al*. 2011) [28]. As is evident from the results obtained during present investigations, data in Table 4 show an enhancement in pectin methylesterase (PME) activity upto 3 DOS from 8.27 to 13.18 units g^{-1} FW at room temperature and upto 9 DOS from 8.27 to 13.23 units g^{-1} FW at low temperature and declined thereafter to 9.24 units g^{-1} FW and 10.83 units g^{-1} FW at 9 DOS and 24 DOS under these conditions. Similar trend was observed in all treated fruits. However, room temperature led to 1.60 fold increase in activity with a mean value of 13.18 units g^{-1} FW at 3 DOS stage whereas increase in PME activity was only 1.15 fold with a value of 9.54 units g^{-1} FW upon storage for same period at low temperature conditions. All the treated fruits showed lower PME activity as compared to the control fruits under both the storage conditions. Fruits treated with chitosan alone and in combination with $CaCl_2$ showed lower PME activity of 9.69 and 9.30 units g^{-1} FW at room temperature and 10.62 and 10.09 units g^{-1} FW at low temperature storage as compared to respective controls which had mean PME activity of 10.53 units g^{-1} FW and 11.36 units g^{-1} FW at room and low temperature storage conditions., the activity of PME increased upto 3 DOS at room temperature while the same level of activity increased at 9 DOS at low temperature thereafter gradually decreased on storage under both conditions (Table 4). Results reported for guava (Gill *et al*. 2014) corroborate our findings. Pre-treatment of guava fruits with chitosan and $CaCl_2$ resulted in reduced activities of cell wall degrading enzymes (PG, PME and cellulase) at both storage conditions but 1.5% chitosan with 1.5% $CaCl_2$ was found to be the most effective treatment. Data on PG (Table 5) and cellulase (Table 6) revealed that activities of cell wall degrading enzymes decreased throughout the storage at both room temperature and low temperature. The best effect on cellulase activity was observed in fruits given treatment of chitosan+$CaCl_2$ and stored at low temperature which showed 20% reduction in activity in comparison to treated fruits stored at room temperature.

Results are in accordance with those reported by Siddiqui *et al*. (2004) [23] in apple and Praduman (2010) [22] in ber fruits during storage. However, low temperature was effective in slowing down the activities of PG and cellulase as compared to room temperature storage suggesting lower degradation of cell wall components. The fruits treated with chitosan showed lower PG activity as reported earlier in banana (Maqbool *et al*. 2016) [18]. The levels of cellulase activity in strawberry fruit treated with chitosan showed lower value than that of control during storage (Gol *et al*. 2013) [10]. Similar results were reported in pear fruit (Zhou *et al*. 2011) [28]. The effect of chitosan on PG activity might be attributed to the fact that chitosan is a negatively charged polysaccharide and is likely to bind directly to the pectins and forms the chitosan-pectin complex and thereby preventing the access of pectinolytic enzymes such as PG to substrate of the cell wall and help in maintenance of fruit firmness. Chitosan also has the ability to inhibit the activities of several enzymes thus preventing deterioration of fruits (Bhaskar-Reddy *et al*. 2000) [3]. Calcium possesses a distinguishable role in reducing the PG and PME activities. Post-harvest treatments of $CaCl_2$ allows the formation of salt-bridge cross-links between Ca^{2+} and COO^- groups of the pectin. This makes cell wall to become less accessible to the enzymes that cause softening. Since PG only hydrolyses homogalacturonan regions whose uranic acid residues have been previously demethylated by PME (de Assis *et al*. 2001) [7], the negative charges generated by PME are necessary for Ca to bind onto the cell wall and to bring out firming effects of Ca. Thus, the Ca application to the fruit can significantly contribute to the texture retention in fruit.

Table 1: Effect of chitosan and calcium chloride treatments on cellulose content in guava fruit during storage at room temperature (A) and low temperature (B)

(A) Cellulose content (% DW)					
Treatment	**Days of storage**				
	0	**3**	**6**	**9**	**Mean**
Control	23.37	18.96	14.41	11.17	16.98
1.5% chitosan	23.37	20.2	17.53	15.34	19.11
1.5% $CaCl_2$	23.37	19.28	15.3	12.42	17.59
1.5% chitosan + 1.5% $CaCl_2$	23.37	21.2	19.75	18.56	20.72
Mean	23.37	19.91	16.75	14.37	
CD ($p \leq 0.05$) a (Treatments): 2.042; b (Days of storage): 2.042; Interaction (a×b): NS					

(B) Cellulose content (% DW)										
Treatment	**Days of storage**									
	0	**3**	**6**	**9**	**12**	**15**	**18**	**21**	**24**	**Mean**
Control	23.37	21.59	20.11	18.84	17.63	16.45	15.34	14.32	13.34	17.89
1.5% chitosan	23.37	21.9	20.66	19.56	18.5	17.48	16.47	15.5	14.58	18.67
1.5% $CaCl_2$	23.37	21.8	20.41	19.19	18.01	16.88	15.79	14.76	13.82	18.23
1.5% chitosan+ 1.5% $CaCl_2$	23.37	21.99	20.81	19.84	18.92	18.03	17.19	16.38	15.59	19.12
Mean	23.37	21.82	20.50	19.36	18.27	17.21	16.20	15.24	14.33	
CD ($p \leq 0.05$) a (Treatments): NS; b (Days of storage): 2.301; Interaction (a×b): NS										

Table 2: Effect of chitosan and calcium chloride treatments on hemicellulose content in guava fruit during storage at room temperature (A) and low temperature (B)

(A) Hemicellulose content (% DW)					
Treatment	**Days of storage**				
	0	**3**	**6**	**9**	**Mean**
Control	10.08	7.39	5.02	3.55	6.51
1.5% chitosan	10.08	8.55	6.96	5.78	7.84
1.5% $CaCl_2$	10.08	7.93	6.16	4.73	7.23

1.5% chitosan + 1.5% $CaCl_2$	10.08	7.92	7.09	6.33	7.86
Mean	10.08	7.95	6.31	5.10	
CD ($p \leq 0.05$) a (Treatments): 0.844; b (Days of storage): 0.844; Interaction (a×b): NS					

(B) Hemicellulose content (% DW)										
Treatment	**Days of storage**									
	0	**3**	**6**	**9**	**12**	**15**	**18**	**21**	**24**	**Mean**
Control	10.08	9.21	8.42	7.81	7.27	6.47	5.73	5.06	4.51	7.17
1.5% chitosan	10.08	9.40	8.79	8.30	7.88	7.42	7.15	6.89	6.60	8.06
1.5% $CaCl_2$	10.08	9.40	9.28	8.25	7.71	7.27	6.70	6.21	5.71	7.85
1.5% chitosan+ 1.5% $CaCl_2$	10.08	9.66	8.81	8.44	8.65	8.07	7.51	7.00	6.67	8.38
Mean	10.08	9.42	8.83	8.20	7.88	7.31	6.77	6.28	5.87	
CD ($p \leq 0.05$) a (Treatments): 0.390; b (Days of storage): 0.585; Interaction (a×b): NS										

Table 3: Effect of chitosan and calcium chloride treatments on pectin content in guava fruit during storage at room temperature (A) and low temperature (B)

(A) Pectin content (% DW)					
Treatment	**Days of storage**				
	0	**3**	**6**	**9**	**Mean**
Control	12.43	10.87	8.23	4.42	8.99
1.5% chitosan	12.43	11.25	9.04	5.57	9.57
1.5% $CaCl_2$	12.43	11.03	8.53	4.91	9.23
1.5% chitosan + 1.5% $CaCl_2$	12.43	11.41	9.37	6.05	9.82
Mean	12.43	11.14	8.79	5.24	
CD ($p \leq 0.05$) a (Treatments): NS; b (Days of storage): 2.138; Interaction (a×b): NS					

(B) Pectin content (% DW)										
Treatment	Days of storage									
	0	3	6	9	12	15	18	21	24	Mean
Control	12.43	11.05	10.91	10.82	10.63	9.18	8.35	7.43	5.14	9.55
1.5% chitosan	12.43	11.86	11.41	11.05	10.93	10.04	8.62	8.13	6.1	10.06
1.5% $CaCl_2$	12.43	11.51	11.17	10.93	10.71	9.56	8.39	7.62	5.93	9.81
1.5% chitosan + 1.5% $CaCl_2$	12.43	12.02	11.54	11.39	11.07	10.66	9.43	8.32	6.54	10.41
Mean	12.43	11.61	11.26	11.05	10.84	9.86	8.69	7.88	5.93	
CD ($p \leq 0.05$) a (Treatments): NS; b (Days of storage): 1.97; Interaction (a×b) : NS										

Table 4: Effect of chitosan and calcium chloride treatments on pectin methylesterase activity in guava fruit during storage at room temperature (A) and low temperature (B)

(A) Pectin methylesterase (units g^{-1} FW)					
Treatment	**Days of storage**				
	0	**3**	**6**	**9**	**Mean**
Control	8.27	13.18	11.42	9.24	10.53
1.5% chitosan	8.27	11.57	10.40	8.51	9.69
1.5% $CaCl_2$	8.27	12.78	11.35	9.19	10.40
1.5% chitosan + 1.5% $CaCl_2$	8.27	10.84	9.77	8.32	9.30
Mean	8.27	12.09	10.74	8.82	
CD ($p \leq 0.05$) a (Treatments): NS; b (Days of storage): 1.087; Interaction (a×b): NS					

(B) Pectin methylesterase (units g^{-1} FW)										
Treatment	Days of storage									
	0	3	6	9	12	15	18	21	24	Mean
Control	8.27	9.54	11.22	13.23	12.94	12.56	12.12	11.54	10.83	11.36
1.5% chitosan	8.27	9.36	10.61	12.02	11.83	11.55	11.18	10.70	10.09	10.62
1.5% $CaCl_2$	8.27	9.42	10.85	12.63	12.40	12.09	11.67	11.14	10.46	10.99
1.5% chitosan + 1.5% $CaCl_2$	8.27	9.05	10.04	11.20	11.07	10.86	10.56	10.15	9.59	10.09
Mean	8.27	9.34	10.68	12.27	12.06	11.77	11.38	10.88	10.24	
CD ($p \leq 0.05$) a (Treatments): 0.320; b (Days of storage): 0.480; Interaction (a×b): NS										

Table 5: Effect of chitosan and calcium chloride treatments on polygalacturonase activity in guava fruit during storage at room temperature (A) and low temperature (B)

(A) Polygalacturonase (units g^{-1} FW)					
Treatment	**Days of storage**				
	0	**3**	**6**	**9**	**Mean**
Control	2.05	4.23	7.01	10.38	5.92
1.5% chitosan	2.05	4.09	6.48	9.51	5.53
1.5% $CaCl_2$	2.05	4.16	6.72	9.93	5.72
1.5% chitosan + 1.5% $CaCl_2$	2.05	4.01	6.07	9.02	5.29
Mean	2.05	4.12	6.57	9.71	
CD ($p \leq 0.05$) a (Treatments): 0.273; b (Days of storage): 0.273; Interaction (a×b): NS					

(B) Polygalacturonase (units g^{-1} FW)										
Treatment	Days of storage									
	0	3	6	9	12	15	18	21	24	Mean
Control	2.05	2.32	2.81	3.39	4.03	4.69	5.71	6.90	8.13	4.45
1.5% chitosan	2.05	2.26	2.68	3.19	3.72	4.29	5.18	6.21	7.29	4.10
1.5% $CaCl_2$	2.05	2.28	2.75	3.28	3.87	4.51	5.48	6.60	7.77	4.29
1.5% chitosan + 1.5% $CaCl_2$	2.05	2.24	2.58	3.00	3.48	4.01	4.86	5.83	6.86	3.88
Mean	2.05	2.28	2.71	3.22	3.78	4.38	5.31	6.39	7.51	
CD ($p \leq 0.05$) a (Treatments): 0.130; b (Days of storage): 0.195; Interaction (a×b): 0.391										

Table 6: Effect of chitosan and calcium chloride treatments on cellulase activity in guava fruit during storage at room temperature (A) and low temperature (B)

(A) Cellulase (units g^{-1} FW)					
Treatment	**Days of storage**				
	0	**3**	**6**	**9**	**Mean**
Control	1.84	3.02	4.80	7.17	4.21
1.5% chitosan	1.84	2.88	4.27	6.46	3.86
1.5% $CaCl_2$	1.84	2.95	4.51	6.72	4.01
1.5% chitosan + 1.5% $CaCl_2$	1.84	2.81	3.87	6.03	3.64
Mean	1.84	2.92	4.36	6.59	
CD ($p \leq 0.05$) a (Treatments): 0.062; b (Days of storage): 0.062; Interaction (a×b): 0.125					

(B) Cellulase (units g^{-1} FW)										
Treatments	**Days of storage**									
	0	**3**	**6**	**9**	**12**	**15**	**18**	**21**	**24**	**Mean**
Control	1.84	1.96	2.30	2.73	3.22	3.73	4.40	5.14	5.91	3.47
1.5% chitosan	1.84	1.90	2.17	2.53	2.91	3.33	3.87	4.55	5.28	3.15
1.5% $CaCl_2$	1.84	1.92	2.24	2.62	3.06	3.55	4.17	4.87	5.69	3.33
1.5% chitosan + 1.5% $CaCl_2$	1.84	1.88	2.07	2.34	2.67	3.05	3.55	4.17	4.85	2.94
Mean	1.84	1.92	2.20	2.56	2.97	3.42	4.00	4.68	5.43	
CD ($p \leq 0.05$) a (Treatments): 0.049; b (Days of storage): 0.074; Interaction (a×b): 0.148										

Conclusion

In this study, the effectiveness of post-harvest treatments using chitosan, calcium chloride (CaCl2), and their combination on the cell wall components and hydrolyzing enzymes of guava (Hisar Surkha) was evaluated. Guava fruits were treated and stored at room temperature (26.3°C) and in the refrigerator (5±2°C). Results showed that both cell wall components (cellulose, hemicellulose, and pectin) decreased over time in all treatments, but the rate of degradation was slower under low temperature. The combination of 1.5% chitosan and 1.5% CaCl2 was most effective in retaining cell wall integrity, reducing enzyme activity, and extending fruit quality. This combined treatment at low temperature best preserved the guava's quality throughout storage. Chitosan and CaCl2 treatments individually also improved retention of cell wall components compared to the control. Thus, using chitosan and CaCl2 together, particularly under refrigeration, enhances the shelf-life and quality of guava by mitigating the effects of cell wall degradation and enzymatic activity.

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