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CHARACTERIZATION AND CONTROL OF CHILLING INJURY OF SOME HORTICULTURAL COMMODITIES

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Abstract

Fruits of green pepper and eggplant when stored at 5°C for a long duration have showed severe chilling injury (CI) symptoms. The severity of that physiological disorder varies with the commodity as well as with the cultivar. For green pepper, fruits of 'Pacific' are more sensitive to low temperature than those of others varieties 'Drago' or 'Zarco'. The most visible symptoms observed on the fruit skin are pitting and decay. For eggplant, chilling symptoms are characterized by weight loss, superficial pitting, pulp and seed browning and dryness as well as decay of the calyx. Ion leakage measurements were found to be very high in almost all tested fruits of these two commodities, especially on eggplant fruits, when exposed to 5°C. Prestorage heat treatment and exposure to ethanol vapor have reduced CI symptoms to some extent in all fruits. These physical treatments have also preserved fruit organoleptic and sensorial quality parameters in comparison to non treated fruits quality.

1. INTRODUCTION

Economic losses due to physiological disorders are greater than is generally realized mainly in developing countries. To a certain level low temperatures are considered the most effective method for keeping postharvest quality of various commodities. The effect of temperature is related to its direct effect on lowering fruit respiration, ethylene production and fruit metabolism in general (Hardenburg et al., 1986). However, for several tropical and subtropical commodities, chilling temperatures can be detrimental. The physiological disorder associated with low but not freezing temperatures is commonly known as chilling injury (CI) . The sensitivity of different commodities constitutes a limitation for storage and transport for long journeys at low temperatures. CI symptoms are generally characterized by surface pitting (citrus, eggplant, cucumber, papaya, pomegranate, sweet pepper etc.), soaked appearance and abnormal ripening (banana, tomato, melon, avocado), internal breakdown (apples, pears, melon), loss of flavor and decay development of several commodities. The extent of CI symptoms are related to commodity, ripening stage, pre-harvest factors (Bramlage, 1982 ; Wang, 1982, 1994), temperature and storage duration (Kasmire, 1985). All fruits are programmed to ripen and senesce differently as do their susceptibility to decay and physiological disorders (Brady,

1987). Therefore, it is difficult to find a single technology for delaying these processes that will suit all crops, or even a single crop under different climate conditions. Chilling injury contribute very much in increasing postharvest losses of several commodities. This aspect should be looked at in order to find means of controlling postharvest losses in abroad sense.

Several post-harvest treatments to reduce the incidence of CI have been reported and described. These approaches include chemical treatments (Temkin-Gorodeiski et al. 1993), coatings (Forney and Lipton, 1990 ; Fallik et al. 1995), and physical treatments (Klein and Lurie, 1990); Klein and Lurie,1992;Lurie and Klein,1991; Lafuente et al., 1991; Mougni et al., 1996).

The present report summarizes results of several experiments conducted on different horticultural commodities in order to ameliorate chilling injury occurring during exposure to low temperature during storage and subsequent transfer to relatively warm temperature. Only results of green pepper and eggplant fruits are presented and commented herein.

2. MATERIALS AND METHODS

Eggplant (*Solanum melongena* L.) fruits cv 'Baluroi and sweet green pepper (*Capsicum annuum* L.) cvs 'Drago', 'Zarco' and 'Pacific' were used in these experiments. Fruits were harvested at desired physiological stages of maturity from commercial farms in the area of Souss-Massa. These crops were produced under plastic greenhouses with appropriate cultural practices. Fruits were transported to the Postharvest Laboratory at IAV Hassan II. After washing, cleaning and sorting to desired weight and color, only sound fruits were used.

After harvest, fruits of the 3 cultivars of sweet green pepper were divided into 2 groups. One group composed of 105 fruits from each cultivar is divided into 7 lots ; 3 lots have received an immersion in hot water of 40°C for durations of 10, 20 and 30 min. Three other lots were exposed to hot air of 40°C and high relative humidity (>95%) for 12, 24 and 36 hours. The last lot is used as a control. The second group is composed of 108 fruits for each variety. Each group was divided into 4 lots of 27 fruits each. One lot was used as control and 3 lots were exposed to different concentrations of ethanol of 0.05, 0.1 and 0.2% for 12 or 36 hours. Fruits were then stored at 5°C for 20 days before transfer to 20°C for an additional 2 days.

Three large groups of eggplant fruits have been used. One group of 90 fruits was separated onto 6 lots of 5 fruits each with 3 replicates. Three lots were kept at 5°C and the others at 20°C. Chilling symptoms were evaluated after 5, 10 and 15 days of storage followed by an additional 3 days at 20°C. The second group composed of 234 fruits was divided into two lots of 117 fruits each. One lot was dipped in hot water of 45, 50 and 55°C for 1, 2 and 3 min and the second was conditioned in hot air of 40°C for 4, 8 and 16 hours. Relative humidity was kept as high as possible to about 96-97% during prestorage heating to avoid any desiccation of fruits. The third lot of fruits was exposed to 0.05, 0.1 and 0.2% of ethanol for 0, 6

and 12 hours prior to storage. After application of different treatments, fruits were kept at 5°C and evaluated after 7 and 14 days of storage.

Different parameters were measured at regular intervals during storage. Chilling injury was assessed by counting the number of fruits with symptoms and evaluating subjectively the rate of injury. Respiration rate of the product was determined using a gas chromatograph, Model Carle 100 Series with a TCD and an FID detectors. Helium was used at a rate of 30ml/min as a carrier and other gases ; oxygen ; nitrogen and hydrogen were used when needed according to the required and indicated flow rates. Fruits were placed in a tightly closed jar for few hours. Air from the headspace was removed 2 x 1 ml and injected into a gas chromatograph. Weight loss (%) was determined by weighing fruits at regular intervals during storage at both temperatures. External color as well as internal (seed and internal color) changes was determined using a Minolta Colorimeter, model CR-200 and 300. Superficial pitting (%) was evaluated based on an arbitrary scale of 0 (no pitting), 1 (trace), 2 (slight), 3 (moderate) and 4 (severe symptoms). Electrolyte leakage (%) was determined using disks taken from fruits and corresponding CI index was calculated based on the method adopted from Coté et al. (1993). Fruit elasticity was measured using an elastimeter. Other parameters include dryness of eggplant fruit calyx, (%) decay and internal quality indices (acidity and total soluble solids). Only data of few parameters will be presented and discussed in this presentation .

3. RESULTS

Green pepper fruits continuously maintained at 20°C tended to lose their chlorophyll content faster than fruits kept at 5°C. Fruits of 'Zarco' started to develop a yellow color after only 5 days at 20°C. During storage at 5°C or after transfer to warm temperatures for an additional 2 days, no significant color change was observed. When the fruits are heat treated or exposed to ethanol before storage, their color was not affected during storage.

Most symptoms of pitting became visible within 2 days of transfer from chilling temperature to 20°C. The severity of pitting increased with storage at 5°C. Fruits of 'Pacific' were found to be more sensitive to chilling temperature than the two other varieties 'Drago' and 'Zarco'. Fruits of the cultivar 'Pacific' showed skin pitting within only 5 days of storage at 5°C. After 15 days, less than 20% of fruits of 'Pacific' remained sound. Figure (1) showed the data of total CI index calculated from chilling symptoms observed on fruits of each cultivar. Heat treated fruits showed less symptoms even after a long storage period at 5°C (Table 1). In general, all the treatments have reduced the incidence of pitting. Nevertheless, short periods of exposure to hot water for 10 min or to hot air for 12 hours were sufficient to reduce by 50% or more the importance of pitting on green pepper fruits.

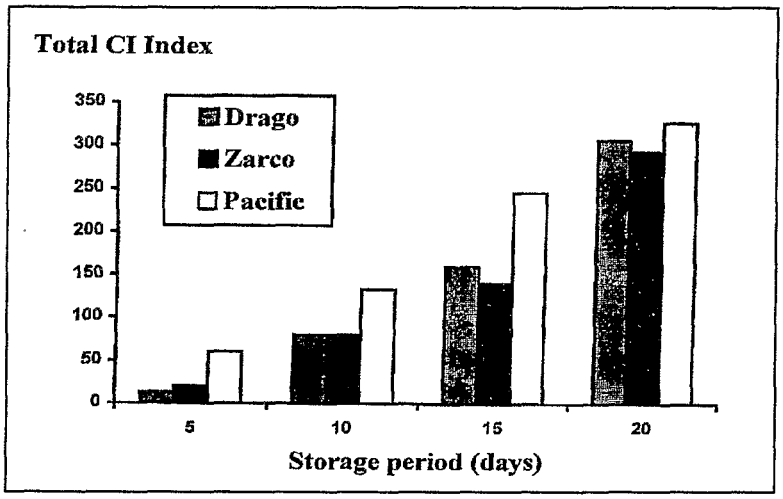

Fig 1. Total chilling injury index on fruit of 3 cultivars of green pepper following storage at 4 °C for different periods and subsequent 2 days at 20 °C.

Table 1. Effect of heat treatment at 40°C by immersion in hot water or exposure to hot air on CI index on fruits of 3 varieties of green pepper after 20 days storage at 5°C plus an additional 2 days at 20°C

		Cultivar		
Treatment	Duration	Drago	Zarco	Pacific
Control	0 min	306 a[z]	293 a	327 a
Hot Water	10 min	160 b	147 b	187 b
	20 min	167 b	147 b	193 b
	30 min	160 b	140 b	200 b
Hot air	12 hrs	114 b	127	133 b
	24 hrs	107 b	126 b	133 b
	36 hrs	120 b	126 b	140 b

[z] For a given treatment, values within columns followed by the same letter are not significantly different at 5% following mean separation method of Newman & Keuls.

A sharp decline in the CI index was also obtained following ethanol treatments. Effect of concentration as well as exposure period were significant (Table 2). With very low concentrations of ethanol (0.05%), longer periods were necessary for good protection. At 0.10%, the effect on CI index increases with the time of exposure. A significant difference was observed between the treatments mainly for Drago but not on Zarco. However, for concentrations greater than 0.1%, there was no difference between 3 or 12 hours treatment on chilling symptoms for all varieties (Table 2).

Table 2. Effect of ethanol concentration and exposure time on total chilling index after 20 days storage at 5°C followed by 2 days at 20°C.

		Concentration of ethanol (%)		
Variety	Exposure time	0.05	0.10	0.20
Drago	0	266 a[z]	266 a	266 a
	3	187 b	141 b	113 b
	6	114 c	100 d	100 b
	12	106 c	114 c	120 b
Zarco	0	240 a	240 c	120 b
	12	120 c	87 c	121 b

[z] For a given treatment and cultivar values within columns followed by the same letter are not significantly different at 5% following mean separation test of Newman & Keuls.

Weight loss of fruit increased during storage for the three varieties. Fruits continuously kept at 20°C or transferred to 20°C after storage at 5°C lost more weight than fruits continuously maintained at 5°C (data not shown). In association with weight loss, fruits showed skin shrinking, depending on the severity of weight loss. Regarding cultivar effect, fruits of 'Pacific' lost less weight than fruits of other varieties. When fruits were heat treated at 40°C either by dip in hot water or exposure to hot air, before storage at low temperature, the % of weight loss was not affected after 20 days of storage at 5°C plus an additional 2 days at 20°C (Table 3). The same effect was also obtained when fruits of green pepper were exposed to Ethanol pretreatment prior to storage at 5°C (data not presented).

Table 3. Effect of heat treatment at 40°C (water dip or air exposure) on weight loss (%) of fruits of three varieties of green pepper after 20 days storage at 5°C followed by an additional 2 days at 20°C.

		Cultivar		
Treatment	Duration	Drago	Zarco	Pacific
Control	0 min	8.08 a[z]	8.98 a	5.93 a
Hot Water (40° C)	10 min	7.86 a	9.0 a	6.04 a
	20 min	8.62 a	8.53 a	5,91 a
	30 min	8.14 a	8.64 a	5.95 a
Hot air (40° C)	12 hrs	8.02 a	8.73 a	6.00 a
	24 hrs	8.64 a8.95 a	8.95 a	5.63 a
	36 hrs	8.65 a8.45 a	8.45 a	5.77 a

[z] For a given cultivar, values within colums followed by the same letter are not significantly different at 5% following mean separation method of Newman & Keuls.

Electrolyte leakage from disks of fruits stored at 5°C for 5 and 20 days exhibited a substantial upsurge when measured on fruits after 2 additional days at 20°C in comparison to fruits continuously maintained at 5 or 20°C. The electrolyte leakage increased with storage duration. After 20 days of storage at 5°C plus an additional 2 days at 20°C, fruits of 'Pacific' showed 50 to more than 100% increase in electrolyte leakage compared to fruits kept continuously at 20°C. Fruits of the other two cultivars showed a similar trend during the same storage period (Table 4).

Table 4. Electrolyte leakage (%) of disks of pericarp tissue from fruits of 3 green pepper cultivars after storing for different periods at 5°C and 20°C.

		Storage period (days)			
Cultivar	Temperature (oC)	5	10	15	20
Drago	5	10.87 a[z]	12.81 a	12.08 a	13.17 a
	+ 2 days at 20	18.69 b	21.53 b	27.53 a	27.84 a
	20	8.77 b	10.50 b	13.90 a	15.90 a
Zarco	5	8.75 a	11.50 a	10.90 a	8.97 a
	+ 2 days at 20	16.45 b	18.38 a	19.68 a	21.00 a
	20	9.33 b	13.21 a	13.34 a	14.56
Pacific	5	11.80 a	12.60 a	13.40 a	13.60 a
	+ 2 days at 20	17.43 c	21.25 b	28.13 a	29.12 a
	20	12.04 b	15.95 a	14.34 a	16.25

[z] For each cultivar values within lines followed by the same letter are not significantly different at 5% following mean separation method of Newman & Keuls.

Total soluble solids (TSS) and acid (A) content of green pepper fruits showed an increase during storage conditions. This increase was observed for the three varieties and at different temperatures. Nevertheless, heat treatments with water immersion or hot air exposure for various periods have not affected the acid nor the sugar content of the fruits during storage (Table 5). Results of these two parameters did not show any difference in comparison to control. Similar results were obtained following treatments with d.ifferent concentrations of ethanol (data not shown).

Table 5. Effect of heat treatment at 40°C (by water immersion or hot air) and ethanol concentrations on acid (%) and total soluble solids (°Brix) after 20 days storage at 5°C followed by 2 additional days at 20°C.

Treatment	Treatment Duration	Green pepper varieties					
		Drago		Zarco		Pacific	
		TSS	A	TSS	A	TSS	A
Control	0 min	5.73	0.157	5.95	0.140	5.33	0.212
Water Dip	10 min	5.58	0.155	5.87	0.147	5.47	0.192
(40° C)	20 min	5.60	0.167	5.88	0.147	5.35	0.177
	30 min	5.54	0.155	6.17	0.135	5.50	0.177
Significance[z]		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hot air	12 hrs	6.06	0.149	6.35	0.148	5.67	0.174
(40° C)	24 hrs	6.11	0.151	6.32	0.133	5.68	0.174
	36 hrs	6.35	0.130	6.35	0.130	5.88	0.168
Significance[z]		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

[z] n.s : not significant at $P < 0.05$

Eggplant fruits are more sensitive to low temperature-induced physiological disorders. The severity of symptoms increased with the duration of storage. After 7 days of storage at 5°C, more than 60% of fruits have expressed superficial pitting, and after 14 days, all fruits presented CI symptoms and 40% of them with large surfaces invaded by *Alternaria* and *Rhizopus* pathogens.

Table 6. Effect of prestorage heat treatment by air at 40°C or water dip at 50°C on total CI index[z] of fruits of eggplant stored at 5°C followed by 2 additional days at 20°C.

		Storage period (days)	
Treatment	Duration	7	14
Control	0 hrs	94 a	302 a
Air (40 °C)	4 hrs	46 b	102 b
	8 hrs	27 c	88 b
	16 hrs	27 c	88 b
Water (50 °C)	1 min	108 a	271 a
	2 min	102 a	166 b
	3 min	80 a	164 b

[z] Values within columns followed by the same letter are not significantly different at 5% following mean separation method of Newman & Keuls.

Heating fruits by immersion in hot water or by exposure to hot air treatments for different periods have significantly reduced the severity of chilling. In fact, 4 hours exposure to 40°C air or 2 min dip in 50°C water are sufficient to reduce chilling

injury symptoms by 50% (Table 6). The same Table shows that air treatment was more efficient than water treatment in reducing chilling disorders of eggplant fruit. Increasing the temperature of water bath to 55°C did not significantly ameliorated the severity of CI of fruits (Table 7).

Table 7. CI index of eggplant fruits following a prestorage water dip at different temperatures before storage at 5°C and subsequent 2 additional days at 20°C.

	Storage period (days)	
Water Temperature	7	14
Non treated	94 a[z]	302 a
Temperature		
45 °C	113 a	226 b
50° C	102 a	166 c
55° C	95 a	160 c

[Z] Values within columns followed by the same letter are not significantly different at 5% following mean separation method of Newman & Keuls.

Prestorage treatment with ethanol vapor was efficient in ameliorating pitting disorders of eggplant fruits. After 7 days of storage at 5°C, treated fruits with 0.1 and 0.2% of ethanol have not shown any symptom of pitting. After 14 days of storage, the severity of chilling was reduced by 60 to 70%, respectively, for 6 and 12 hours exposure to 0.05%. At 0.1% or higher concentration, short periods of 6 hours were found more efficient in reducing pitting symptoms (Table 8.). The dryness of the fruit calyx was controlled by associating a polysacharide ester coating (Semperfresh) with ethanol vapor treatment at a rate of 1.7%. This coating has preserved significantly the green color of the calyx during storage. In other studies, a fungicide was also included in order to prevent any pathologen development.

Table 8. Effect of ethanol concentration and exposure duration on pitting index on eggplant fruits after storage at 5°C and subsequent 2 days at 20°C.

Ethanol concentration (%)	Exposure duration (hours)	Storage period at 5°C (days) 7	14
Control	-	94 a[z]	302 a
0.05	6	13 b	137 b
	12	0 b	106 b
0.1	6	0 b	54 b
	12	0 b	88 b
0.2	6	0 b	39 b
	12	0 b	74 b

[z]Values within columns followed by the same letter are not significantly different at 5% following mean separation method of Newman & Keuls.

Electrical conductivity measured from disks of fruits at 20°C was 7.10 and remained unchanged for 14 days of storage. Meanwhile, fruits stored at 5°C and then transferred to 20°C, showed a significant increase as illustrated in Table (9). Heat treatment reduced ion leakage and maintained EC low during the storage.

Table 9. Ion leakage of prestorage heat treated fruits of eggplant at 40°C before storage at 5°C for 7 and 14 days and 2 subsequent days at 20°C.

Treatment	Storage period at 5°C (days) 0	7	14
Control	7.14	10.90 a	16 a
4 hrs	-	6.67 b	14.65 b
8 hrs	-	7.85 b	10.43 c
16 hrs	-	7.50 b	9.87 c

[z] Values within columns followed by the same letter are not significantly different at 5% following mean separation method of Newman & Keuls.

4. DISCUSSION

Pitting, weight loss and shriveling constitute some of the most visible symptoms of chilling injury of fruits of both green pepper and eggplant when exposed to chilling temperature of 5°C for several days. The severity of symptoms depended on fruit cultivar and storage duration. The necrotic areas observed on chilled tomato peel are correlated with changes in phospholipid content of the membrane. The decrease in phospholipid is accompanied by an increase in membrane tissue leakage (Lurie et al. 1995). Ion leakage is also considered as an interesting indicator of CI on these crops. However, for green pepper, electrolyte leakage increased in both fruits continuously stored at 20°C or prestored at 5°C and then transferred to 20°C. These results showed that senescent fruits of pepper can also exhibit an important increase in their electrolyte leakage. These results agreed with the conclusions of Côté et al. (1993) that electrolyte leakage can often mislead in evaluating CI incidence. In contrast, ion leakage measured on eggplant fruits tended to increase with the importance of CI as well as of the weight loss. Prestorage heat treatment was reported to act as an inhibitor of the synthesis of some enzymes responsible for the phospholipid degradation in the peel tissue of tomato fruits (Lurie et al. 1995).

The beneficial effect of heat treatment which confers greater thermotolerance of fruits to low temperatures have been reported on tomato (Lafuente et al., 1991 ; Lurie and Klein, 1991 ; Lurie et al. 1995), on cucumber (Imani et al. 1995), on green pepper (Mougni et al. 1996). The response of the plant tissue or organ to heat exposure is the induction and accumulation of a specialized group of proteins termed heat-shock proteins (Lurie and Klein, 1991). Moreover, postharvest heat treatment conditions for different commodities are diverse and vary within cultivars and within individual commodities (Whitaker, 1994).

Postharvest treatments with exogenous ethanol have a positive effect in reducing chilling injury on green pepper and eggplant fruits. Very low concentrations of the product are sufficient in inhibiting chilling injury symptoms. The reduction of CI with ethanol treatment may be related to its effect on inhibition or delaying maturation (Kelly and Saltveit, 1988; Saltveit and Mencarelli, 1988) or may be by increasing the defense mechanism of tissues to different attack. Several other methods and compounds such as polyamines, antioxidants, intermittent warming, controlled atmosphere, temperature conditioning and others, were found to have a positive

effect in reducing CI in different crops (Wang, 1994). Despite numerous reports on these techniques, commercial application in postharvest of these methods is not yet well established and necessitate further investigations.

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