ISSN 1110-0419

Annals Of Agric. Sc., Moshtohor,
Vol. 44(3): 1239-1252, (2006).

EFFECT OF DIFFERENT METHODS AND CONCENTRATIONS OF PROLINE PREHARVEST AND POSTHARVEST APPLICATION ON THE CONTROL OF CHILLING INJURY OF BANZAHIR LIMES AND MARSH GRAPE FRUITS DURING COLD STORAGE

BY

Ekbal, Z. Aly
Sabahia Research Station, Alexandria, Ministry of Agriculture, Egypt

ABSTRACT

The results of 2004 and 2005 seasons revealed that all proline treatments significantly reduced chilling injury, weight loss percentage and percent of unmarketable fruits during cold storage i.e. eliminated fruit surface pitting. This effect was clear in Banzahir limes than that of marsh grapefruit.

In both seasons, peel proline free amino acids significantly increased with proline treatments and with increasing cold storage duration for both Banzahir limes and marsh grapefruit, whereas, peel total sugars decreased with proline application.

All proline treatments caused a pronounced increase in fruit juice and ascorbic acid, while acidity was decreased, TSS were increased with storage time.

We can noticed that proline foliar treatments had better result compared with proline fruit dipping treatments.

Key words: proline, citrus fruits, cold storage injury

INTRODUCTION

Storage of citrus fruits at relatively high temperatures might be considered unappropriate due to the high incidence of fungal attack and rapid fruit deterioration. On the other hand, being of tropical and subtropical regions, citrus fruits are potentially chilling sensitive and susceptible to low storage temperatures injure (El-Helaly, 2002). Chandler (1985), Davis and Harding (1995), as well as, numerous of other investigators had reported that fruit peel pitting, a form of rind breakdown, occurs frequently on citrus fruits under cold storage. Differences between citrus species and varieties in the development of peel pitting; the chilling injury symptom, were quite noticed. Purvis (1980) and Chandler (1985) stated that lime and grapefruit rinds, certainly, tended to be more resistant to chilling injury such as pitting than other citrus cvs. Nevertheless, Purvis (1980) compared the respiration rate of chill-sensitive grapefruit flavedo and that of limes, the more chill-resistant and found a break in Arrhenius plot of CO_2 evolution and O_2 uptake by grapefruit flavedo at 12°C or lower. He pointed out that oxidase activity, membrane proteins and/or change in the substrate respired at chilling temperatures may have a role in the development of peel chilling injury. Furthermore, Purvis (1981), Purvis and Yelenosky (1982) and Nordby *et al.* (1987) found that during cold acclimation of young grapefruit trees considerable change occurred in proline and soluble carbohydrates of fruit peel flavedo tissue. They suggested that these changes might be in direct relation with the susceptibility of grapefruit rind to chilling injury. Purvis *et al.* (1997) found that fruits harvested during mid-season (February – March) are generally, less susceptible to Cl than fruits harvested earlier in the season (October- January). He mentioned that proline levels were high in March as a result of low temperatures and reach a minimum in January.

Syvertsen and Smith (1993) pointed out that proline is synthesized in leaves and transported to fruit, and they indicated that the concentration of proline did not change appreciably neither in lime nor in grapefruit peel during the storage period. Therefore, this investigation carried out to study the effect of preharvest and postharvest proline application on minimizing chilling injury of Banzahir limes and Marsh grapefruits.

MATERIALS AND METHODS

This investigation was carried out during 2004 and 2005 seasons in order to study the effect of pre harvest foliar spray of proline and postharvest fruit dipping treatments in proline (amino acid) at different concentrations in eliminating chilling injury on Banzahir limes and Marsh grapefruit during cold storage at 5°C and RH (85–90). The Banzahir lime trees were 18 years old and grapefruit trees were 25 years old, grown in a private orchard at El-Taarh village, El-Behera Governorate.

The trees were budded on sour orange rootstock and spaced at 5 meters apart. The orchard soil was clay loam. The trees were flood irrigated with Nile water, fertilized with 19 cubic meters of organic manure per feddan in winter, and with ammonium nitrate (33% N) at a rate of 2.5 kg per tree, a three equal doses, March, may and July. Twenty eight nearly uniform trees were selected from both species for the study. Sixteen trees from the selected trees were arranged in a randomized complete block design and were sprayed with water only (control), 0.4, 0.8 and 1.2% proline each spray treatment was replicated 4 times. In both experimental seasons the trees were sprayed three times, at full bloom, fruit set and four weeks before harvested. Fruit of both species were harvested during the second of November of both 2004 and 2005 from the experimented trees. From all selected trees (both citrus species), 150 round fruits free of rind punctures and of similar size were chosen from each replicate (tree).

For fruit dipping treatments in proline, fruit from other 12 trees (non sprayed trees) from both citrus species were also, picked and 150 fruits were taken from each replicate (tree). Fruits were dipped in 2% tween 80 + proline at 0.4, 0.8 and 1.2 % (for 5 minutes) and the dipping treatments were repeated 3 times at one hour intervals, then fruits were allowed to dry. All treated fruits were held in plastic nets, then stored at 5°C with 85–90% relative humidity for 12 weeks. The storage temperature was proposed by Purvis and Grierson (1982) to maximize the development of fruit peel pitting and the symptoms of chilling injury were recorded at 3 weeks interval.

Four fruit samples (replicates) of 10 fruits each, were taken at random from both fruit species and fruit were periodically weighed and the loss in weight loss percentage was calculated.

The number of unmarketable fruits due to chilling injury, decay and shrinkage was recorded and calculated as percentage from the total number of each sample.

Four fruit samples (replicates) of 10 fruits in each, were taken at random from both fruit species. The pitted areas on the peel of each fruit were measured by tracing the outlines of all the pitted spots onto tracing paper (Ahmed and Ismail, 2000).

In the peel tissue (flavedo and albedo) of each sample soluble sugar were extracted by 80% ethanol. The total soluble sugars of the extract was determined before and after hydrolysis with HCl by the Nelson arseno-Molybdate colorimetric method, as described by Malik and Singh (1980). Total free alpha amino acids were directly determined in the sugar alcoholic extract using Lee and Takahashi (1966) method. Proline of the flavedo was determined as described by Bates *et al.* (1973).

The juice was extracted from eight fruits of each sample (Rep.). It was analyzed for total soluble solids, acidity and vitamin C. The percentage of total soluble solids in the juice was determined by a hand refractometer. Acidity of juice was determined by titration with 0.1 N sodium hydroxide. The percentage of acid (expressed as citric acid) was then calculated. Ascorbic acid (Vitamin C)was determined by titration with 2, 6-dichlorophenol indophenol dye in the juice and expressed as mg/100 ml juice. According to A.O.A.C (1990) The data collected throughout the course of this study was statistically analyzed using the randomized complete block design with 7 treatments and 4 replicates as a method described by Snedecor and Cochran (1990). The L.S.D method at 0.05 level was used to compare the effect of treatments (T), date of sampling by weeks (W)and their interaction (T x W).

RESULTS AND DISCUSSION

Chilling injury symptoms

The data presented in Tables (1 and 2) showed that in both seasons of study the symptoms of chilling injury were significantly reduced by all proline treatments compared to control. It was clear from the pitted area (PA) data that e it was almost eliminated. In the meantime, the data indicated that, the two foliar applications of proline at (0.8 and 1.2%) significantly had the best result in this respect compared with other proline treatments in both citrus species.

The effect of proline treatments in eliminating fruit surface pitting symptoms of Cl, was clear in Banzahir limes than that of marsh grapefruit. Purvis (1980) pointed out that limes and orange were more resistant or less susceptible to chilling injury compared to grapefruit. Moreover, it was noticed that the development of surface pitting was delayed towards the end of the storage period. This argument was more pronounced in Banzahir limes. These results were in line with those obtained by Purvis (1980).

Peel composition

The data of the present investigation, generally, indicated that in both seasons of study, All proline treatments significantly reduced pitting and fruit had higher proline concentration in the peel of both types of fruit as compared with the control fruits. In the meantime, the proline foliar application at (0.8 and 1.2%) induced resulted in higher peel proline content than other proline application treatments in both types of fruits. Data, also indicated that the concentration of proline in the peel of either Banzahir limes or Marsh grapefruit tended to show a marked increase with increasing cold storage duration. This increment, however, was supported by statistical significancy in either years of study and or with both citrus species (Table 1 and 2). These results appeared to agree with those reported by other numerous investigators, Purvis and Yelenosky (1982), Syvertsen and Smith (1983) as well as, Nordby *et al.* (1987)who reported that proline apparently increased in grapefruit flavedo or peel tissues in response to low temperatures. The concentration of free amino acids in the peel of both fruit species was significantly higher in all proline applications as compared with the control. In the meantime free amino acids in peel of fruit were higher in fruits treated with foliar application at (0.4, 0.8 and 1.2%) than fruit treated with other applications in both years of study (Tables 1 and 2).

Moreover, a general increase of peel free amino acid content in all treated fruits with proline was noticed with storage duration in both seasons of study. This trend is in agreement with the findings of Leopold and Kriedmann (1981) who reported that subjecting substropical fruits to chilling temperatures from 0°C to 10°C associated with a matching increase in amino acids. Moreover, Levitt (1969) added that protein hydrolysis under cold storage conditions increased substantially 4 to 9 times than the normal rate .

Table (1): Effect of proline treatments on eliminating chilling injury and peel proline and free amino acid contents of Banzahir limes during cold storage in 2004 and 2005 seasons

Treatments	Stored period (Weeks)											
	2004						2005					
	Pitting area (cm²/fruit)											
	0	3	6	9	12	Mean	0	3	6	9	12	Mean
Control	0.00	0.49	1.32	2.40	3.20	1.48	0.00	0.56	1.82	2.90	3.81	1.82
Proline (S) 0.4%	0.00	0.20	0.36	1.00	1.77	0.67	0.00	0.00	0.30	0.89	1.72	0.58
Proline (S) 0.8%	0.00	0.00	0.23	0.65	1.32	0.44	0.00	0.00	0.19	0.30	0.91	0.28
Proline (S) 1.2%	0.00	0.00	0.18	0.66	1.40	0.448	0.00	0.00	0.11	0.43	1.16	0.34
Proline (D)0.4%	0.00	0.26	0.42	1.18	1.86	0.744	0.00	0.29	0.46	1.29	1.90	0.79
Proline (D) 0.8%	0.00	0.00	0.31	1.03	1.83	0.634	0.00	0.00	0.35	1.23	1.85	0.71
Proline (D) 1.2%	0.00	0.00	0.20	0.90	1.55	0.530	0.00	0.00	0.26	0.96	1.77	0.60
Means	0.00	0.14	0.43	1.12	1.85		0.00	0.12	0.50	1.14	1.87	
$L.S.D._{0.05}$	T= 0.42		W= 0.33		T×W= 2.13		T= 0.45		W= 0.34		T×W= 2.23	
	Proline (mg/ 100g peel)											
Control	7.96	8.40	9.80	9.98	10.66	9.36	7.88	8.20	9.40	9.72	10.20	9.08
Proline (S) 0.4%	12.01	12..66	12.80	13.06	13.80	12.87	13.00	13.59	13.96	14.38	14.88	13.96
Proline (S) 0.8%	13.69	13.87	14.05	15.96	16.11	14.74	13.96	14.50	14.89	15.33	15.92	14.92
Proline (S) 1.2%	13.00	14.31	15.83	16.70	16.95	15.36	14.40	14.78	15.15	15.75	16.27	15.27
Proline (D 0.4%	7.96	11.70	12.00	12.30	12.92	11.38	7.88	11.40	11.78	12.18	12.63	11.17
Proline (D) 0.8%	7.96	11.92	12.19	12.65	12.93	11.53	7.88	11.71	12.00	12.45	12.80	11.37
Proline (D) 1.2%	7.96	12.09	12.36	12.58	12.98	11.59	7.88	11.93	12.17	12.41	12.85	11.45
Means	10.08	12.14	12.72	13.32	13.76		10.41	12.30	12.76	13.17	13.65	
$L.S.D._{0.05}$	T= 0.91		W= 0.68		T×W= 4.57		T= 0.96		W= 0.71		T×W= 4.87	
	Free amino acids (mg/ 100g peel)											
Control	23.18	28.30	33.91	30.33	29.00	28.94	25.11	29.60	35.11	34.13	33.19	31.43
Proline (S) 0.4%	30.88	35.10	39.28	43.18	46.40	38.97	31.13	36.19	40.50	44.60	47.71	40.03
Proline (S) 0.8%	34.25	39.92	45.11	46.12	46.30	42.34	35.28	40.35	46.01	47.12	46.98	43.15
Proline (S) 1.2%	35.40	38.18	44.01	44.15	44.18	41.18	36.23	39.28	45.00	46.17	47.66	42.87
Proline (D 0.4%	23.18	33.00	37.28	37.49	37.66	33.71	25.11	33.80	39.19	39.31	39.79	35.24
Proline (D) 0.8%	23.18	35.13	36.95	38.18	38.36	34.36	25.11	35.40	37.44	38.79	39.61	35.27
Proline (D) 1.2%	23.18	37.10	37.46	37.79	38.32	34.77	25.11	37.98	38.18	38.67	39.44	35.89
Means	27.61	35.25	39.14	39.61	40.03		29.01	36.09	40.06	41.26	42.05	
$L.S.D._{0.05}$	T= 0.94		W= 0.69		T×W= 4.72		T= 0.98		W= 0.73		T×W= 5.11	

Table (2): Effect of proline treatments on eliminating chilling injury and peel proline and free amino acid contents of Marsh grapefruit during cold storage in 2004 and 2005 seasons

Treatments	Stored period (Weeks)											
	2004						2005					
	Pitting area (cm^2/fruit)											
	0	3	6	9	12	Mean	0	3	6	9	12	Mean
Control	0.00	2.96	5.30	8.60	13.42	6.06	0.00	2.98	5.60	9.01	14.00	6.32
Proline (S) 0.4%	0.00	1.80	3.80	5.90	9.49	4.20	0.00	1.60	3.50	5.41	9.30	3.96
Proline (S) 0.8%	0.00	0.00	2.00	3.30	5.06	2.07	0.00	0.00	1.93	3.00	4.86	1.96
Proline (S) 1.2%	0.00	0.00	1.98	3.10	5.02	2.02	0.00	0.00	1.80	2.91	4.69	1.88
Proline (D) 0.4%	0.00	1.90	4.00	5.98	9.60	4.30	0.00	1.81	3.96	5.89	9.40	4.21
Proline (D) 0.8%	0.00	1.87	3.85	5.96	9.55	4.25	0.00	1.85	3.83	5.91	9.49	4.23
Proline (D) 1.2%	0.00	0.00	3.82	5.93	9.52	3.85	0.00	0.00	3.82	5.90	9.50	3.84
Means	0.00	1.22	3.54	5.54	8.81		0.00	1.18	3.49	5.43	8.75	
L.S.D.$_{0.05}$	T= 0.35		W= 0.24		T×W= 1.78		T= 0.33		W= 0.22		T×W= 1.62	
	Proline (mg/ 100g peel)											
Control	4.00	4.30	4.79	5.60	5.91	4.92	4.11	4.42	4.86	6.18	6.99	5.31
Proline (S) 0.4%	6.09	6.75	7.15	8.39	8.79	7.43	6.25	6.96	7.35	8.11	8.39	7.41
Proline (S) 0.8%	8.29	8.67	9.29	9.13	9.55	8.99	8.41	8.87	9.45	9.76	9.93	9.28
Proline (S) 1.2%	8.92	9.18	9.59	9.72	9.98	9.48	9.03	9.30	9.74	9.83	10.21	9.62
Proline (D) 0.4%	4.00	6.60	6.99	7.93	8.61	6.83	4.11	6.13	6.54	7.08	8.06	6.38
Proline (D) 0.8%	4.00	6.89	7.42	8.09	8.51	6.98	4.11	6.43	6.95	7.72	8.34	6.71
Proline (D) 1.2%	4.00	7.13	7.89	8.27	8.70	7.20	4.11	6.26	6.98	7.50	8.24	6.62
Means	5.61	7.07	7.59	8.16	8.58		5.73	6.91	7.41	8.03	8.59	
L.S.D.$_{0.05}$	T= 0.75		W= 0.32		T×W= 3.78		T= 0.69		W= 0.46		T×W= 3.48	
	Free amino acids (mg/ 100g peel)											
Control	22.10	25.01	25.11	27.42	28.01	25.65	22.51	25.41	25.99	28.00	28.77	26.14
Proline (S) 0.4%	25.91	27.19	28.93	29.31	30.93	28.45	26.11	27.40	29.50	29.85	30.91	28.75
Proline (S) 0.8%	26.99	27.98	29.88	31.92	33.01	29.96	27.07	28.11	30.00	32.02	33.42	30.12
Proline (S) 1.2%	27.90	28.42	29.81	32.80	33.67	30.52	28.00	28.71	30.09	32.96	34.00	30.75
Proline (D) 0.4%	22.10	26.20	27.13	28.03	29.91	26.67	22.51	26.73	27.75	28.81	30.62	27.28
Proline (D) 0.8%	22.10	27.00	29.01	30.09	30.30	27.70	22.51	27.14	28.81	30.89	31.02	28.07
Proline (D) 1.2%	22.10	27.60	28.29	30.16	30.40	27.71	22.51	27.83	28.45	30.55	31.01	28.07
Means	24.17	27.06	28.39	29.96	30.89		24.46	27.33	28.66	30.44	31.39	
L.S.D.$_{0.05}$	T= 0.68		W= 0.44		T×W= 3.45		T= 0.72		W= 0.52		T×W= 3.66	

The content of total sugars was significantly higher in the control fruits compared with fruits of proline foliar treatments (Tables 3 and 4). No significant differences were found in total sugars in all proline treatments. The higher content of total sugars in the control might be due to the extreme severity of the CI symptoms leading to an early senescence of the fruit peel. This may led to increase the degradation of the cell wall carbohydrate polymers (Burns, 1990) and consequently an increase in the total soluble sugars.

Data also showed that in both experimental seasons, it was noticed that the total sugars had no constant trend through the storage period for both citrus species. For sugars, it was suggested that an indirect evidence indicated that leaves are the source of soluble carbohydrates accumulating in flavedo during cold acclimation since both starch and starch degrading enzymes were not detected in the flavedo tissue (Purvis and Grierson, 1982). Additionally, citrus fruits are strong sinks of photosynthesis

To explain the role of total sugars and proline in CI reduction(as shown in Tables 1, 2, 3 and 4) soluble carbohydrates (including reducing sugars) influence the mechanism of chilling resistance in several ways. They reduce the water loss by reducing the cell wall potential stabilize cell membranes and enzymes, and serve as energy source for cells (Purvis, 1990). Based on this, soluble carbohydrates play a positive role in reducing the chilling injury symptoms. Similarly soluble sugars and proline play a beneficial role in CI resistance which directly or indirectly several functions were suggested such a osmoticum, desiccation protectant, a nitrogen and reducing power sits during stress and a source of energy (Purvis, 1981).

Concerning free amino acids, there was an argument that the role of free amino acids was hard to interpret that it linked to sugars, it may be necessary to comment on its role. Steponkus (1971) indicated that higher concentration of free amino acids have sugar-binding capacity, which protects protein from being denaturated at low temperatures. He also, added that higher free amino acid concentration, provides the necessary conditions to synthesise new proteins. This leads to more protection to cell walls and hence, reduction of cell membrane damage or cell collapse.

Finally, it may be concluded that the CI resistance mechanism probably involves proline, sugars and free amino acids working simultaneously together.

Juice composition

The juice quality of the same fruit used for peel analysis was evaluated by determining the contents of ascorbic acid, total soluble solids and acidity.

Examining the data in Tables (5 and 6) pertaining to juice quality, it was found that ascorbic acid (v.c) content was significantly higher in all proline treatments than in control and foliar spray – fruits had highest v.c. content in both citrus species and in both seasons of study.

Table (3): Effect of proline treatments on peel total soluble sugars content of Banzahir limes during cold storage in 2004and 2005 seasons

Treatments	Stored period (Weeks)					
	2004					
	Total sugars (mg/ 100 peel)					
	0	3	6	9	12	Mean
Control	4.10	4.44	4.51	4.69	4.90	4.53
Proline (S) 0.4%	3.86	4.35	4.46	4.48	4.56	4.34
Proline (S) 0.8%	4.09	4.16	4.23	4.25	4.29	4.20
Proline (S) 1.2%	4.05	4.14	4.32	4.41	4.46	4.28
Proline (D) 0.4%	4.10	4.36	4.48	4.52	4.75	4.44
Proline (D) 0.8%	4.10	4.25	4.41	4.54	4.69	4.40
Proline (D)1.2%	4.10	4.29	4.35	4.49	4.53	4.35
Means	4.06	4.28	4.39	4.48	4.60	
L.S.D.$_{0.05}$	T= 0.23		W= 0.16		T×W= 1.28	
	2005					
Control	4.25	4.43	4.51	4.58	4.76	4.51
Proline (S) 0.4%	4.00	4.35	4.39	4.45	4.47	4.33
Proline (S) 0.8%	4.19	4.26	4.33	4.41	4.47	4.33
Proline (S) 1.2%	4.76	4.28	4.39	4.45	4.45	4.45
Proline (D) 0.4%	4.25	4.34	4.37	4.39	4.35	4.33
Proline (D) 0.8%	4.25	4.35	4.37	4.48	4.48	4.39
Proline (D)1.2%	4.25	4.32	4.34	4.41	4.50	4.36
Means	4.27	4.33	4.39	4.45	4.33	
L.S.D.$_{0.05}$	T= 0.21		W= 0.14		T×W= 1.18	

T . Treatments W . Storage period (weeks) T x W Interaction
Proline (S) : proline foliar spray (Treatments) Proline(D) : proline dipping

It may be suggested here that proline treatments reduced chilling injury (Pitted Area "PA") in comparison to control where CI symptoms were severe, that leads to an early senescence of the fruit.

Ascorbic acid decreased with advanced storage time. This may be attributed to its oxidation with time (Kays, 1991; Salukhe *et al.*, 1991).

Data also showed that the total soluble solids content in the juice of the control fruit was significantly higher than that of the all proline treatments (Tables 6 and 7). The higher content of TSS in the control fruits was probably affected by water loss and by cell wall breakdowns due to senescence, both of which would lead to apparent increase in TSS. Within the treatments, TSS content, also tended to increase with storage duration (Echeverria and Ismail, 1990) which may be explained by the same reasoning above. The acidity of fruit juice was significantly higher in the control compared to the other treatments. However, as pointed out for the TSS, the differences were found due to water loss that caused concentrating effect in control treatment. The acidity declined with storage time, which may be attributed to the use of acids as substrates for respiration (Echeverria and Valich, 1989).

Table (4): Effect of proline treatments on peel total soluble sugars content of Marsh grapefruit during cold storage in 2004 and 2005 seasons

Treatments	Stored period (Weeks)					
	2004					
	Total sugars (mg/ 100 peel)					
	0	3	6	9	12	Mean
Control	5.46	5.42	5.55	5.46	4.68	5.31
Proline (S) 0.4%	4.56	4.62	4.66	4.80	4.92	4.71
Proline (S) 0.8%	4.54	4.79	4.75	4.79	4.87	4.75
Proline (S) 1.2%	4.59	4.72	4.77	4.73	4.82	4.73
Proline (D) 0.4%	5.46	4.44	4.84	4.57	4.64	4.79
Proline (D) 0.8%	5.46	4.52	4.84	4.47	4.56	4.77
Proline (D1.2%	5.46	4.66	4.78	4.46	4.55	4.78
Means	5.08	4.78	4.88	4.75	4.72	
L.S.D.$_{0.05}$	T= 0.31		W= 10.89		T×W= 1.59	
	2005					
Control	5.50	5.78	5.81	5.45	5.25	5.64
Proline (S) 0.4%	4.59	4.62	4.91	4.71	4.67	4.70
Proline (S) 0.8%	4.64	4.66	4.71	4.95	4.64	4.70
Proline (S) 1.2%	4.74	4.83	4.32	4.80	4.59	4.66
Proline (D) 0.4%	5.50	4.93	4.96	4.98	4.68	5.01
Proline (D) 0.8%	5.50	5.02	5.26	4.99	4.80	5.11
Proline (D1.2%	5.50	5.02	5.32	4.77	4.76	5.07
Means	5.14	4.98	5.04	5.01	4.77	
L.S.D.$_{0.05}$	T= 0.34		W= 0.26		T×W= 1.90	

Fruit weight loss and unmarketable fruit percentage

The data in Table (7) clearly showed that control treatment had significantly higher fruit weight loss and unmarketable fruits percentage comparing with all proline treatments. In the meantime, proline foliar spray treatments had lower fruit weight loss and unmarketable fruits percentage than proline fruit dipping treatments in both citrus species and in both seasons of study during cold storage. For fruit weight loss, it may suggested here that proline treatments Eleminimized chilling stress in comparison to control where the severity CL symptoms leads to an early senescence of fruits (Salunkhe, 1991). Fruit weight loss and unmarketable fruits percentage tended to increase with storage duration in line with these results those reported by (El Helaly, 2002).

We can conclude from above results that it can be recommended to supply proline as foliar application treatments (1.2, 0.8 and 4.0%) and fruit dipping treatments (1.2, 0.8 and 4.0%) respectively, as a good treatments for reducing chilling injury, fruit weight loss percentage and unmarketable fruits percentage in Banzahir limes and Marsh grapefruit during storage at low temperature (5°C) cold storage and the treatments increased juice v.c. and reduced TSS and acidity content compared with control treatment.

Table (5): Effect of proline treatments on Banzahir limes juice TSS, acid and V.C. content during cold storage in 2004 and 2005 seasons

Treatments	Stored period (Weeks)											
	2004						2005					
	TSS%											
	0	3	6	9	12	Mean	0	3	6	9	12	Mean
Control	9.35	9.43	9.71	9.98	10.15	9.72	9.00	9.20	9.45	9.78	9.96	9.48
Proline (S) 0.4%	9.00	9.19	9.39	9.65	9.79	9.40	8.77	8.96	9.18	9.30	9.55	9.15
Proline (S) 0.8%	8.84	9.00	9.28	9.42	9.67	9.24	8.66	8.85	9.00	9.22	9.43	9.03
Proline (S) 1.2%	8.95	9.20	9.45	9.63	9.72	9.39	8.46	8.59	8.83	9.00	9.20	8.32
Proline (D) 0.4%	9.35	9.40	9.61	9.79	9.99	9.63	9.00	9.13	9.21	9.45	9.67	9.29
Proline (D) 0.8%	9.35	9.39	9.56	9.70	9.88	9.58	9.00	9.11	9.18	9.35	9.56	9.24
Proline (D) 1.2%	9.35	9.38	9.52	9.65	9.79	9.54	9.00	9.08	9.14	9.25	9.48	9.19
Means	9.17	9.28	9.50	9.69	9.86		8.84	8.99	9.14	9.34	9.55	
L.S.D.$_{0.05}$	T= 0.2		W= 0.16		T×W= 1.1		T= 0.18		W= 0.14		T×W= 0.92	
	Acid %											
Control	11.07	10.89	10.61	10.24	10.00	10.56	10.90	10.76	10.45	10.10	9.86	10.41
Proline (S) 0.4%	10.96	10.65	10.38	10.08	9.81	10.38	10.70	10.40	10.15	9.90	9.70	10.17
Proline (S) 0.8%	10.91	10.60	10.30	10.00	9.75	10.31	10.55	10.29	10.04	9.76	9.52	10.03
Proline (S) 1.2%	10.85	10.40	10.06	9.96	9.67	10.19	10.36	10.15	9.92	9.65	9.38	9.39
Proline (D) 0.4%	11.07	10.72	10.53	10.03	9.84	10.44	10.90	10.60	10.22	9.82	9.48	10.20
Proline (D) 0.8%	11.07	10.63	10.50	9.98	9.90	10.42	10.90	10.58	10.20	9.80	9.45	10.19
Proline (D) 1.2%	11.07	10.60	10.48	9.88	9.72	10.35	10.90	10.55	10.18	9.77	9.42	10.16
Means	11.00	10.64	10.41	10.02	9.81		10.74	10.48	10.17	9.83	9.54	
L.S.D.$_{0.05}$	T= 0.61		W= 0.44		T×W= 0.32		T= 0.56		W= 0.41		T×W= 2.84	
	V.C. (mg/ 100 ml juice)											
Control	60.03	58.00	55.04	51.00	47.15	54.24	57.33	56.03	54.88	53.00	51.92	54.63
Proline (S) 0.4%	68.21	66.91	65.70	63.90	59.01	64.75	65.41	64.91	63.00	61.66	60.00	63.00
Proline (S) 0.8%	68.60	67.00	65.99	64.15	59.90	65.13	65.94	65.21	63.60	62.00	61.79	63.71
Proline (S) 1.2%	68.71	59.54	66.11	64.75	60.55	56.53	66.18	56.91	64.20	62.96	62.03	64.26
Proline (D) 0.4%	60.03	59.50	57.00	54.01	50.00	56.11	57.33	56.80	55.01	53.40	52.17	54.94
Proline (D) 0.8%	60.03	59.61	57.14	54.29	50.40	56.29	57.33	57.00	56.89	53.60	52.83	55.53
Proline (D) 1.2%	60.03	59.68	57.29	54.44	50.66	56.42	57.33	57.11	56.93	54.00	53.01	55.68
Means	63.66	62.61	60.61	58.08	53.95		60.98	60.42	59.22	57.23	56.25	
L.S.D.$_{0.05}$	T= 0.63		W= 0.47		T×W= 3.19		T= 0.57		W= 0.47		T×W= 2.83	

Table (6): Effect of proline treatments on Marsh grapefruit juice TSS, acid and V.C. content during cold storage in 2004 and 2005 seasons

Treatments	Stored period (Weeks)											
	2004						2005					
	TSS %											
	0	3	6	9	12	Mean	0	3	6	9	12	Mean
Control	8.11	8.23	8.34	8.45	8.66	8.36	8.25	8.37	8.49	8.62	8.75	8.50
Proline (S) 0.4%	8.00	8.06	8.20	8.31	8.50	8.27	8.08	8.16	8.30	8.42	8.59	8.31
Proline (S) 0.8%	7.90	8.00	8.10	8.22	8.41	8.13	7.96	8.09	8.19	8.32	8.40	8.19
Proline (S) 1.2%	7.80	7.93	8.07	8.16	8.34	8.06	7.90	8.01	8.11	8.44	8.50	8.19
Proline (D) 0.4%	8.11	8.24	8.28	8.38	8.55	8.31	8.25	8.30	8.40	8.50	8.63	8.42
Proline (D) 0.8%	8.11	8.18	8.24	8.34	8.53	8.28	8.25	8.27	8.36	8.44	8.60	8.38
Proline (D) 1.2%	8.11	8.15	8.20	8.30	8.45	8.24	8.25	8.20	8.30	8.36	8.53	8.33
Means	8.02	8.11	8.20	8.31	8.49		8.13	8.20	8.31	8.44	8.57	
L.S.D.$_{0.05}$	T= 0.12		W= 0.09		T×W= 0.6		T= 0.14		W= 0.11		T×W= 0.71	
	Acid %											
Control	1.46	1.36	1.29	1.23	1.18	1.30	1.54	1.45	1.38	1.29	1.23	1.38
Proline (S) 0.4%	1.50	1.42	1.38	1.30	1.24	1.37	1.60	1.54	1.47	1.40	1.35	1.47
Proline (S) 0.8%	1.54	1.45	1.39	1.34	1.28	1.40	1.66	1.58	1.51	1.44	1.39	1.52
Proline (S) 1.2%	1.56	1.48	1.41	1.36	1.30	1.42	1.70	1.64	1.58	1.50	1.45	1.57
Proline (D) 0.4%	1.46	1.38	1.32	1.28	1.22	1.33	1.54	1.49	1.42	1.32	1.28	1.41
Proline (D) 0.8%	1.46	1.39	1.34	1.29	1.24	1.34	1.54	1.51	1.44	1.35	1.30	1.43
Proline (D) 1.2%	1.46	1.41	1.36	1.32	1.26	1.36	1.54	1.53	1.45	1.37	1.34	1.45
Means	1.49	1.41	1.36	1.30	1.25		1.59	1.53	1.46	1.38	1.33	
L.S.D.$_{0.05}$	T= 0.02		W= 0.014		T×W= 0.10		T= 0.03		W= 0.02		T×W= 0.18	
	V.C. (mg/ 100 ml juice)											
Control	43.18	39.19	32.98	26.13	24.03	33.09	44.25	40.00	33.11	27.15	24.55	33.81
Proline (S) 0.4%	50.51	43.96	36.01	30.90	26.95	37.67	52.77	49.18	42.30	36.56	33.90	42.94
Proline (S) 0.8%	48.33	42.95	35.91	32.90	27.93	37.60	50.89	46.55	40.11	34.25	34.00	41.16
Proline (S) 1.2%	47.69	42.80	34.60	31.00	28.06	36.83	50.15	45.91	40.35	35.00	34.81	41.24
Proline (D) 0.4%	43.18	41.05	33.45	31.89	23.98	34.71	44.25	41.01	34.92	28.21	26.01	34.88
Proline (D) 0.8%	43.18	41.35	33.92	32.23	24.18	34.97	44.25	42.11	35.00	29.10	27.00	35.49
Proline (D) 1.2%	43.18	41.36	33.97	32.39	24.42	35.06	44.25	42.61	35.40	29.58	27.43	35.85
Means	45.61	41.81	34.40	31.06	25.65		47.26	43.91	37.31	31.39	29.67	
L.S.D.$_{0.05}$	T= 0.05		W= 0.037		T×W= 0.26		T= 0.07		W= 0.04		T×W= 0.38	

Table (7): Effect of proline treatments on fruit weight loss and unmarketable fruits percentage of Banzahir limes and Marsh grapefruit during cold storage in 2004 and 2005 seasons

Treatments	Storage period (weeks)															
	2004								2005							
	0	6	12	mean	0	6	12	mean	0	6	12	mean	0	6	12	mean
	Banzahir limes				Marsh grapefruit				Banzahir limes				Marsh grapefruit			
	Weight loss %															
Control	0.00	14.50	18.70	11.07	0.00	13.11	17.92	10.34	0.00	13.40	19.03	10.81	0.00	12.70	17.50	10.07
Proline (S) 0.4%	0.00	6.00	9.93	5.31	0.00	5.41	9.13	4.87	0.00	5.91	10.00	5.30	0.00	6.18	9.77	5.32
Proline (S) 0.8%	0.00	5.96	8.16	4.71	0.00	4.91	5.19	4.37	0.00	6.13	8.50	4.88	0.00	7.00	10.11	5.70
Proline (S) 1.2%	0.00	4.98	6.92	3.97	0.00	4.14	8.00	4.05	0.00	5.04	7.06	4.03	0.00	6.13	9.06	5.06
Proline (D) 0.4%	0.00	7.80	12.27	6.69	0.00	7.19	12.30	6.50	0.00	8.11	12.66	6.92	0.00	9.13	13.40	7.51
Proline (D) 0.8%	0.00	7.00	10.95	5.98	0.00	6.91	10.72	5.88	0.00	6.94	11.13	6.02	0.00	7.13	12.05	6.39
Proline (D)1.2%	0.00	6.91	10.15	5.69	0.00	6.00	9.83	5.28	0.00	7.10	11.01	6.04	0.00	6.98	11.93	6.30
Means	0.00	7.59	11.01		0.00	6.81	10.44		0.00	7.52	11.34		0.00	7.89	11.97	
	T=1.73	W=0.75	T×W= 5.16		T=1.50	W=0.66	T×W= 4.5		T=1.80	W=0.79	T×W= 5.42		T=1.91	W=0.83	T×W= 5.75	
	Unmarketable fruits %															
Control	0.00	20.0	40.10	20.0	0.00	25.0	42.00	22.33	0.00	30.01	48.11	26.07	0.00	25.00	47.60	24.20
Proline (S) 0.4%	0.00	7.01	11.13	6.05	0.00	6.90	11.00	5.97	0.00	7.18	13.19	6.79	0.00	8.21	14.05	7.42
Proline (S) 0.8%	0.00	6.71	9.15	5.29	0.00	7.13	10.0	5.71	0.00	7.12	10.01	5.71	0.00	8.11	10.18	6.10
Proline (S) 1.2%	0.00	4.96	8.72	4.63	0.00	5.14	9.01	4.72	0.00	5.14	9.13	4.76	0.00	6.02	9.20	5.07
Proline (D) 0.4%	0.00	7.18	15.30	7.49	0.00	8.00	16.11	8.06	0.00	8.10	16.11	8.07	0.00	7.11	15.40	7.50
Proline (D) 0.8%	0.00	6.88	14.00	6.96	0.00	7.17	14.60	7.26	0.00	9.18	15.06	8.08	0.00	6.77	14.12	6.96
Proline (D)1.2%	0.00	6.11	12.20	6.10	0.00	7.41	13.91	7.11	0.00	7.41	14.10	7.17	0.00	6.00	13.11	6.37
Means	0.00	8.41	15.83		0.00	9.54	16.66		0.00	10.59	17.96		0.00	9.60	17.67	
	T=2.10	W=0.94	T×W= 6.33		T=2.18	W=0.97	T×W= 6.60		T=2.25	W=1.16	T×W= 6.79		T=2.20	W=0.99	T×W= 6.82	

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تأثير إضافة البرولين بطرق ومستويات مختلفة قبل وبعد الحصاد للسيطرة على أضرار البرودة فى الليمون البنزاهير والجريب فروت مارش أثناء التخزين.

إقبال زكريا علي أحمد
محطة بحوث البساتين بالصبحية – الإسكندرية – مركز البحوث الزراعية – مصر.

أظهرت نتائج عامي ٢٠٠٤، ٢٠٠٥ أن جميع معاملات البرولين قلل معنويا من أضرار البرودة ومن نسبة الفقد في الوزن ونسبة الثمار غير القابلة للتسويق خلال التخزين المبرد على درجة ٥°م وكان هذا التأثير اكثر وضوحا في ثمار الليمون البنزهير عن ما في ثمار الجريب فروت مارش.

زاد محتوى القشرة من البرولين والأحماض الأمينية الحرة معنويا نتيجة الرش بالبرولين وأيضا نتيجة زيادة فترة التخزين لكل من الليمون البنزهير وجريب فروت مارش. بينما قل محتواها من السكريات الكلية.

أدت جميع المعاملات بالبرولين إلى زيادة واضحة في محتوى العصير من فيتامين ج بينما زاد محتواه من المواد الصلبة الذائبة الكلية مع التخزين.

ولاحظنا أن معاملات الرش بالبرولين أعطت نتائج افضل من معاملات غمر الثمار بالبرولين.