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Benzyl-aminopurine (BAP) treatments delay cell wall degradation and softening, improving quality maintenance of refrigerated summer squash

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ABSTRACT

Round summer squash are harvested before reaching full maturity and even though they are highly perishable, fruit postharvest handling is mostly based on storage at non-chilling temperatures. Finding complementary treatments minimizing deterioration and reducing postharvest losses would be extremely useful. In this work we evaluated the effect of postharvest cytokinin (CK) treatments on refrigerated round soft rind squash. Fruit were harvested at commercial maturity and sprayed with 1 mmol L^{-1} benzylaminopurine (BAP) or water (control) prior to storage at 5 °C for 0, 13 or 25 days. Quality was assessed upon removal from cold storage as well as after a 2 day shelf-life period at 20 °C. CK-treated fruit showed slower deterioration and dehydration and remained firmer than the control. BAP sprays did not affect color, respiration or sugar-acid balance. The treatments prevented phenolic compound accumulation, and decreased pectin solubilization. By the end of the storage period BAP-treated squash had higher levels (45%) of tightly-bound polyuronides than untreated controls, indicating a substantial delay in cell wall dismantling. CK sprays also reduced neutral sugar solubilization from pectin-rich fractions, but no changes were found in the cross-linking glycans or cellulose. To our knowledge, this is the first work showing that CK can regulate pectin disassembly in developing fruit. Postharvest BAP sprays preventing texture deterioration may be a simple treatment to complement refrigeration of round, soft rind, summer squash.

1. Introduction

Immature round summer squash [*Cucurbita maxima* var. Zapallito (Carr.) Millan] have a relative short postharvest life. In contrast to the Italian summer squash (zucchini), which has been studied in detail (Wang, 1996; Gualanduzzi et al., 2009) other soft rind squash have received very little attention. Low temperature storage is recommended to extend their postharvest life. However, refrigeration just above the freezing point is not possible since the fruit are chilling sensitive (Massolo et al., 2013). Modified atmosphere offers few benefits to summer squash quality maintenance (Suslow and Cantwell, 2013) and finding treatments to complement refrigeration and maximize fruit storing capacity would be extremely useful. Previous work reported that 1-methylcyclopropene (1-MCP) treatments maintained quality and reduced chilling injury (CI), indicating that in spite of its immature stage and non-climacteric ripening pattern, ethylene contributes to fruit deterioration (Massolo et al., 2013). The role of other hormones in summer squash ripening, senescence and quality maintenance has not been studied to date.

Cytokinins (CKs) play a crucial role in plant cell division and differentiation (Peleg and Blumwald, 2011; Hwang et al., 2012) and have been used to delay senescence in a number of crops (Gan and Amasino, 1996). Applications of the synthetic cytokinin N6-benzylaminopurine (BAP) reduced the expression of chlorophyll-degrading genes (Gomez-Lobato et al., 2012) and delayed chloroplast dismantling and yellowing in leaves (Ben-Yaakov

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et al., 2006; Zhang et al., 2011) and broccoli florets (Clarke et al., 1994; Costa et al., 2006).

In fruit, CKs have been mostly used before harvest. Sprays with the CK analogs 1-(2-chloro-pyridyl)-3-phenylurea (CPPU) induced tomato set and parthenocarpy and delayed grape rachis browning (Ding et al., 2013; Raban et al., 2013). However, CKs are most commonly used as thinning agents in apples (Petri et al., 2006; Cin et al., 2007; Schröder et al., 2012).

In harvested commodities, CKs are employed for postharvest short-term pulses or directly in flower vase solutions to delay deterioration (Chen et al., 2013). Strikingly, direct evidence regarding the role of CKs in fruit ripening and senescence is scarce. Breaker *rin* tomato mutants have higher amounts of CKs than normal ripening fruit (McAtee et al., 2013). CKs decrease in mango, grape and orange before ripening (Chen, 1983; Miñana et al., 1989; Bottcher et al., 2011). In contrast, in kiwifruit CKs increase dramatically during development (Pilkington et al., 2013). Preharvest CPPU sprays have been reported to increase fruit size and modify sugar content and acidity (Nickell, 1986; Ogata et al., 1988). However, the observed effects of fruit composition may have been indirect due to modifications in the growth rate and sink-source balance. Few studies have evaluated the effect of postharvest CK treatments on fruit quality. Moreover, the results reported show large variations depending on the species considered. In litchi, BAP postharvest applications reduced the metabolic rate and delayed ripening (Jiang and Fu, 1998), while in green olives similar treatments induced respiration and accelerated color development (Tsantili et al., 2002).

Although CKs are on the United States Environmental Protection Agency (US-EPA) approved biopesticide list for postharvest applications, the number of studies evaluating their effects in ripening, senescence and storage disorders of fruit is limited. Particularly in immaturely harvested fruit, almost no research has been conducted. A recent study has shown that BAP may be useful to reduce chlorophyll turnover in cucumber (Chen and Yang, 2013). Whether or not CKs can regulate other central ripening processes is almost unexplored. The aim was to determine the effect of BAP postharvest sprays on cell wall metabolism, softening and quality maintenance of refrigerated summer squash.

2. Materials and methods

2.1. Reagents

Benzylaminopurine (BAP), galacturonic acid, ethylene-diaminetetracetic acid (EDTA), Na_2CO_3, $NaBH_4$, glucose, sodium borate, anthrone were purchased from Sigma (St Louis, USA). Ethanol sulfuric acid, Folin–Ciocalteu reagent and NaOH were obtained from Anedra (Argentina) and *m*-phenyl phenol from, Fisher Scientific (NH, USA). Chemicals and solvents used were reagent grade and used without further purification unless otherwise specified.

2.2. Plant material treatments and storage

Round summer squash [*Cucurbita maxima* var. Zapallito (Carr.) Millan] produced in La Plata (Argentina) were harvested at commercial maturity based on size and rind appearance (140–190 g and having a glossy and soft rind) and transported to the laboratory within 1 h from harvest. Fruit with defects or bruises were eliminated. The squash ($n = 200$) were divided into 4 groups and sprayed to run-off with solutions containing 0, 0.1, 1.0 or 10 mmol L^{-1} BAP. After the treatment, the fruit were held at room temperature for 10 min and packed in groups of 4 in plastic trays covered with perforated PVC. Samples were stored at 5 °C for 25 days and during storage fruit deterioration was visually assessed. The whole experiment was performed in duplicate. Based on the results obtained (data not shown) CK concentrations of 1.0 mmol L^{-1} BAP were selected for further evaluation.

In a second group of experiments, summer squash ($n = 250$) were harvested at commercial maturity and immediately transported to the laboratory. Fruit were divided into two groups which were either sprayed with 1.0 mmol L^{-1} BAP or distilled water (control). After 5–10 min at room temperature, fruit were placed in plastic trays and wrapped with perforated PVC. The trays were stored at 5 °C for 0, 13 or 25 days and then transferred to 20 °C for 2 days (13 + 2 and 25 + 2). At each sampling date, 25 fruit from both control and BAP sprayed groups were taken for quality evaluation. Fruit were immediately analyzed or otherwise frozen in liquid N_2 and stored at −80 °C until use. The whole experiment was performed in duplicate.

2.3. Deterioration index

Fruit deterioration was visually assessed as previously reported (Massolo et al., 2013). Twenty five fruit were evaluated for each treatment and storage time.

2.4. Weight loss

Fruit were individually weighed before packing and during storage. Weight loss was calculated from initial (IW) and final weights (FW) as:

$$\text{Weight loss}\,(\%) = \frac{\text{IW} - \text{FW}}{\text{FW}} \times 100$$

2.5. Respiration rate

Fruit were placed in groups of three in a glass flask. The flask was tightly sealed and incubated at 20 °C for 10 min. Carbon dioxide concentration was determined with an IR sensor, Alnor Compuflow (Model 8650, Alnor CA, EEUU). Results were expressed as mmol of CO_2 produced per kilogram of fresh fruit in 1 h. Three measurements were done for each treatment and storage time.

2.6. Titratable acidity and pH

Longitudinal wedges from different fruit were frozen in liquid nitrogen and ground in a mill. Ten grams of the resulting powder were added to 100 mL of water. Fruit acidity was evaluated titrimetrically with NaOH (0.025 mol L^{-1}) until pH 8.2 (AOAC, 1980). Four samples were evaluated for each treatment and storage time. Results were expressed as $[H^+]$ mmol kg^{-1}.

2.7. Soluble sugars

Frozen fruit was processed in a mill as indicated in Section 2.6 and 1 g of the resulting powder was extracted with 5 mL of ethanol. The mixture was vortexed and centrifuged at 13,000 × *g* for 10 min at 4 °C. The supernatant was taken to 150 mL with distilled water. Total sugars were measured according to Greenfield and Southgate (1992). Briefly 1 mL of 0.5 g L^{-1} anthrone, prepared in 98% (w/w) H_2SO_4, was added slowly to the test tubes in a water-ice bath containing 100 μL of sample extract and 100 μL of distilled water. Samples were boiled at 100 °C for 10 min, cooled in water and the absorbance at 620 nm was measured in a spectrophotometer (Beckman Model UV Mini-1240, CA, USA). Four measurements were done for each treatment and storage time. Glucose was used as a standard and results were expressed as grams of glucose equivalents per kilogram of fresh fruit

2.8. Skin color

Fruit color was measured with a colorimeter (Minolta, Model CR-400, Osaka, Japan). The L^*, a^* and b^* chromaticity values were obtained and the hue angle and chroma were calculated (McGuire, 1992). Twenty five fruit were evaluated for each treatment and storage time and two measurements were done on each fruit.

2.9. Phenolic compounds

Ethanol soluble phenolics were determined according to Singleton et al. (1999). Fruit was frozen in liquid N_2, ground in a mill and approximately 1 g of the resulting powder was added to 5 mL of ethanol. The suspension was vortexed and centrifuged at 13,000 × *g* for 10 min at 4 °C. Fifty microlitres of 1 N Folin–Ciocalteu reagent were pipetted into test tubes containing 100 μL of sample extracts and taken to 1.4 mL with water. Samples were vortexed and after 3 min at 20 °C 100 μL of 20% (w/v) Na_2CO_3 dissolved in 0.1 mol L^{-1} NaOH were added. Samples were vortexed and incubated at 20 °C for 1 h and the absorbance at 760 nm was subsequently measured. For each treatment and storage time, two extracts were prepared and each one was measured in duplicate. A standard curve with chlorogenic acid (ChA) was performed and results were expressed as grams of ChA equivalents per kilogram of fresh weight.

2.10. Texture

Firmness was measured in a texture analyzer (TA.XT2, Stable Micro Systems Texture Technologies, Scarsdale, NY, USA) fitted with a 3 mm flat probe. Each fruit was compressed 10 mm at the equatorial zone at a rate of 1.0 mm s^{-1} and the maximum force (firmness), distance to tissue failure (DF) and resistance to penetration (RP, initial slope of the test curve), were registered. Two measurements were done on each fruit and 25 fruit were analyzed for each treatments and storage time. Results were expressed in newtons (N).

2.11. Cell wall isolation

Thirty grams of fruit were placed in 100 mL ethanol and were homogenized in an Ultraturrax® (IKA Works, Brazil), for the extraction of low molecular weight solutes and boiled for 20 min to ensure the inactivation of enzymes. The insoluble material was vacuum-filtered and sequentially washed with 40 mL ethanol, 40 mL ethanol:chloroform (1:1, v/v), and 40 mL acetone and dried at 37 °C, yielding the alcohol insoluble residue, AIR. The dried residue was weighed. Two independent extractions were made for each treatment and storage time. Results were expressed as grams of AIR 100 g^{-1} of fresh fruit.

2.12. Cell wall fractionation

Fractions of different cell wall components were obtained by sequential chemical extraction of the cell wall material (AIR). Approximately 40 mg of AIR residue from each sample were suspended in 10 mL of water and stirred at room temperature for 12 h with shaking, then centrifuged at 13,000 × *g* per 10 min, and the supernatant obtained was vacuum filtered. The filtrate was taken to 15 mL with water and designated water soluble fraction (WSF). The insoluble pellet was then extracted with 10 mL of 50 mmol L^{-1} EDTA at room temperature for 4 h and the supernatant obtained after centrifugation at 13,000 × *g* per 10 min was taken to 15 mL and designated EDTA soluble fraction (ESF). Subsequently, the residue was extracted with 10 mL of 0.1 mol L^{-1} Na_2CO_3 containing 20 mmol L^{-1} $NaBH_4$ and stirred for 1 h at 4 °C with shaking. The slurry was centrifuged as described above and the supernatant was taken to 15 mL with water and designed as Na_2CO_3 soluble fraction (NSF). The insoluble pellet was then extracted with 10 mL of 1 mol L^{-1} KOH containing 20 mmol L^{-1} $NaBH_4$ for 1 h at 4 °C with shaking centrifuged and the extracted solution was designated as the 1 M KOH-soluble fraction (1KSF). The residue was finally re-extracted with 10 mL of 4 mol L^{-1} KOH containing 20 mmol L^{-1} $NaBH_4$ for 1 h at 4 °C and after centrifugation the supernatant containing 4 M KOH-soluble material was obtained (4KSF). Two cell wall samples were analyzed for each treatment and storage time analyzed and each sample was extracted in duplicate. Samples of the different fractions obtained were assayed for neutral sugar (NS) and uronic acids (UA).

2.13. Uronic acids (UA)

UA were measured according to Blumenkrantz and Asboe-Hansen (1973). Aliquots of the different cell wall fractions (100 μL) were pipetted into test tubes and taken to 200 μL with distilled water. After that 1 mL of 98% (w/w) H_2SO_4 containing 75 mmol L^{-1} sodium borate was added in an ice water bath. Samples were vortexed and incubated at 100 °C for 10 min. After boiling the reaction mixtures were cooled in a water ice bath and 20 mL of 0.15% w/v *m*-phenylphenol dissolved in 0.5% w/v NaOH were added. After vortexing the absorbance at 520 nm was measured in a spectrophotometer (Beckman Model UV Mini-1240, CA, USA). A standard curve was generated with galacturonic acid (GalA) and results were expressed as mg GalA per g AIR. Two independent samples were analyzed for each treatment and storage condition and measurements were done in duplicate.

2.14. Neutral sugars (NS)

NS were measured in the different cell wall fractions by the anthrone method as indicated in Section 2.7. Two independent samples were analyzed for each treatment and storage condition and measurements were done in duplicate.

2.15. Statistical analysis

Experiments were performed in a factorial design being the factors treatment (Control or BAP treated fruit) and storage regime (0, 13; 13 + 2; 15 and 15 + 2). Data were analyzed by ANOVA with the PC-SAS software package (SAS Institute Inc., Cary, NC, USA). The model assumptions of homogeneity of variance and normality were probed by means of Levene's and Shapiro–Wilk's tests, respectively. Means were compared by a Fisher test at a significance level of 0.05.

3. Results and discussion

3.1. Fruit deterioration index, weight loss and respiration rate

Fruit deterioration was significantly reduced by pre-storage BAP sprays (Fig. 1 A and B). After 13 d at 5 °C the DI reached values of 0.4 and 0.05 in control and CK-treated fruit, respectively. After 25 days CK treated fruit showed a similar DI than the control at day 13. During the 2 day shelf-life period after both 13 and 25 d at 5 °C BAP-treated fruit also showed lower deterioration than the corresponding controls.

Previous studies have indicated that soft rind squashes are highly sensitive to chilling (Wang, 1996). Storage at 0 °C resulted in severe fruit damage and temperatures 5–10 °C are currently recommended to maximize fruit postharvest life (Suslow and Cantwell, 2013). Most data available are based on studies conducted in zucchini which are particularly susceptible to chilling damage. Wang (1996) showed that storage at 5 °C just for 4 d resulted in extensive CI. In contrast in the present work very low CI incidence was found

Table 1
Acidity, soluble sugars, 1 M KOH soluble cross-linking glycans (1KSF), 4 M KOH soluble cross-linking glycans (4KSF), and α-cellullose in control and BAP-sprayed (1 mM) summer squashes stored for 0, 13, and 25 days at 5 °C and subsequently transferred to 20 °C for 2 days ('13 + 2' and '25 + 2').

		Storage regime				
		0	13	13 + 2	25	25 + 2
Acidity (mmol kg^{-1})	Control	7.3^{cd}	9.5^{bc}	13.4^{a}	8.4^{cd}	10.8^{b}
	BAP	6.5^{d}	7.4^{cd}	13.6^{a}	8.0^{d}	12.8^{ab}
Sugars (g kg^{-1})	Control	33.6^{a}	33.4^{a}	29.8^{cd}	26.4^{e}	30.1^{bc}
	BAP	33.1^{ab}	30.1^{bc}	27.2^{de}	29.1^{cde}	28.1^{cde}
1KSF (mg g^{-1})	Control	1.0^{a}	0.7^{a}	0.7^{a}	0.6^{a}	1.0^{a}
	BAP	0.9^{a}	0.8^{a}	0.5^{a}	0.7^{a}	0.6^{a}
4KSF (mg g^{-1})	Control	0.9^{bc}	0.7^{d}	0.7^{d}	0.8^{cd}	1.1^{a}
	BAP	1.0^{ab}	0.8^{cd}	0.8^{cd}	1.0^{ab}	0.8^{cd}
α-Cellulose (mg g^{-1})	Control	0.24^{a}	0.13^{b}	0.18^{ab}	0.13^{b}	0.18^{ab}
	BAP	0.17^{ab}	0.21^{ab}	0.17^{ab}	0.15^{ab}	0.15^{ab}

Values with different letters are significantly different based on a Fisher's least significant difference (LSD) test at a level of significance of $P \leq 0.05$.

throughout the 25 d storage period. Although fruit with similar pre- and postharvest operations should be evaluated to unequivocally determine the chilling sensitivity of different summer squashes, this suggests that round "calabacines" are less chilling sensitive than zucchini squash. In previous study storage of round summer squash at 0 °C for 10 days resulted in marked chilling damage (Massolo et al., 2013). Interestingly, the present study shows that the fruit store well at 5 °C.

Decay incidence was 17 and 2% for control and BAP-treated fruit respectively, at the end of the experiment (data not shown). Beno-Moualem et al. (2001) reported that cytokinins may increase resistance to fungal decay. No evident yellow zones on the fruit surfaces were detected even after 25 d at 5 °C, though green color intensity decreased gradually during storage (Fig. 1A). Differently to what has been reported in other organs such as leaves and flowers (Costa et al., 2006; Chen et al., 2013), CK did not contribute to surface color retention in summer squash.

Pre-storage BAP sprays decreased squash weight loss throughout the storage period (Fig. 1C). After 25 + 2 d weight loss was 5.2 and 3.9% in control and treated squash respectively. Fruit respiration rate increased slightly immediately after the treatments (Fig. 1D). Until 13 + 2 d of storage and according to a non-climacteric behavior, the respiration rate of both control and treated squash declined. Subsequently, CO_2 production increased in both control and BAP-treated fruit. Exacerbated RR in chilling-sensitive species could be the result of prolonged refrigerated storage (Wang, 1982; Lee et al., 2001). Treated fruit showed slightly higher RR than the control at day 25, but no differences were detected afterwards. Overall, data indicated that pre-storage 1 mM BAP sprays may be useful to complement refrigeration, decreasing weight loss and decay.

3.2. Color, sugars, acids, surface and phenolic compounds

After 13 d lightness (L^*) increased in both control and treated fruit (Fig. 2A). No further changes were recorded until day 25, but after the last shelf-life period the fruit became significantly lighter. Changes in *hue* angle indicated a shift from dark to light green tones (Fig. 2B). The reduction of fruit *hue* angle was delayed after 13 + 2 d in BAP-treated squash, but no differences were detected afterwards. Fruit *chroma* increased in the first 13 d of storage but dropped at the last sampling date (Fig. 2C). Endogenous CKs in developing kiwifruit are implicated in maintaining fruit flesh chlorophyll levels (Pilkington et al., 2013). Cytokinin treatments have been also shown to delay chlorophyll degradation and even induce re-greening in leaves (Zavaleta-Mancera et al., 1999). In contrast, results from the present work showed that BAP treatments did not contribute to green color retention in summer squash fruit. Although CKs are known to act as negative senescence regulators in plants, it has been shown that senescence of fruit and vegetative organs do not share all regulatory events (Wagstaff et al., 2009).

Fruit titratable acidity did not show marked changes during refrigerated storage. An increasing trend was evident after fruit transfer to 20 °C, but no differences were found between control and treated fruit (Table 1). Sugar content was close to 3.3% at harvest and showed a slight reduction during postharvest storage. Antognozzi et al. (1996) showed that CPPU treatments increased sugar content. However, in this study the CK were applied in the field and in a starch accumulating fruit species such as kiwifruit. As for acidity, postharvest CK sprays did not affect fruit sugar content of summer squash (Table 1).

Ethanol soluble phenolics were *ca.* 500 mg kg^{-1} in both control and treated fruit. After storage for 13 d the concentration of phenolics increased in the control, remaining unchanged in BAP-treated squash (Fig. 2D). Further increases were detected in untreated fruit during storage at 20 °C. In contrast phenolics remained unchanged in CK-sprayed squash. At the end of the storage period, the controls accumulated 40% higher contents of phenolic compounds than treated squashes. Though the basis of the marked accumulation of phenolics depicted in control squash is unknown, the fact that it occurred at both 5 and 20 °C indicates that is not a consequence of chilling stress and rather is a developmental response. Since in our study only detached fruit were studied, it is not possible to rule out that it is a normal senescence response being delayed by BAP application. Tissue antioxidants are known to decline before tissue death. However they have been shown to accumulate during early senescence (Cavaiuolo et al., 2013). Total phenolics increased dramatically in senescent detached organs (Kar and Mishra, 1976) and CK treatments have been reported to prevent phenolic compounds accumulation (Parthasarathy et al., 2002).

3.3. Fruit texture and cell wall contents

To further characterize the effect of BAP sprays on summer squash we evaluated the changes in fruit firmness, distance to tissue failure and compression resistance. The three parameters showed distinctly different patterns during storage as well as between control and CK exposed squashes. Fruit firmness decreased significantly during 13 d of storage at 5 °C in the controls, but remained unchanged in CK-sprayed fruit (Fig. 3A). However, after 25 days of storage no significant differences were recorded between both groups of fruit. At the end of the shelf-life periods, treated squash remained significantly firmer than control fruit. The

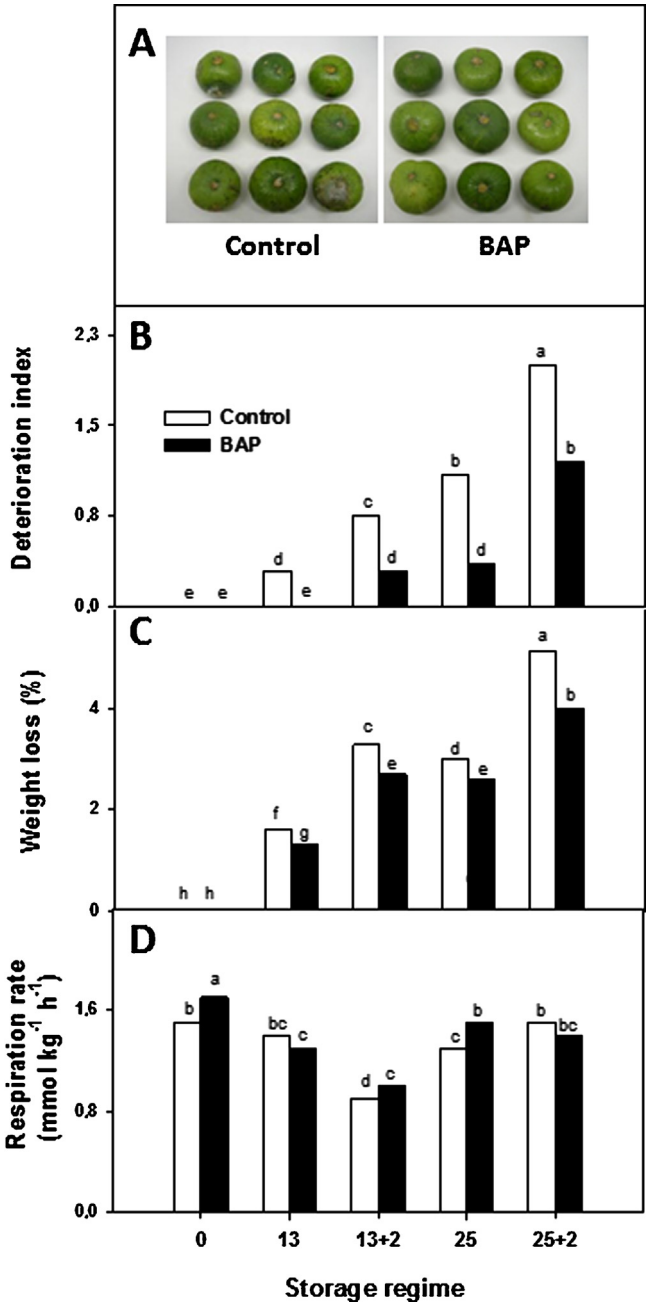

Fig. 1. (A) Appearance, (B) deterioration index, (C) weight loss and (D) respiration rate in control and BAP-sprayed (1 mM) summer squash stored for 0, 13, and 25 days at 5 °C and subsequently transferred to 20 °C for 2 days ('13 + 2' and '25 + 2'). Values with different letters are significantly different based on a LSD test at a significance level of $P < 0.05$.

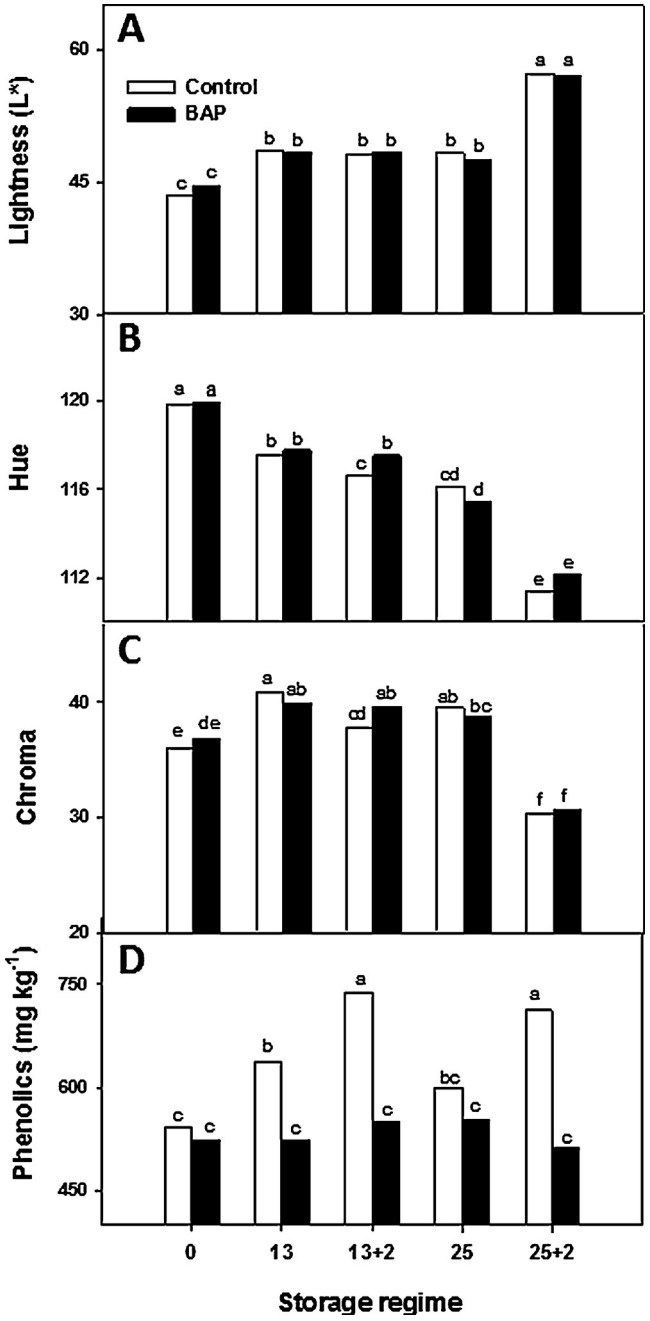

Fig. 2. (A) Lightness, (B) hue, (C) chroma and (D) phenolic compounds in control and BAP-sprayed (1 mM) summer squash stored for 0, 13, and 25 days at 5 °C and subsequently transferred to 20 °C for 2 days ('13 + 2' and '25 + 2'). Values with different letters are significantly different based on a Fisher's LSD test at a significance level of $P < 0.05$.

distance to tissue mechanical failure will, in these cases, provide useful complementary information to characterize fruit textural modifications. Fruit DF increased during storage, but BAP-treated squash maintained lower values than the control at all sampling dates. Interestingly, the differences depicted in DF between control and treated fruit were relatively higher than those recorded in maximum force (Fig. 3A and B). Fruit DF was more highly dependent on storage temperature than firmness. Finally, we determined fruit initial compression resistance which may best represent usual consumer squeezing tests. Fruit squeezing resistance decreased during storage and also in this case BAP-treated fruit had higher values at all sampling dates (Fig. 3C).

Beno-Moualem et al. (2001) found that benzyladenine treatments delayed softening in avocado *cv*. Ettinger. In contrast, early work in non-climacteric olives showed that the CK kinetin did not affect firmness (Shulman and Lavee, 1973). The present results indicate that CK sprays can prevent texture deterioration in harvested summer squash.

Textural changes in fruit have been attributed to different factors such as excessive water loss leading to reduced cell turgor pressure as well as to disassembly of cell wall polysaccharides (Vicente et al., 2007a). The effects of BAP on squash firmness maintenance may have been due to changes in tissue susceptibility to dehydration. It would be useful to determine whether the

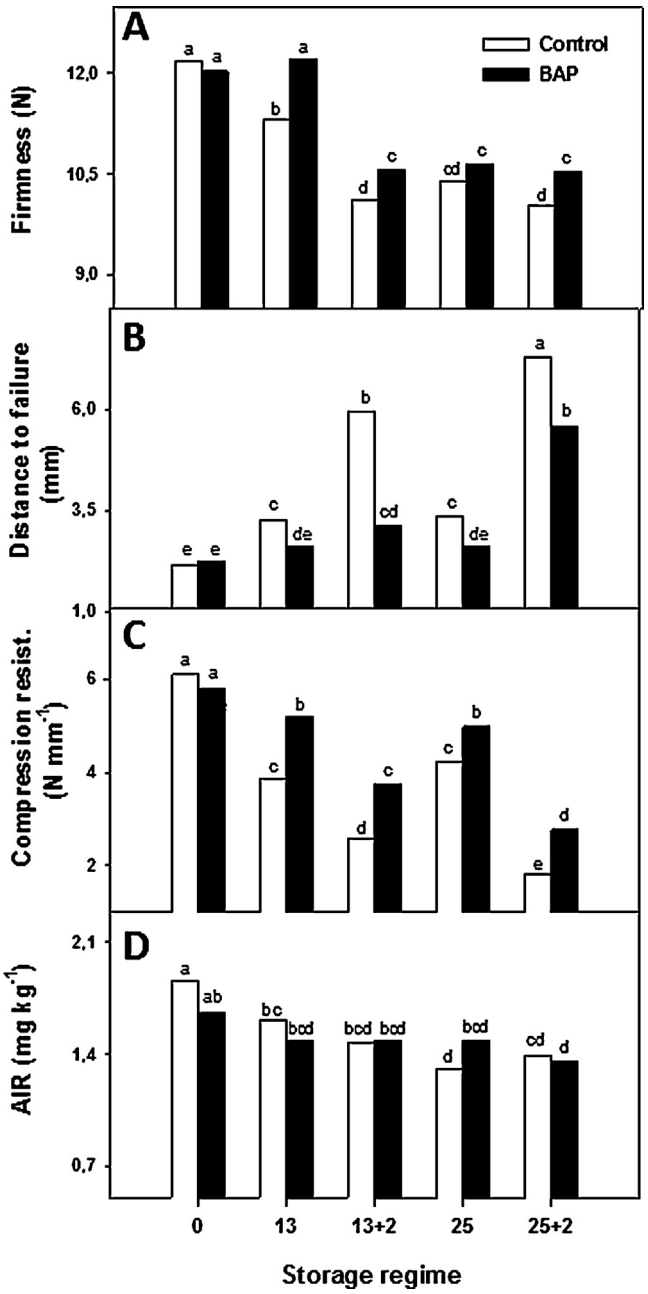

Fig. 3. (A) Firmness, (B) distance to tissue failure, (C) resistance to penetration and (D) cell wall material (AIR) in control and BAP-sprayed (1 mM) summer squash stored for 0, 13, and 25 days at 5 °C and subsequently transferred to 20 °C for 2 days ('13 + 2' and '25 + 2'). Values with different letters are significantly different based on a Fisher's LSD test at a significance level of $P < 0.05$.

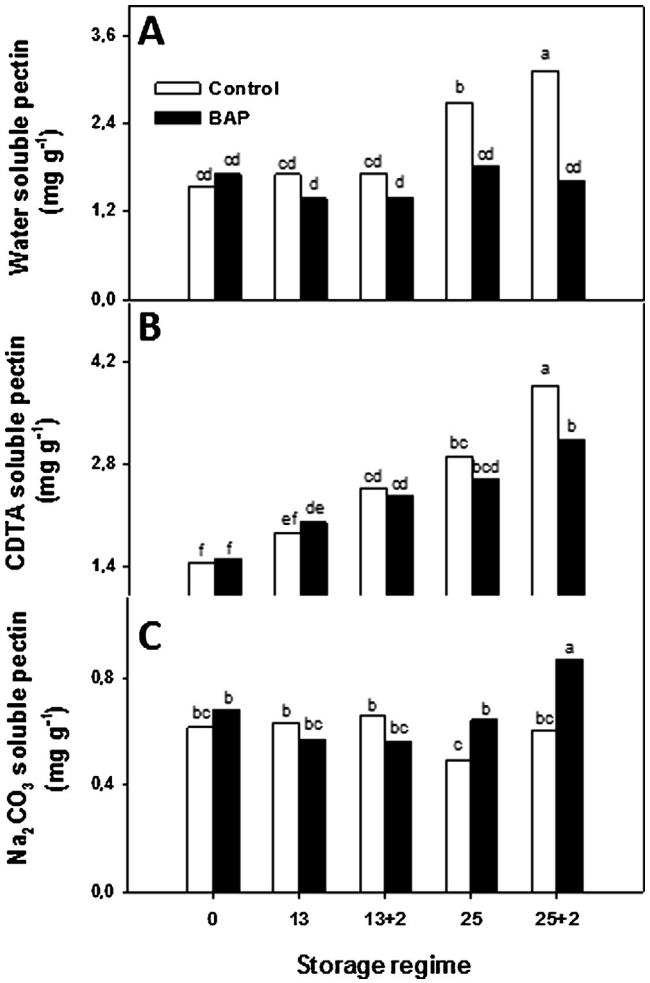

Fig. 4. (A) Water soluble pectin (WSP), (B) EDTA soluble pectin (ESP) and (C) Na_2CO_3 soluble pectin (NSP) in control and BAP-sprayed (1 mM) summer squash stored for 0, 13, and 25 days at 5 °C and subsequently transferred to 20 °C for 2 days ('13 + 2' and '25 + 2'). Values with different letters are significantly different based on a Fisher's LSD test at a significance level of $P < 0.05$.

treatments may affect incipient on-going cuticle development in developing fruit. In apples, BAP treatment did not change cuticle thickness, but was shown to affect fruit epidermal structure (Stern et al., 2013). Further analyses to evaluate this in more detail are encouraging. The lower fruit softening observed may have been associated with a delay of fruit cortex primary cell wall disassembly. However, modifications in the peel may have also contributed to part of the textural differences observed. We extracted the cell wall material from control and treated squash. The AIR decreased during storage accompanying softening, but no differences in total wall contents were found between control and treated fruit (Fig. 3D).

3.4. Solubilization of cell wall components

To further test whether or not BAP treatments resulted in specific changes in cell wall turnover, we fractionated the AIRs to segregate the major polysaccharide groups based on their solubility. Water soluble pectin showed no changes after 13 or 13 + 2 days. However, a large increase in WSP was detected in control squash after 25 days of refrigerated storage (Fig. 4A). Water soluble uronic acids further increased during the last shelf-life period at 20 °C. BAP-treated fruit maintained markedly lower WSP than the control. Ionically-bound polyuronides increased progressively during storage both control and treated fruit (Fig. 4B). As for WSP lower CDTA–soluble pectins were detected in CK-treated summer squash at the end of the storage period. Control fruit showed no marked changes in sodium carbonate soluble polyuronides (Fig. 4C). In contrast, NSP increased in BAP sprayed fruit. Higher levels of tightly bound pectin than the control were detected after 25 and 25 + 2 d. The increase of water and ionically-bound pectins has been shown in softening fruit (Brummell, 2006). Results show that CK delay UA solubilization. In contrast to our findings, in some fruit species the rise in loosely-bound pectin has been associated with a concomitant decrease in NSP (Vicente et al., 2007b). This difference suggests that in squash, uronic acids are being mainly solubilized from cellulose and or cross linking glycan-associated wall material. Extensive

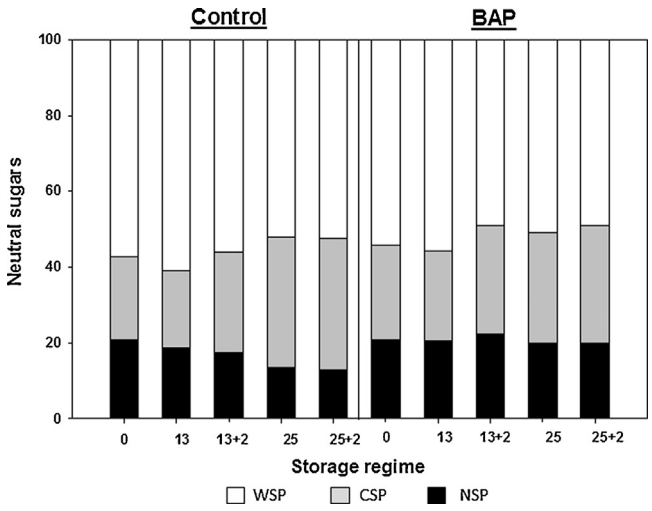

Fig. 5. Relative distribution of neutral sugars based on their solubility in water (WSP), EDTA (ESP) and Na_2CO_3 (NSP) in control and BAP-sprayed (1 mM) summer squash stored for 0, 13, and 25 days at 5 °C and subsequently transferred to 20 °C for 2 days ('13 + 2' and '25 + 2').

association between pectins and other wall polymers is known to exist (Popper and Fry, 2005; Zykwinska et al., 2005). Reduced neutral sugars solubilization was also found in pectin-rich fractions of BAP-treated squash (Fig. 5). Tightly-bound neutral sugars represented by the NSP steadily decreased in the controls but remained unchanged in fruit treated with BAP. CK have been shown to induce cell wall biosynthesis (Robertson et al., 1999), but to our knowledge this is the first report indicating that CK may regulate pectin turnover in harvested fruit. It would be interesting to determine the specific wall degrading enzymes that may be affected by BAP. Finally, no changes were detected in α-cellulose and cross-linking glycans either during storage or between control and treated fruit (Table 1). The fact that the differences in softening between control and CK-treated squash were already detected before wall modifications suggests that the initial effect of CK on firmness retention is more likely related to differences in water loss. Larger textural differences at long times may result, at least in part, from reduced solubilization of wall pectins.

4. Conclusions

The present work showed that round soft rind summer squash are quite tolerant to low temperature storage and could be stored at 5 °C for 25 days without marked CI. Pre-storage BAP sprays reduced fruit deterioration mainly decreasing dehydration, softening and decay but, in contrast to what has been established for vegetative and floral tissues, did not affect green color retention. CKs prevented some major metabolic changes occurring in detached summer squash namely phenolic compound accumulation and pectin catabolism. The main effects observed were delayed solubilization of both uronic acids and neutral sugars in pectin-rich fractions. Instead, the hormonal treatments did not affect cellulose-cross linking glycan solubilization. Overall, results indicate that cytokinin treatments (1 mM, BAP) delayed wall degradation and softening and may be useful to maintain quality of refrigerated summer squash. To our knowledge this is among the first reports indicating that CKs may affect cell wall degradation in detached fruit.

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