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Effect of different chemical treatments on enzymes activity of Kinnow fruits

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Abstract
Kinnow mandarin is a perishable fruit and liable to be spoiled under ambient conditions. The postharvest losses can be minimized through checking the rate of transpiration, respiration, microbial infection and protecting membranes from disorganization by using semi-permeable coatings. Present investigation was conducted to study the effect various concentrations of Gum Arabic, Calcium Lactate and Glycerin and their different combination on enzymes activity of Kinnow fruits during storage at room temperature. Activity of polygalacturonase, pectin methyl esterase and cellulase enzymes increased during storage period upto 49th day in all treatments. All coated fruits showed lower activities of polygalacturonase, pectin methyl esterase and cellulase enzymes throughout the storage period. Gum Arabic (10%) + Glycerin (2.5%) coated fruits had minimum activities of cell wall degrading enzymes as compared to control fruits.

Keywords: Kinnow, coating, Gum Arabic, cellulase, polygalacturonase

Introduction
Kinnow mandarin is a hybrid of king and willow leaf mandarins (*Citrus nobilis* × *C. deliciosa*). The mandarin having largest area and maximum production constitutes about 26.54% of total area under citrus. It is an important fruit of India and commercially grown in the arid irrigated and sub-mountainous tract of India. Kinnow was introduced in India in early nineteen forties and since then it has become the most flavored winter season fruit. Kinnow mandarin bears highest place in production, productivity, juice content and fruit quality. Kinnow mandarins are rich in ascorbic acid (13–54 mg per 100 g of edible portion) and calcium (25–46 mg per 100 g of edible portion). The postharvest losses can be minimized by extension of shelf life through checking the rate of transpiration, respiration, microbial infection and protecting membranes from disorganization (Bisen & Pandey, 2008) [5]. The foresaid objectives can be achieved to some extent by use of edible coatings, gel, oil, lipid, starch, packaging and wrapping materials and different type of storage used as postharvest treatments. Edible coatings are applied as thin coating that forms a protective barrier around fruit and can be consumed along with the coated product**.** Coatings make good oxygen and lipid barrier at low to intermediate relative humidity because the polymers can effectively make hydrogen bonds (Sihag *et al.,* 2005) [14].
The softening and textural changes that occur during fruit ripening are characteristic of particular species, and are due to differences in cell wall thickness, composition and activity of fruit wall degrading enzymes at different extent (Harker *et al.,* 1997) [10]. Modification of the cell wall is believed to underlie changes in firmness and texture, but the type and magnitude of the alterations carried out during ripening vary considerably. However, no systematic work has been done to study the comparative effect of gum Arabic, calcium lactate and glycerin alone as well as in combination on activity of enzymes in kinnow fruits, so, present experiment was conducted to find out the effective post harvest treatments of gum arabic, calcium lactate and glycerin or in proper concentration to check the activity of enzymes of Kinnow fruits.

Materials and methods
Present experiment was conducted in the laboratory of department of Botany & and Plant Physiology, CCS Haryana Agricultural University, Hisar. The experiment was designed in completely randomized design. Mature Kinnow fruits of uniform size were harvested with the help of secateurs keeping small intact pedicel with each fruit from the orchard of the department of Horticulture, CCS Haryana Agricultural University, Hisar. Kinnow fruits were cleaned with muslin cloth and were dipped in aqueous solutions of Gum Arabic (5%), Gum Arabic (10%), Calcium lactate (1%), Calcium Lactate (3%), Glycerin (2.5%), Gum Arabic (5%)+Glycerin (2.5%), Glycerin (2.5%)+ Glycerin (2.5%), Calcium lactate (1%)+ Glycerin

(2.5%) & Calcium lactate (3%)+ Glycerin (2.5%) for 10 minutes and were dried in shade thereafter were wrapped in cling film. Two kg fruits were packed in each cardboard box and all the treatments were replicated four times. Fruits of all treatments were stored at room temperature. Fruits at random were taken from each treatment for analysis of enzymes such as cellulase, pectin methylesterase and polygalacturonase in fresh fruits and at seven days of interval up to 49th day.

Pectin methylesterase enzyme activity

Pectin methylesterase was extracted and assayed by the method of Hagerman & Austin (1986) [9].

Extracts Preparation

Fresh fruit peel (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

One hundred µl enzyme extract was mixed with 2.5 ml 0.5 % (w/v) apple pectin in buffer (2 mM Tris-HCl, pH 7.5) and 0.4 ml 0.01 % (w/v) bromothymol blue in the same buffer. The absorbance at 620 nm was measured immediately and after 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 condition 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 of galacturonic acid/30min.

Polygalacturonase enzyme activity

Extracts Preparation

Polygalacturonase was extracted according to the method of Singh & Singh (1993) [15]. Fruit peel (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 4°C. Supernatant obtained was dialysed against 0.01 M sodium acetate buffer (pH 5.2) for 4 h by changing buffer every h.

Assay

The enzyme was assayed according to the method of Ahmed & Labavitch (1980) [2]. The assay mixture (1 ml) contained 0.2 ml enzyme extract mixed with 0.2 ml cold sodium acetate buffer (0.1 M, pH 5.2), 0.5 ml 0.3 % (w/v) polygalacturonic acid and 50 µl containing 125 µg each of chloramphenicol and cycloheximide. The mixture was incubated at 37°C 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 following method using galacturonic acid as standard (20-100 µg). Reducing sugars were determined by the method of Somogyi (1952) [16].

Reagents

Copper reagent A: Anhydrous Na_2CO_3 (25 g), Rochelle salt (25 g), Sodium bicarbonate (25 g), Anhydrous sodium sulphate (200 g). Final volume made 1lit.

Copper reagent B: $CuSO_4$. $5H_2O$ (15g), Conc. H_2SO_4 (1-2 drops. Final volume made 100 ml.

Alkaline copper reagent: Copper reagent A and Copper reagent B were mixed in 25:1(prepared fresh before use)

Preparation of arsenomolybdate reagent

Ammonium molybdate (25 g) was dissolved in 450 ml distilled water and 21 ml conc. H_2SO_4 was added to it. Then 3.0 g disodium hydrogen arsenate was dissolved in 25 ml distilled water and added to the acidified molybdate solution with constant stirring. The volume was made to 500 ml with distilled water. Solution was kept in an incubator at 37°C for 24 h. This reagent was stored in glass stopped brown bottle.

Analytical procedure

Above prepared assay mixture was taken in a 25 ml graduated test tube. One ml alkaline copper reagent was added, mixed well and heated for 20 min in the boiling water bath. Then tubes were cooled and 1 ml arsenomolybdate reagent was added, mixed thoroughly and diluted to 25 ml. A stable blue colour quickly appeared, absorbance of which was read at 520 nm on spectrophotometer. Concentration of reducing sugars was calculated from the standard curve of glucose (10-100 µg)) prepared simultaneously. One enzyme unit was defined as the amount of enzyme required to release 1 mg of galacturonic acid/20 h at 37°C.

Cellulase Enzyme Activity

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 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 in polygalacturonase using glucose (20-100 µg) as the standard. One enzyme unit was expressed as the amount of enzyme required to release 1 mg of glucose released/20 h at 37°C.

Results and Discussion

Cellulase

The increase in cellulase activity of Kinnow fruits during storage as depicted in table 1. The cellulase activity increased with increase in storage period from 0.086 units/ g FW on 0th day of storage to 0.174 units/ g FW on 49th day of storage. Cellulases break down the cellulose molecule into simple sugars this could result in loss of firmness as well as influences the quality and shelf life of kinnow. There was direct correlation between firmness and cellulase activity in fruits. As firmness decreased, increase in cellulase activity was observed. These results are in agreement with the findings in Tomato (Barka *et al.* 2000; Ali & Abu-Goukh, 2005) [4,3] and in Guava (Abu- Goukh & Bashir, 2003; Mondal, 2005; Carvalho *et al.* 2009) [1,12,6]. Minimum cellulase activity was recorded in Gum Arabic (10%) + Glycerin (2.5%) coated fruits i.e. 0.130 units/ g FW followed by Gum Arabic (5%) + Glycerin (2.5%) treated fruits i.e. 0.132 units/ g FW. Maximum cellulase activity was recorded in control fruits (0.140 units/ g FW) followed by Glycerin 2.5% treated fruits (0.136 units/ g FW). The lower activity in coated Kinnow might be due to the inhibition of the cellulase activity by these coatings. Similar results were reported by Gol *et al.* (2013) [7] in Strawberries.

Table 1: Effect of different treatments on Cellulase activity (units/ g FW) in Kinnow fruits during storage at room temperature

Treatments	Days of storage								
	0	**7**	**14**	**21**	**28**	**35**	**42**	**49**	**Mean**
Gum Arabic (5%)	0.086	0.100	0.117	0.134	0.143	0.155	0.166	0.173	0.134
Gum Arabic (10%)	0.086	0.098	0.116	0.132	0.142	0.153	0.164	0.172	0.133
Calcium Lactate (1%)	0.086	0.102	0.118	0.135	0.141	0.155	0.164	0.173	0.134
Calcium Lactate (3%)	0.086	0.100	0.116	0.134	0.140	0.151	0.166	0.175	0.134
Glycerin (2.5%)	0.086	0.105	0.119	0.137	0.142	0.155	0.167	0.175	0.136
Gum Arabic (5%) + Glycerin (2.5%)	0.086	0.096	0.114	0.132	0.139	0.151	0.161	0.175	0.132
Gum Arabic (10%) + Glycerin (2.5%)	0.086	0.095	0.113	0.130	0.136	0.144	0.163	0.169	0.130
Calcium Lactate (1%) + Glycerin (2.5%)	0.086	0.098	0.116	0.133	0.142	0.157	0.169	0.174	0.134
Calcium Lactate (3%) + Glycerin (2.5%)	0.086	0.097	0.115	0.131	0.141	0.156	0.167	0.173	0.133
Control	0.086	0.106	0.119	0.138	0.147	0.163	0.174	0.183	0.140
Mean	0.086	0.100	0.116	0.134	0.141	0.154	0.166	0.174	
CD at 5%	T=0.003 D=0.003 T×D=0.050								

Pectin Methyl Esterase (PME)

Pectin methyl esterase enzyme degrades the pectin fibre which ultimately leads to fruit softening. Pectin methyl esterase increased progressively with increase in storage period (Table 2). It increased from 0.185 units/ g FW (0^{th} day of storage) to 0.348 units/ g FW (49^{th} day of storage). Pectin methyl esterase is responsible for deesterification of pectin and its activity increased as the storage period increased. So increased activity of PME resulted in cell wall softening in kinnow fruits. A continuous increase in PME activity during storage was reported by Barka *et al.* (2000) [4] in Tomato, Goulao *et al.* (2007) [8] in Apple, Carvalho *et al.* (2009) [6] in Guava & Yadav *et al.* (2012) [18] in Ber fruit. Coated kinnow fruits showed linear enhancement in PME activity but the level of increase was less as compared to control. Among treatments, minimum PME activity was observed in Gum Arabic (10%) + Glycerin (2.5%) coated fruits i.e. 0.264 units/ g FW followed by Gum Arabic (5%) + Glycerin (2.5%) treated fruits i.e. 0.265 units/ g FW and highest PG activity was recorded in control fruits (0.127 units/ g FW) followed by Glycerin 2.5% treated fruits (0.125 units/ g FW). This may be because of the reason that these coatings might have suppressed the enzymatic activity of pectin methyl esterase. Similar results were reported by Zhou *et al.* (2011) [19] and Gol *et al*. (2013) [7].

Table 2: Effect of different treatments on Pectin methyl esterase (units/ g FW) in Kinnow fruits during storage at room temperature

Treatments	Days of storage								
	0	**7**	**14**	**21**	**28**	**35**	**42**	**49**	**Mean**
Gum Arabic (5%)	0.185	0.206	0.236	0.269	0.274	0.307	0.325	0.348	0.269
Gum Arabic (10%)	0.185	0.205	0.234	0.268	0.272	0.306	0.323	0.347	0.268
Calcium Lactate (1%)	0.185	0.203	0.235	0.266	0.272	0.304	0.322	0.345	0.267
Calcium Lactate (3%)	0.185	0.203	0.231	0.263	0.270	0.303	0.320	0.343	0.266
Glycerin (2.5%)	0.185	0.208	0.240	0.272	0.277	0.309	0.327	0.350	0.271
Gum Arabic (5%) + Glycerin (2.5%)	0.185	0.207	0.238	0.265	0.267	0.302	0.32	0.338	0.265
Gum Arabic (10%) + Glycerin (2.5%)	0.185	0.206	0.235	0.262	0.269	0.3	0.318	0.336	0.264
Calcium Lactate (1%) + Glycerin (2.5%)	0.185	0.205	0.237	0.268	0.273	0.306	0.324	0.347	0.268
Calcium Lactate (3%) + Glycerin (2.5%)	0.185	0.206	0.235	0.265	0.271	0.305	0.322	0.346	0.267
Control	0.185	0.208	0.242	0.275	0.278	0.309	0.329	0.352	0.272
Mean	0.185	0.206	0.236	0.268	0.274	0.306	0.324	0.348	
CD at 5%	T=0.004 D=0.004 T×D=0.005								

Polygalacturonase (PG)

Data presented in table 3 indicated that PG activity increased with the increase in period of storage. PG activity increased from 0.079 units/ g FW on initial day of storage to 0.181 units/ g FW on 49^{th} day of storage. This might be correlated with the decrease in firmness as polygalacturonase enzyme hydrolyses pectin (major component of cell wall) which results in softening of fruits. So, with the decrease in firmnesss the activity of PG increases. These results are in agreement with the findings in Ber (Sharma & Siddiqui, 2004; Jawanda *et al.* 2009; Yadav *et al.* 2012) [13,11,18], Apple (Goulao *et al.* 2007) [8] and Peach (Ullah *et al.* 2013) [17]. The Kinnow fruits coated with different concentration of Gum Arabic, Calcium Lactate and Glycerin and their combination showed progressive increase in PG activity during storage but the increase was much higher in control during the complete storage period. Maximum PG activity was found in control (0.127 units/ g FW) Glycerin 2.5% treated fruits (0.125 units/ g FW). Gum Arabic 10% + Glycerin 2.5% coated fruits showed minimum PG activity i.e. 0.120 units/ g FW. The lower activity of polygalacturonase in coated fruits might be due to the reason that the coating effectively prevent the access of pectinolytic enzymes to the substrate of the cell wall and thus help maintain fruit firmness. Similar results were reported by Zhou *et al* (2011) [19] in Pears.

Conclusion

It maybe concluded that all coatings were effective to check the activity of the fruits wall degrading enzymes and decreased the softening process as a result of which firmness of the kinnow fruits maintained for longer times. However, Gum Arabic 10% + Glycerin 2.5% coating was the most effective to minimize the activity of enzymes and maintained the firmness.

Table 3: Effect of different treatments on Polygalacturonase activity (units/g FW) in Kinnow fruits during storage at room temperature

Treatments	Days of storage								
	0	**7**	**14**	**21**	**28**	**35**	**42**	**49**	**Mean**
Gum Arabic (5%)	0.079	0.083	0.095	0.115	0.127	0.142	0.165	0.183	0.124
Gum Arabic (10%)	0.079	0.082	0.094	0.114	0.126	0.141	0.164	0.181	0.123
Calcium Lactate (1%)	0.079	0.083	0.096	0.117	0.127	0.144	0.166	0.183	0.124
Calcium Lactate (3%)	0.079	0.084	0.093	0.115	0.126	0.143	0.164	0.182	0.123
Glycerin (2.5%)	0.079	0.085	0.096	0.118	0.128	0.145	0.167	0.185	0.125
Gum Arabic (5%) + Glycerin (2.5%)	0.079	0.080	0.092	0.112	0.124	0.140	0.163	0.178	0.121
Gum Arabic (10%) + Glycerin (2.5%)	0.079	0.079	0.092	0.110	0.123	0.138	0.162	0.176	0.120
Calcium Lactate (1%) + Glycerin (2.5%)	0.079	0.082	0.094	0.115	0.127	0.143	0.165	0.181	0.123
Calcium Lactate (3%) + Glycerin (2.5%)	0.079	0.081	0.093	0.113	0.125	0.142	0.163	0.180	0.122
Control	0.079	0.085	0.097	0.120	0.131	0.146	0.169	0.186	0.127
Mean	0.079	0.082	0.094	0.115	0.126	0.142	0.165	0.181	
CD at 5%	T=0.002 D=0.002 T×D=0.010								

References

1. Abu- Goukh AA, Bashir HA. Changes in pectic enzymes and cellulase activity during guava fruit ripening. Journal of Food Chemistry 2008; 83:213-218.
2. Ahmed AE, Labavitch JM. Cell wall metabolism in ripening fruits. II. Changes in carbohydrate-degrading enzymes in ripening 'Bartlett' pears. Plant Physiology. 1980; 65:1014-1016.
3. Ali MB, Abu-Goukh AA. Changes in pectic substances and cell wall degrading enzymes during tomato fruit ripening. Journal of Agricultural Sciences. 2005; 13:202-223.
4. Barka EA, Kalantati S, Makhlouf J, Arul J. Impact of UV-C irradiation on the cell wall degrading enzymes during ripening of tomato (*Lycopersican esculentum* L.) fruit. Journal of Agricultural Food Chemistry. 2000; 48: 667-671.
5. Bisen A, Pandey SK. Effect of post harvest treatment on biochemical composition and organoleptic quality in Kagzi lime fruit during storage. Journal of Horticulture Science. 2008; 3:53-56.
6. Carvalho AB, Assis SAD, Leite KMSC, Bach EE, Oliveria OMMF. Pectin methyl esterase activity and ascorbic acid content from guava fruit, *cv.* Predilecta, in different phases of development. International Journal of Food Sciences and Nutrition. 2009; 60:255-265.
7. Gol NB, Patel PR, Ramana Rao TV. Improvement of quality and shelf-life of strawberries with edible coatings enriched with chitosan. Postharvest Biology and Technology. 2013; 85:185-195.
8. Goulao LF, Santos J, Sousa ID, Oliveira CM. Pattern of enzymatic activity of cell wall modifying enzymes during growth and ripening of apples. Postharvest Biology and Technology. 2007; 43:307-318.
9. Hagerman AE, Austin PJ. Continuous spectrophotometric assay for plant pectin methyl esterase. Journal of Agricultural Food Chemistry. 1986; 34:440-444.
10. Harker FR, Redgwell RJ, Hallett IC, Murray SH, Carter G. Texture of fresh fruit. Horticultural reviews. 1997; 20: 121-224.
11. Jawandha SK, Mahajan BVC, Gill PS. Effect of preharvest treatments on the cellulase activity and quality of ber fruit under cold storage conditions. Notulae Scientia Biologicae. 2009; 1:88-91.
12. Mondal K. Antagonistic effect of polyamines on ripening related biochemical changes in guava (*Psidium guajava* L.), PhD Thesis, CCS Haryana Agricultural University, Hisar, India, 2005.
13. Sharma RK, Siddiqui S. Ripening of ber (*Ziziphus mauritiana* L.) fruits on tree and during storage: study on pulp firmness and cell wall composition. Indian Journal of Horticulture. 2004; 61:308-311.
14. Sihag RP, Behiwal LS, Mehta PK. Effect of post harvest application of potassium permanganate on shelf life of Peach fruit. Haryana Journal of Horticulture Science. 2005; 34:259-260.
15. Singh A, Singh M. Cell wall degrading enzymes in *Orobanche aegyptiaca* and its host *Brassica campestris*. Physiologia Plantarum. 1993; 89:177-181.
16. Somogyi M. Notes on sugar determination. Journal of Biological Chemistry. 1952; 195:19-23.
17. Ullah S, Khan AS, Malik AU, Shahid M. Cultivar and harvest location influence fruit softening and antioxidative activities of peach during ripening. International Journal of Agriculture and Biology. 2013; 15:1059-1066.
18. Yadav P, Kumar S, Jain V, Malhotra SP. Cell wall metabolism of two varieties of ber (*Ziziphus mauritiana* Lam.) fruit during ripening. Food Technology and Biotechnology. 2012; 50:467-472.
19. Zhou R, Li Y, Yan L, Xie J. Effect of edible coatings on enzymes, cell membrane integrity, and cell-wall constituents in relation to brittleness and firmness of Huanghua pears (*Pyrus pyrifolia Nakai* cv. Huanghua) during storage. Food Chemistry. 2011; 124:569-575.