

Full Length Research Paper

An assessment of the effect of pre-harvest treatments on the cellulase activity and quality of ber fruit under cold storage conditions

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Studies were carried out to find out the effect of various pre-harvest treatments: CaCl_2 (@ 0.5, 1.0 and 2.0%), $\text{Ca}(\text{NO}_3)_2$ (@0.5, 1.0 and 2.0%), GA_3 (@ 20, 40 and 60 ppm) and Bavistin (@ 0.1%) on the cellulase activity and quality of 'Umran' ber fruits during cold storage. Marked trees were sprayed at colour break stage with the test chemicals. Fruits were packed in CFB boxes and placed in cold storage (3 - 5°C and 85 - 90% RH) for 30 days. The fruits were evaluated after 10, 20 and 30 days interval for various parameters such as cellulase activity, phenolic content, palatability rating and rotting percentage. Cellulase activity registered a gradual increase up-to 20 days of storage thereafter a decline was noted in all the treatments. The palatability rating increased up to 10 days of storage in all the treatments, except control but subsequently it decreased with longer in storage periods. Among the various pre-harvest treatments CaCl_2 (2%) recorded minimum cellulase activity and rotting percentage and registered high palatability rating and phenolics content during cold storage conditions. Studies showed that pre-harvest application of CaCl_2 (2%) maintained very good fruit quality and prolonged shelf-life for 20 days under cold storage conditions.

Key words: Ber, storage, cellulase, calcium, GA_3 .

INTRODUCTION

Ber (*Zizyphus mauritiana* Lamk) is a hardy fruit, which can thrive well under adverse edaphic and climatic conditions. It is a nutrition rich fruit, containing high levels of calcium, phosphorus and vitamin C but comparatively low market price and therefore, referred as poor man's apple. It is an ideal fruit for cultivation in the arid and semi arid zones of northern India, as it has the negligible irrigation requirement throughout hot and dry period of May and June. High yielding cultivar 'Umran' is commercially grown in Northern India. During its peak harvesting season there is usually a glut in the market with a crash in its market price and low returns to the growers. Like most of other fruits, ber is perishable in nature and cannot be stored for long periods under ambient conditions (Salunkhe and Kadam, 1995). During storage the rotting of fruit depends upon the activity of cellulase enzyme. Higher activity of this enzyme results in fruit softening which subsequently leads to the decay of the fruits. Cellulase is considered to be responsible for the hydrolysis of cellulose fibrils of the cell wall (Babbita et al., 1973). Calcium compound extend the shelf-life of several fruits by maintaining fruit firmness, minimizing rate of respi-

ration, protein breakdown and disease incidence (Gupta et al., 1984). Calcium is important in the maintenance of cell wall integrity in plants. Heavy influx of external or internal calcium inhibits ripening process due to reduction in enzymatic activity. Similarly, certain growth regulators like Gibberellic acid are known to promote the shelf life of fruits. Mehta et al. (1986) reported that GA_3 @ 100 ppm significantly suppresses the succinate activities of malatedehydrogenase during post-harvest ripening of papaya and thus retard ripening. The growers can greatly benefit if storage health is maintained by application of these compounds. Therefore, the present studies were conducted to investigate the effect of pre-harvest application of various chemicals on cellulase activity and fruit quality during cold storage.

MATERIALS AND METHODS

The present investigations were carried out in the department of Horticulture Punjab Agricultural University Ludhiana, India during 2002 - 2003 on commercially bearing Umran trees of similar age maintained under similar cultural practices. The chemicals viz; CaCl_2 (0.5, 1.0 and 2.0%), $\text{Ca}(\text{NO}_3)_2$ (0.5, 1.0 and 2.0 %) and GA_3 (20, 40 and 60 ppm) were sprayed on the trees at the colour break stage. The solutions of CaCl_2 and $\text{Ca}(\text{NO}_3)_2$ were prepared by dissolving the salts in irrigation water and sprayed after cooling at

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Table 1. Cellulase activity in ber fruit influenced by pre-harvest chemical treatments and storage during 2002 and 2003.

Treatment	Cellulase activity (Percent reduction in viscosity)										
	2002					2003					
	Days after storage (DAS)					Days after storage (DAS)					
	0	10	20	30	Mean (Treatment)	0	10	20	30	Mean (Treatment)	
CaCl ₂ 0.5%	0.88	1.15	2.20	1.54	1.44	0.85	1.17	1.97	1.53	1.38	
CaCl ₂ 1%	0.79	1.06	1.88	1.56	1.32	0.72	1.04	1.89	1.57	1.31	
CaCl ₂ 2%	0.70	0.92	1.78	1.73	1.28	0.65	0.80	1.80	1.76	1.26	
Ca(NO ₃) ₂ 0.5%	0.88	1.20	2.26	1.40	1.43	0.86	1.17	2.07	1.49	1.40	
Ca(NO ₃) ₂ 1%	0.82	1.16	2.03	1.50	1.38	0.72	1.07	1.98	1.54	1.33	
Ca(NO ₃) ₂ 2%	0.74	1.01	1.85	1.60	1.30	0.68	0.96	1.92	1.61	1.29	
GA ₃ - 20 ppm	0.85	1.10	2.21	1.50	1.41	0.70	1.02	2.16	1.56	1.36	
GA ₃ - 40 ppm	0.70	1.02	1.85	1.60	1.29	0.65	0.96	1.90	1.64	1.28	
GA ₃ - 60 ppm	0.66	0.94	1.80	1.70	1.28	0.63	0.88	1.82	1.72	1.26	
Bavistin 0.1%	0.88	1.23	2.27	1.38	1.44	0.86	1.20	2.18	1.44	1.42	
Control (untreated)	0.90	1.98	1.63	1.30	1.45	0.89	1.89	1.65	1.32	1.44	
Mean (DAS)	0.80	1.16	1.99	1.53		0.75	1.10	1.94	1.56		
C. D (0.05)											
Treatments (A)	=	0.0580					0.059				
DAS (B)	=	0.0349					0.060				
Interaction (A x B)	=	0.116					0.199				

ambient temperature. Whereas, the GA₃ was initially dissolved in ethyl alcohol before making the final volume with irrigation water. All the experimental trees, except control, were sprayed with Bavistin (0.1%) 15 days before harvesting. Eleven treatments including control were laid out in completely randomized block design with three replications.

Fruits were harvested at optimum maturity from the selected trees. One kilogram fruit from each replication of respective treatment was packed in netlon carriers. Thereafter, the packed fruits were placed in corrugated fibre board (CFB) boxes (5% perforations) with paper lining and kept in cold storage (temperature 3 - 5°C and R.H- 85 - 90%). Fruit samples were analysed for physico- chemical characteristics after 10, 20 and 30 days of storage. Palatability rating (PR) was recorded on the basis of color, taste, flavor and general appearance by a panel of five judges on a score card viz: 1-Poor; 2-Fair; 3-Good; 4-Verygood and 5-Excellent. The percent of rotting fruits (PRF) was calculated by counting the number of healthy fruits retained (F_r) and total number of fruits (F_t) within each storage interval using the equation. The PRF was calculated as $PRF = 100[F_t - F_r] / (F_t)^{-1}$. Phenolics were estimated as total tannins after developing colour with Folin-Denis reagent (AOAC 1980). The absorbance of the developed blue colour recorded in 'Spectronic-20' colorimeter at 660 nm wave length.

Cellulase activity was estimated at various intervals of fruit storage by measuring the per cent reduction in viscosity of substrate. For assay 4 ml of carboxymethyl cellulose (CMC) solution, 1 ml of sodium acetate acetic acid buffer (pH 5.2) and two millilitres of enzyme source (extract) pipetted into the visco-meter. Mixed the contents by drawing air rapidly through the large arm of the viscometer by suction. Then, suction was applied to viscometer through the small arm and the efflux time of the mixture was determined. This was considered as the zero time. Efflux time of the mixture was determined at various intervals and cellulase activity was expressed as per cent reduction in viscosity of the substrate (Mahadevan and

Sridhar, 1982). The data was analyzed using CPCS-1 software package (developed by the Department of Statistics, Punjab Agricultural University, Ludhiana, India).

RESULTS AND DISCUSSION

Cellulase activity varied significantly among different treatments and on storage interval during both years of the study (Table 1). All treatments showed a progressive increase in cellulase activity with the advancement of storage period up to 20 days of storage, but afterwards the enzyme activity decreased towards the end of storage (Table 1). After 20 days of storage, the minimum cellulase activity was recorded in CaCl₂ (2%) treated fruits followed by GA₃ 60 ppm treatment. Heavy influx of external calcium inhibits the ripening process due to reduction of enzyme activity. Presence of calcium ions limits the polyglacturo-nase activity in the cell wall of the apple fruit skin (Gleen and Pooviah 1986). External application of Ca increased the rigidity of cell wall. At the end of storage, maximum enzyme activity was observed in CaCl₂ (2%) treated fruits due to higher substrate content retained by this treatment. However, in other treatment substrate content for enzyme activity was decomposed within 20 days of storage. Similar results were reported by Mahajan (1994) in apple (*M. domestica*).

High phenolic content in fruits is linked with higher resistance from various pathological rots (Khare et al., 2007). Total phenolic content decreased with the advancement of storage period regardless of

Table 2. Total phenolic in ber fruit influenced by pre-harvest chemical treatments and storage during 2002 and 2003.

Treatment	Total Phenolic (%)									
	2002					2003				
	Days after storage (DAS)				Mean (Treatment)	Days after storage (DAS)				Mean (Treatment)
0	10	20	30	0		10	20	30		
CaCl ₂ 0.5%	0.104	0.098	0.088	0.078	0.092	0.103	0.098	0.089	0.076	0.091
CaCl ₂ 1%	0.105	0.098	0.090	0.082	0.094	0.106	0.096	0.091	0.080	0.93
CaCl ₂ 2%	0.108	0.103	0.098	0.090	0.100	0.110	0.107	0.099	0.093	0.102
Ca(NO ₃) ₂ 0.5%	0.102	0.096	0.084	0.066	0.087	0.103	0.098	0.086	0.070	0.089
Ca(NO ₃) ₂ 1%	0.104	0.098	0.087	0.074	0.091	0.105	0.098	0.088	0.076	0.092
Ca(NO ₃) ₂ 2%	0.107	0.103	0.092	0.084	0.097	0.108	0.102	0.092	0.085	0.097
GA ₃ -20 ppm	0.106	0.100	0.085	0.074	0.091	0.107	0.101	0.084	0.075	0.092
GA ₃ -40 ppm	0.107	0.103	0.096	0.084	0.098	0.108	0.105	0.097	0.086	0.099
GA ₃ -60 ppm	0.110	0.104	0.096	0.088	0.099	0.112	0.106	0.098	0.090	0.101
Bavistin 0.1%	0.103	0.096	0.084	0.068	0.087	0.103	0.096	0.082	0.070	0.087
Control (untreated)	0.102	0.086	0.070	0.057	0.078	0.103	0.088	0.062	0.059	0.078
Mean (DAS)	0.105	0.099	0.088	0.076		0.106	0.100	0.088	0.078	
C.D. (0.05)										
Treatments (A)	=	0.0029					0.006			
DAS (B)	=	0.0017					0.0039			
Interaction (A x B)	=	0.0059					NS			

pre-harvest treatment (Table 2). Similar results were reported by Panwar (1981) and Goel and Siddiqui (1999) in Ber fruits. After 30 days of storage fruits treated with CaCl₂ (2%) exhibited maximum phenolic content (0.090 and 0.093 %) while it was minimum in control. Similarly in avocados, calcium compound treatments resulted in suppression of respiration and high phenolic content of the fruits (Rensburg and Engelbrecht, 1986).

At the time of harvesting the untreated fruits showed maximum palatability rating (Table 3). The pre-harvest treatments might have retarded the ripening process of ber fruits which led to low palatability as compared to untreated fruits. But, after 10 days of storage the PR of fruits increased in all the treatments except control, where it decreased. However with the advancement of storage period, there was continuous reduction in PR in all the treatments. At the end of storage, the maximum PR (3.50 and 3.70) was recorded in CaCl₂ (2%) treated fruits which was followed by GA₃ 60 ppm treatment. These pre-harvest treatments of Ca and GA₃ might have delayed senescence which resulted in maintenance of fruit health in storage. Similar results are obtained by Kumar et al. (1994) in 'Banarasi Pawandi' fruits.

After 20 days of cold storage, rotting was noticed in some treatments (control > Bavistin 0.1% > Ca (NO₃)₂ 0.5% > CaCl₂ 0.5%) while fruits from remaining treatments maintained their normal health. At the end of cold storage, the percentage of rotten fruits was significantly

higher in untreated fruits as compared to all other treatments (Table 4). The fruit treated with CaCl₂ (2%) registered minimum rotting followed by GA₃ 60 ppm treatment. An increase in calcium content of fruit has been associated with decreased incidence of physiological disorders, improved storage life, reduced severity of bacterial and fungal rots (Raese 1986; Conway and Sams, 1994) as calcium is known to impart resistance against the attack of infectious pathogens (Bangreth et al., 1972). Calcium compounds, significantly thickened the middle lamella of fruit cells owing to increased deposition of calcium pectate and thereby maintained the cell wall rigidity which inhibits the penetration and spread of pathogens in fruits (Gupta et al., 1987). The prolongation of fruit life due to growth regulators was probably due to effectiveness of these chemicals in retaining of green pigments, retardation of ripening and senescence (Huang, 1974). These results are in line with findings of Gupta et al. (1980) who suggested that the pre-harvest sprays of calcium compounds (Chlorides, Nitrates, Sulphates and Phosphates) reduced the decay loss in ber fruits.

Conclusion

It can be concluded from the study that pre-harvest application of CaCl₂ (2%) is useful to prolong the storage life of ber fruits up to 20 days with very good fruit quality under cold storage conditions.

Table 3. Palatability rating of ber fruit influenced by pre-harvest chemical treatments and storage during 2002 and 2003.

Treatment	Palatability rating (1- 5)									
	2002					2002				
	Days after storage (DAS)				Mean (Treatment)	Days after storage (DAS)				Mean (Treatment)
0	10	20	30	0		10	20	30		
CaCl ₂ 0.5%	4.08	4.50	3.30	2.33	3.55	4.00	4.50	3.25	2.50	3.56
CaCl ₂ 1%	3.91	4.60	3.90	2.80	3.80	3.83	4.60	4.00	3.00	3.86
CaCl ₂ 2%	3.75	4.85	4.50	3.50	4.15	3.80	4.75	4.33	3.70	4.14
Ca(NO ₃) ₂ 0.5%	4.20	4.40	3.25	2.20	3.51	4.25	4.50	3.10	2.33	3.54
Ca(NO ₃) ₂ 1%	4.00	4.50	3.70	2.60	3.70	3.83	4.65	3.58	2.70	3.69
Ca(NO ₃) ₂ 2%	3.80	4.70	4.16	3.20	3.96	3.75	4.70	4.10	3.25	3.95
GA ₃ -20 ppm	4.00	4.66	3.60	2.60	3.71	3.80	4.60	3.50	2.70	3.65
GA ₃ -40 ppm	3.80	4.80	4.00	3.20	3.95	3.70	4.75	4.10	3.30	3.96
GA ₃ -60 ppm	3.70	4.70	4.30	3.40	4.02	3.60	4.70	4.25	3.60	4.04
Bavistin 0.1%	4.25	4.33	3.00	2.10	3.42	4.50	4.25	3.25	2.20	3.55
Control (untreated)	4.66	4.00	2.40	1.60	3.16	4.50	4.00	2.50	2.00	3.25
Mean (DAS)	4.01	4.55	3.67	2.68		3.96	4.54	3.63	2.84	
CD _(0.05)										
Treatments (A)	=	0.161					0.190			
DAS (B)	=	0.097					0.114			
Interaction (A x B)	=	0.323					0.380			

Table 4. Rotting of ber fruit influenced by pre-harvest chemical treatments and storage during 2002 and 2003.

Treatment	Percent rotting							
	2002				2003			
	Days after storage (DAS)			Mean (Treatment)	Days after storage (DAS)			Mean (Treatment)
10	20	30	10		20	30		
CaCl ₂ 0.5%	*-	0.90	2.10	1.0	*-	0.60	1.80	0.80
CaCl ₂ 1%	-	-	1.34	0.44	-	-	1.25	0.42
CaCl ₂ 2%	-	-	0.78	0.26	-	-	0.50	0.17
Ca(NO ₃) ₂ 0.5%	-	1.25	2.63	1.29	-	1.0	2.20	1.07
Ca(NO ₃) ₂ 1%	-	-	1.82	0.60	-	-	1.40	0.47
Ca(NO ₃) ₂ 2%	-	-	1.25	0.41	-	-	1.20	0.40
GA ₃ -20 ppm	-	-	1.92	0.64	-	-	1.50	0.50
GA ₃ -40 ppm	-	-	1.20	0.40	-	-	1.00	0.33
GA ₃ -60 ppm	-	-	1.10	0.36	-	-	0.80	0.27
Bavistin 0.1%	-	1.30	3.74	1.68	-	1.20	3.50	1.57
Control (untreated)	-	3.02	8.53	3.85	-	2.50	8.30	3.60
Mean (DAS)	-	0.59	2.40		-	0.48	2.13	
CD _(0.05)								*- = No rotting
Treatments (A)	=	0.199				0.218		
DAS (B)	=	0.104				0.114		
Interaction (A x B)	=	0.344				0.379		

REFERENCES

- AOAC (1980). Official methods of analysis of analytical chemists. Association of the Official Analytical Chemists, Washington, D.C.
- Babbita JK, Powers MJ, Patterson ME (1973). Effect of growth regulators on cellulase, polygalacturonase, respiration, colour and texture of ripening tomato. J. Am. Soc. Hort. Sci. 98: 71- 81.
- Bangreth F, Dilley DR, Dewey DH (1972). Effect of post-harvest calcium

- treatments of apple on internal breakdown and respiration of Apple fruits. *J. Am. Soc. Hort. Sci.* 97: 679-82.
- Conway WS, Sams CE (1994). Possible mechanism by which postharvest calcium treatment reduces decay in apples *Malus domestica* inoculation with *Penicillium expansum*. *Phyto Pathology*, 74: 208-10.
- Gleen GM, Poovaiah BW (1986). The role of calcium in delaying softening of apples. *Hort. Sci.* 21: 666.
- Goel A, Siddiqui S (1999). Changes in enzyme activities and physico-chemical characteristics of ber (*Zizyphus mauritiana* Lamk) cv. Umran as influenced by harvesting maturity. *Ind. J. Agric. Res.* 33: 209-13.
- Gupta OP, Jindal PC, Singh BP (1980). Effect of pre-harvest spray of calcium nitrate on the storage behaviour of grapes cv. Perlette. *J. Res. Haryana Agric. Univ.* 10: 204-06.
- Gupta OP, Siddiqui S, Chauhan KS (1987). Evaluation of various calcium compounds for increasing the shelf life of ber (*Zizyphus mauritiana* Lamk) Fruits. *Ind. J. Agric. Res.* 21: 65-70.
- Gupta OP, Singh BP, Singh SP, Chauhan KS (1984). Effect of calcium compounds as pre-harvest spray on the shelf life of peach cv. Sharbati. *Punjab Hort. J.* 24: 105-10.
- Huang CC (1974). Maintaining freshness of pineapple fruits for export. *Taiwan Agriculture Quarterly* 10: 103-11.
- Khare V, Mehta P, Kachhwaha M, Mehta A (2007) Role of phenolic substances in pathogenesis of soft rot diseases *J. Basic Microbiol.* 34(5): 323-328.
- Kumar D, Rajput CBS, Singh SP (1994). Effect of calcium chloride and Bavistin on the post-harvest life of jujube fruits (*Zizyphus mauritiana* Lamk) cv. Banarasi Pewandi. *Prog. Hort.* 26: 72-76.
- Mahadevan A, Sridhar R (1982). *Methods in physiological plant pathology*, Sivagami Pub. Madras.
- Mahajan BVC (1994). Biochemical and enzymatic changes in apple during cold storage. *J. Food Sci. Tech.* 31: 142-44.
- Mehta PM, Raj SS, Raju PS (1986). Influence of fruit ripening retardants on succinate and malate dehydrogenase in papaya fruits with emphasis on preservation. *Ind. J. Hort.* 43: 169-73.
- Panwar JS (1981). Post harvest physiology and storage behaviour of ber fruit (*Zizyphus mauritiana* Lamk) in relation to temperature and various treatments cv. Umran and Kaithli. Thesis Abstract : 64-65.
- Raese JT (1986). Nitrogen and calcium important in determining yield, fruit quality and disorders of 'Anjou' pears. In. *Proc. Pac. Northwest Tree Fruit Short Course* pp. 155-68.
- Rensburg E Van, Engelbrecht AHP (1986). Effect of calcium salts on susceptibility to browning of avocado fruit. *J Food Sci.* 51: 1067-68.
- Salunkhe DK, Kadam SS (1995). *Handbook of Fruit Science and Technology* pp. 387. Marcel Dekker Inc., New York.