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A short hot water treatment inhibits ripening-related enzyme activities in Sweet pepper during storage and marketing simulation

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ABSTRACT

The aim was to evaluate the effects of a short hot water rinsing and brushing (HWRB) treatment on ripening -related enzyme activities in association with other quality parameters in two freshly harvested bell pepper cultivars. Application of HWRB treatment at 55°C for about 15s to two bell pepper cultivars ('Parker'-red and 'Nibla'- yellow) significantly reduced water loss, thus maintaining fruit firmness, and significantly reduced decay incidence after subsequent prolonged storage. However, 'Parker' lost less weight , was firmer and had lower decay incidence than 'Nibla' after 21 d at 7°C and additional 3d at 17°C. HWRB treatment also impeded polygalacturonase (PG) and Exo- cellulase(Exo-CMCase) enzyme activities, but not Endo-CMCase activity in fruits, thereby retarding ripening processes and extending the fruit shelf life. PG exhibited the strongest response to HWRB. Controlling physiological and pathological deterioration by introducing the use of HWRB technology to suppress some biochemical pathways involved in fruit ripening and softening could potentially add significant value by extending storability and marketing, and is of great commercial importance.

Keywords: *Capsicum annum*, Hot water rinsing and brushing (HWRB), Postharvest

INTRODUCTION

Fruits and vegetables are not only an enjoyable component of a healthy diet but are also a valuable source of vitamins, minerals, antioxidants and fibers. Fruit ripening involves softening and changes in fruit texture, which predisposes fruits to physiological and pathological deterioration thereby reducing their shelf life (Brummell and Harpster 2001). Slowing the physiological and biochemical processes associated with fruit ripening, such as enzyme activities, should lead to optimum utilization of fresh produce and of the benefits derived from them.

Bell sweet peppers (*Capsicum annuum* L.) are one of the commercially important crops that are classified as vegetable fruits; they are green when unripe, and may be purple, red, yellow, orange or brown when ripe. The texture, in particular the crispness, of pepper is important to consumers as a quality attribute. Bell peppers are also becoming increasingly popular decorative items (Frank *et al.*, 2001). The major postharvest problem with this crop is excessive softening, which may cause shrinkage, drying and pathological disorders, all of which severely reduce the quality and acceptability of the product (Lowlands *et al.*, 1993;Priya-Sethu *et al.*,1996).

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A short hot water rinsing and brushing (HWRB) treatment has been developed for simultaneously cleaning and disinfecting fresh agricultural produce (Fallik *et al.*, 1999). The overall quality of the treated fresh produce was found to be significantly higher than that of the untreated fruits, in terms of general appearance, significantly less weight loss, greater firmness and less decay incidence (Fallik, 2004). HWRB treatment also enhanced fruit resistance to chilling injury and decay development in several commodities (Porat *et al.*, 2000; Pavoncello *et al.*,2001; Fallik *et al.*, 2002).

The present study was conducted to evaluate the effects of the HWRB treatment on ripening-related enzyme activities and other quality parameters in freshly harvested bell peppers of new cultivars in order to light more shed on the mode-of-action of HWRB technology.

Abbreviations

Decay(D);Endo-cellulase (endo-CMCase); Exo-cellulase (exo-CMCase); Firmness(F); Hot water rinsing and brushing (HWRB); Relative humidity (RH); Polygalacturonase (PG); Round per minute (RPM); Weight loss (WL);

MATERIALS AND METHODS

Plant Materials

Fruits of two commercial cultivars of sweet bell peppers (*Capsicum annuum* L.), 'Parker' (red colour, DeRuiter, Holland) and 'Nibla' (Yellow colour, DeRuiter, Holland) were harvested from an unheated commercial plastic greenhouse in the Arava Valley in the south of Israel, once a month, for three months during 2003.

Transplants approximately six weeks old were transferred to a non-heated plastic house in the middle of August and were grown at a density of 38,000 plants ha^{-1} The plastic house was covered with translucent plastic (0.12 mm thick, "Infra Red-Ginegar Plastic Ltd, Kibbutz Ginegar, Israel).Climatic conditions during the experimental season were: January-average temperatures: min 7°C/max 20°C, with ~11.0 h day length and average RH 25%; February-average temperatures: min 5°C/max 18°C, with ~11.5 h day length and average RH 25%. March-average temperatures: min 15°C/max 27°C with ~12.0 h day length and average RH 20%.

All treatments were carried out within 24 h after harvest. Fruits were rinsed and brushed in hot water at 55±2°C for 15 s and dried out inside a 3m long tunnel equipped with 3 forced air fans as described by Fallik *et al.*, (1999). Fruits were then packed in corrugated cartons, 4-5 Kg per carton. The control treatments comprised rinsing fruits under tap water (~22°C) and brushing them.

Quality traits

Fruit quality was assessed at the end of 21d storage at 7°C,plus 3d of shelf life at 17°C (to simulate marketing conditions), as follows: *Weight loss (WL):*Fruits were weighed daily and WL was expressed as a percentage of the initial weight of five fruits per carton. *Fruit firmness(F):*This was evaluated according to Ben-Yehoshua *et al.*,(1983).Fruits with 0-2.5 mm residual deformation were considered very firm; 2.6-4.0 mm-firm;4.1-5.5 mm-soft;>5.6 mm-very soft. Decay (D): A fruit was considered decayed once fungal mycelia appeared on the peel or calyx. Decay was expressed as percentage of the initial total of fruits that were decayed.

Preparation of pericarp tissue for enzyme assay

Pericarp tissue (~150g) was excised from five fruits per cultivar, 6h after the HWRB treatment, after 21 d storage at 7°C(storage) and after 21 d storage at 7°C plus 3 d at 17°C (marketing). Pericarp tissue that was excised from fruit treated with tap water brushing served as the control. The tissue was cut into pieces, throughly mixed, subdivided into 25-g portions, frozen in liquid nitrogen and stored at -80°C pending further analysis.

Enzyme extraction

Pericarp tissue (25 g) was homogenized in 50ml of cold distilled water for 3 min in a Waring Laboratory blender and centrifuged for 10 min at 15,000 rpm in an RC-5B refrigerated super-speed centrifuge (Sorvall, Newtown, USA).The supernatant was filtered through miracloth and the pellet was recovered, and washed in 50 ml cold distilled water. The centrifugation and washing of the pellet were repeated twice more. The pellet that was recovered at the end of the third washing was divided into two equal parts, one for extraction of the polygalacturonase (PG),and the other for extraction of the Endo-cellulase and Exo-cellulase (endo and exo-CMCase) enzymes. PG was extracted in 10 ml of 0.1 M sodium acetate buffer,pH5, and 10 ml of 0.2 M NaCl (in a ratio of 1:1), stirred for 30 min at room temperature. CMCase was extracted in a 1:1 mixture of 1M NaCl and sodium acetate buffer, pH 5,which was centrifuged for 10 min at 15,000 rpm, and the supernatant (i.e.,the enzyme extract) was filtered through miracloth, and held on ice pending further analysis.

Enzyme assay (PG and Exo-CMCase)

To determine the PG activity a 100-µl aliquot of enzyme extract and 1 ml of 0.5% polygalacturonic acid (PGA) were added to 900 µl of a 1:1 mixture of 0.1M sodium acetate buffer, pH5, and 0.2M NaCl (sodium acetate buffer : NaCl). For the Exo-CMCase assay,1 ml of enzyme extract and 1 ml of 0.5% carboxymethyl cellulose were added to the Na-acetate buffer: NaCl mixture. The reaction was terminated after 1-24 h incubation at 30°C, by boiling 0.2 ml of reaction mixture,1 ml of 0.2 M borate buffer, pH 0.9, and 0.2 ml of 1% cyanoacetamide for 15 min. The UV spectra of the reaction products were measured at 276 nm, with the blank as the control. One unit of PG or Exo-CMCase activity was defined as the amount of enzyme capable of catalysing the formation of one nanomole of reducing sugar per hour, per g fresh weight, under the assay condition.

Endo-cellulase assay (endo-CMCase)

A mixture of 3 ml of enzyme solution plus 6 ml of 1.25% caboxymethyl cellulose was incubated at 30°C in an Ostwald viscometer head (150 ml).The endo-CMCase activity was defined as the percentage loss of viscosity in 60 min. The blank contained the boiled enzyme.

Statistical analysis

All the results were subjected to statistical analysis using a one and two-way Anova computer program. (SAS, 1990). The results of the study are presented as the average of those of three experiments conducted during the growing season since similar trend had been shown each month.

Table 1:Quality traits of Parker (red)and Nibla (yellow)after 21d of storage at 7°C plus 3d at 17°C(Shelf life).

Cultivar	weight loss(%)[1]		Firmness(mm)[2]		Decay incidence(%)	
	Control[3]	Washed[4]	Control	Washed	Control	Washed
(a)	*After 21 d of storage at 7°C*					
Parker	2.2Ab[5]	1.9Bb	2.4Ab	2.3Ab	2.0Ab	0.6Aa
Nibla	2.6Aa	2.2Ba	3.1Aa	2.7Aa	4.0Aa	1.2Ba
(b)	*After 21 d storage at 70C+3dat shelf life*					
Parker	3.5Ab	2.5Ba	3.4Ab	2.9Bb	7.0Ab	1.4Ba
Nibla	4.0Aa	2.5Ba	5.0Aa	4.2Ba	10.Aa	2.8Ba

[1] weight loss from the initial level.
[2] Firmness in mm deformation :<2.5=very firm;2.6-4.0=firm;4.1-5.5=soft;>5.6=very soft
[3] Tap water rinsing and brushing
[4] Hot water rinsing and brushing (HWRB)
[5] Values followed by the same capital letter are not significantly different between controls and treated fruits, whereas values followed by the same lower-case letter are not significantly different between the cultivars, at P=0.05 according to Duncan's multiple range test.

RESULTS

After 21 d storage at 7°C,significant differences in weight loss, between HWRB- treated and control fruits, were observed in both cultivars, 'Parker' and 'Nibla' (Table 1a), but no differences were found in fruit firmness between treatments. Decay incidence in both 'Parker' and 'Nibla' was significantly higher in control than in HWRB-treated fruits. Significant differences were observed between the two cultivars in all three-quality parameters assessed (Table 1a): 'Parker' lost less weight, was firmer and had less decay than 'Nibla'.

After storage and shelf life simulation, significant differences were found in all quality traits except decay incidence, between treated and control fruits, and between the cultivars (Table 1b). In general 'Parker' exhibited much better quality traits than 'Nibla' (Table 1).The activity levels of polygalacturonase (PG) immediately after harvest and HWRB or control treatment, after 21 d at 7°C,and after shelf life simulation were significantly higher in control than in HWRB-treated fruits of both cultivars, except in 'Parker' after 3 d at shelf life (Figure 1A). The PG activity was significantly higher in 'Nibla' than in 'Parker', except after 3 d at shelf life (Figure 1A).In general, PG activity was higher immediately after harvest than after 21 d of storage at 7°C. Once fruits were transferred to the shelf life simulation at 17°C,PG activity was slightly alleviated (Figure 1A). PG activity was influenced very highly ($P<0.001$)by the cultivar and by the HWRB treatment immediately after harvest (Table 2a). However, the interaction between cultivar and treatment in their effects on PG activity was very moderate ($P<0.5$) (Table2b). After 3d at 17°C,HWRB treatment influenced PG activity strongly ($P<0.001$),but only a moderate interaction was observed ($P<0.5$) (Table 2c).

The Exo-CMCase activity levels were

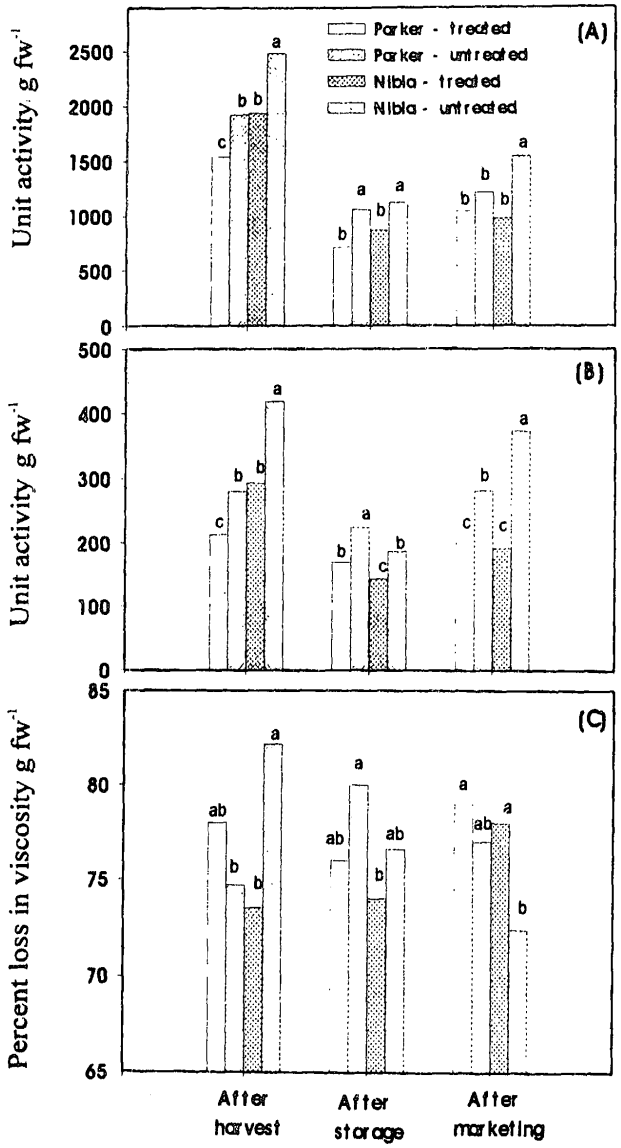

Figure 1. The effect of HWRB on polygalacturonase (A), Exo-CMC (B) and Endo-CMC(C) in Parker (red) and Nibla (Yellow) Cultivars, Immediately after harvest and postharvest treatment, after 21d of storage at 7°C(after storage)and after an additional 3d at 17°C (after marketing). Tap water rinsing and brushing fruits served as controls.

significantly higher in control than in HWRB-treated fruits of both cultivars, immediately after harvest, after 21d at 7°C and after an additional 3 d at 17°C (Figure 1B). The activity was relatively high immediately after harvest, it then declined during storage and rose again during shelf life (Figure 1B). Exo-CMCase activity in HWRB-treated 'Nibla' fruits was significantly higher than in HWRB-treated 'Parker' fruits immediately after harvest and after the additional 3 d at 17°C.However,after 21 days at 7°C, Exo-CMCase activity was lower in 'Nibla' than in 'Parker' (Figure 1B). Immediately after harvest ($p<0.5$) (Table 2a), and no interaction was observed after 21 d of storage at 7°C (Table 2b). After 3 d at 17°C, the Exo-CMCase activity was influenced only very moderately ($P<0.5$) by the cultivar, whereas it was strongly influenced ($P<0.001$) by the treatment and by the interaction between cultivar and treatment (Table 2c).

Immediately after harvest, Endo-CMCase activity was significantly higher in untreated 'Nibla' than in untreated 'Parker' or HWRB-treated 'Nibla' Fruits (Figure 1C). After 21 d of storage at 7°C no differences in Endo-CMCase activity were observed between treated and untreated fruits of either cultivar. At the end of 3 d of shelf life ,no significant differences were observed between treated and untreated fruits of either cultivar. At the end of 3 d of shelf life, no significant differences were observed between HWRB-treated and untreated 'Parker' fruits, whereas there were significant differences between treated and untreated 'Nibla' fruits (Figure 1C). Immediately after harvest, there was no cultivar effect on endo-CMCase activity, but there were very moderate effects of the treatment ($P<0.5$) and of the interaction between cultivar and treatment ($P<0.5$) (Table 2a). After 21d of storage at 7°C,the cultivars and treatment had very moderate effects ($P<0.5$) on Endo-CMCase activity, and no interaction was observed (Table 2b). Similar findings were observed after an additional 3 d at 17°C (Table 2c).

Table 2: Table of variance (P values)of the enzyme activities of Parker (red) and Nibla (yellow)immediately after treatment, after 21d of storage at 7°C,and after an additional 3 d at 17°C

Source	PG	Exo-CMC	Endo-CMC
(a)	*Immediately after treatment*		
Cultivar	***1	***	NS
Treatment	***	***	*
CxT2	*	*	*
(b)	*After 21d storage at 7°C*		
Cultivar	NS	***	*
Treatment	*	***	*
CxT	NS	NS	NS
(C)	*After 21 d storage at 7°C+d at 17°C*		
Cultivar	NS	*	*
Treatment	***	***	*
CxT	*	**	NS

[1]*, **, ***,NS=P<0.05, 0.01, 0.001, not significant, respectively.
[2]C-Cultivar,T-Treatment (hot water rinsing and brushing versus tap water rinsing and brushing [control]

DISCUSSION

In the present study, we have shown the influence of a short hot water rinsing and brushing treatment on several quality and biochemical attributes of two sweet bell pepper cultivars, during a prolonged storage and marketing simulation.

The beneficial effects of HWRB on several quality traits of several crops were reviewed by Fallik (2004). Application of the HWRB treatment to these two commercial cultivars ('Parker'-red and 'Nibla'- Yellow) at 55°C for about 15 s, significantly reduced decay incidence during prolonged storage (Table 1).

Paull and Chen (2000) reported that heat treatment inhibited the ripening of many fruits and vegetables, and other workers reported that HWRB treatment appeared to inhibit certain ripening processes, as indicated by the relatively low respiration rate and ethylene evolution of HWRB-treated fruit (Fallik 2004), or by the slow rate of colour development in HWRB-treated melons (Fallik *et al.*,2000) and tomatoes (Ilic *et al.*, 2001).

The roles of polygalacturonase (PG)and cellulase enzymes (CMCases) in fruit ripening and softening appear to be related to the changes in fruit texture and quality attributes that ultimately lead to fruit tissue disintegration to allow seed dispersal (Kramer *et al.*, 1992; Brummel and Harpster , 2001), but the levels of these activities depend upon the cultivar (Brummel *et al.*, 1997).Increased PG activity has long been associated with fruit ripening, although the amount detected varies widely with species, storage temperature and maturity (Hobson 1962; Ketsa and Daengkanit 1999;Imsabai *et al.*, 2002). Suppression of Pg activity in fruits was found to improve tissue integrity, most probably because of reduced cell separation in the tissue (Langley *et al.*, 1994). HWRB treatment at 55°C for 15 s significantly reduced enzyme activities that are related to fruit ripening and softening, in both 'Parker' and 'Nibla' cultivars, which could also explain the better overall quality of HWRB treated pepper fruits after prolonged storage. Following HWRB or control treatment immediately after harvest, PG and Exo-cellulase (Exo-CMCase) activities were reduced compared with those of untreated fruits, immediately, after 21 d of storage and after shelf life simulation, with PG activity exhibiting the stronger response. The decrease in the activities of PG and Exo-CMCase in HWRB -treated fruits coincided with the slow loss of fruit firmness (Table 1). Such loss of firmness in many ripening fruits is associated with alterations in cell wall and middle lamella structures (Seymour and Gross, 1996).These changes include solubilization of cell wall pectin, which involves the action of cell wall

hydrolytic enzymes: polygalacturonase, pectinesterase, β-galacturonase and cellulase (Huber, 1983). Recently, Rao and Paran (2003) reported that the PG-encoding gene was a good candidate gene for selecting a pepper fruit with a soft phenotype. Lurie *et al.*,(1995) found that the extent to which heat treatment reduced cellulase activity in avocado fruits was correlated with its reduction of their rate of softening.

The inconsistency among the various reports on Endo-CMCase activity after heating was probably due to the instability of the enzyme under different storage conditions after hear treatment. In similar experiments with cherimoya, Sanchez *et al.*, (1998) found that PG activity was more thermostable than cellulase activity. It is also possible that the endo-CMCase activity in pepper is not closely associated with the later stages of the ripening process (Harpster *et al.*, 2002). A similar observation was reported by Ketsa and Daengkanit (1998,1999) in durian fruit.

In general, cv. 'Nibla' has been found more susceptible to physiological and pathological deterioration after prolonged storage than cv. 'Parker'(Maalekuu *et al.*, 2003), and the quality differences between these cultivars could be attributed to differences in some of the physical and morphological characteristics reported by Maalekuu *et al.*, (2003). It is also possible that the higher activities in PG and Exo-CMCase in both washed and control 'Nibla', compared with 'Parker'(Figure 1A, B) could explain the more rapid physiological deterioration -such as ripening and softening- in the yellow cultivar, and its consequently lower durability in storage and marketing.

Controlling physiological and pathological deterioration in bell sweet peppers and other fresh produce by the introduction of HWRB technology to suppress some of the biochemical pathways involved in fruit ripening and softening is an approach that could potentially enhance the value of the fruit significantly by extending its storage and marketing life. Thus, this technology is of great commercial importance.

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