



Postharvest treatments to reduce chilling injury in summer squash (*Cucurbita pepo*) fruits during storage

PANKAJ KUMAR KANNAUJIA¹, RAM ASREY², AWANI KUMAR SINGH³ and ELDHO VARGHESE⁴

ICAR-Central Institute of Post Harvest Engineering and Technology, Abohar, Punjab 152 116, India

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ABSTRACT

The present study was carried out to minimize postharvest problems of chilling injury during cold storage by the application of anti-chilling compounds, viz. salicylic acid (SA), sodium nitroprusside (SNP) and a nitric oxide donor (NO). For minimizing chilling injury symptoms summer squash (*Cucurbita pepo* L.) fruits were treated with salicylic acid (1.0–1.5 mM) and sodium nitroprusside (1.0–1.25 mM) followed by cold storage at 5±2°C and 85–90% relative humidity. Among the used anti-chilling molecules, salicylic acid @1.0 mM provides the best results for minimizing chilling injury score (0.74) and proline content (333.87 µg/g) during cold storage. Fruits treated with 1.5 mM SA and 1.0 mM SNP showed a 37.42% and 27.42% lower electrolyte leakage as compared to control. In the case of malondialdehyde (MDA) content, 1.0 and 1.5 mM SA dose showed a ~39% and ~36% lower content than control. Higher fruit firmness (7.07 N) retention was found in 1.0 mM sodium nitroprusside and followed by 1.0 mM salicylic acid (6.96 N) treated fruits. Overall 1.0 mM salicylic acid followed by 1.0 mM sodium nitroprusside proves better for prevention of chilling injury and lower production of malondialdehyde content.

Key words: Chilling injury, Electrolyte leakage, Malondialdehyde content, Salicylic acid, Sodium nitroprusside

Summer squash (*Cucurbita pepo* L.) is an annual vegetable crop which belongs to Cucurbitaceae family. It is the main source of carbohydrates, dietary fibers, minerals (Ca, Mg, P and Zn) and many important vitamins. Yellow to pink fruit flesh is richer in vitamin A than green-fleshed varieties. In India, there are limited varieties which are commercially available in summers like Australian Green, Pusa Alankar and Patty Pan. Summer squash is considered as an important vegetable across the globe due to being a commercial crop for both field and greenhouse conditions. It is grown as spring and summer crop in India. Squashes are quick growing, short duration (from flowering to harvest 45 to 60 days) vegetables and that's why it is the most popular crop among the farmers practicing protected cultivation. Due to short shelf-life and quality deteriorative phenomenon, it is mainly cultivated in peri-urban areas. Compared with other squashes, it is mostly consumed at the immature stage for culinary purposes as whole tender fruit before seeds begin to enlarge and harden.

Summer squash is very sensitive to low-temperature storage and optimum storage range varies between 5–10°C depending on cultivar and production season. Summer squash CI symptom includes peel discoloration, surface pitting, browning and water-soaked appearance. Generally, these symptoms become more pronounced when fruits are taken out to ambient temperature. Malondialdehyde is an oxidative stress indicator which enhance plant membrane integrity losses at low temperature.

Salicylic acid is a ubiquitous plant-based phenolic compound which regulates physiological activities and hormonal balance in plants. Besides, it also plays an important role in minimizing chilling injury by inducing expression of antioxidant and reactive oxygen species scavenging genes (Asghari and Aghdam 2010). Nitric oxide (NO), a free radical based reactive gas which acts as a multi-functional signaling molecule in physiological processes of plants. NO also plays an important role in alleviating chilling injury symptoms by suppressing generation of reactive oxygen species (Flores *et al.* 2008).

MATERIALS AND METHODS

The experiment was conducted at research farm of ICAR-Indian Agricultural Research Institute (IARI), New Delhi during 2015–16. Fruits of summer squash cultivar Pusa Alankar and Australian Green were obtained from the centre for protected cultivation technology farm, IARI,

Present address: ¹Scientist (pankajkannaujia@ymail.com), Horticultural Crop Processing Division, ICAR-CIPHET, Abohar, Punjab; ², ³Principal Scientist (ramu_211@yahoo.com, singhawani5@gmail.com), ICAR-IARI, New Delhi; ⁴Scientist (eldhoiasri@gmail.com), Fishery Resources Assessment Division, ICAR-CMFRI, Kochi.

New Delhi. Chemicals and reagents used for the study were procured from Merck India Ltd. A comprehensive study was carried out to observe the effect of different concentrations of salicylic acid and sodium nitroprusside on summer squash fruits during storage at $5\pm 2^{\circ}\text{C}$ and 85–90% relative humidity in a temperature-controlled chamber. Physical, physiological and biochemical changes in summer squash were recorded at 3 days interval with three replications. The treatment doses of the different anti-chilling molecule were; control, salicylic acid-1.0 mM, salicylic acid-1.5 mM, sodium nitroprusside-1.0 mM and sodium nitroprusside-1.25 mM.

The incidence of chilling injury (CI) was determined by using a rating scale of 0–4 based on dark colouration and skin area of the affected fruit. The scale used was: 0, nil rind damage; 1, trace; 2, slight; 3, moderate and 4, severe. The CI index was calculated by the following formula:

$$[\Sigma(A \times B)/C]$$

where, A, injury score of individual fruit; B, fruit affected; C, number of fruit recorded.

To determine the rate of electrolyte leakage, the method described by McCollum and McDonald (1991) was used and was expressed as percentage. Pectin methylesterase activity (PME) in squash fruit was measured by the method of Hagerman and Austin (1986) with minor modifications. PME activity was expressed as $\Delta A_{620} \text{ min/g/fruit weight (FW)}$.

Fruit firmness was determined by using a texture analyzer (model TA+Di, Stable Micro Systems, UK) by compression test. The sample fruits were compressed using a cylindrical probe (2 mm diameter) by pre and post-test speed of 5 mm/s and 10 mm/s respectively. First peak force (N) in the force-deformation curve was taken as firmness of the sample. Hardness was defined as the maximum force (kgf) during the compression, which was expressed in Newtons (N). Lipid peroxidation in terms of malondialdehyde (MDA) production was measured by following the method of Eum *et al.* (2009) and expressed as $\mu\text{g/g FW}$. Proline contents were measured by following the method of Bates *et al.* (1973) with minor modifications and expressed as $\mu\text{g/g FW}$.

The experiment was conducted under a completely randomized design setup with two factors, viz. five treatments (including control) and five storage intervals. Two-way analysis of variance was performed on the data using PROC GLM of SAS 9.3 software and significant effects ($P < 0.05$) was noted. Further, a significant difference amongst the means was determined by Tukey's HSD test.

RESULTS AND DISCUSSION

Chilling injury index: Chilling injury in summer squash vegetable during storage increased rapidly with the advancement of the storage period in all the treatments (Table 1). Control fruits exhibited much faster development of chilling injury symptoms compared to treated summer squash, which increased faster after 6 days of storage. However, SNP and SA treated fruits showed a significantly lower level of CI as compared to the control. After 12

days of storage, minimum CI (0.74 score) was observed in fruits treated with 1.0 mM SA followed by 1 mM SNP (0.85), while it was highest (1.68 score) in control. This indicates that lower doses (1.0 mM) of SA and SNP proved most suitable for alleviating the chilling injury symptom as compared to higher doses. At the end of storage summer squash treated with 1.0 mM SA and 1.0 mM SNP showed a $\approx 56\%$ and $\approx 50\%$ lower chilling injury score respectively, as compared to control.

Salicylic acid is an endogenous signaling molecule, which plays an important role in response to stressful environmental conditions. During chilling injury, a huge amount of ROS is produced which increases the oxidative stress and causes CI symptoms. SA alleviated CI symptom by inducing the expression of ROS avoidance genes and ROS scavenging genes thus increasing the antioxidant capacity of the plant cells against the ROS (Asghari and Aghdam 2010). SA treatments also induce synthesis and accumulation of heat shock proteins (HSPs) which protect the horticultural produce against chilling injury (Tian *et al.* 2007). On the other side SNP, might have down-regulated the generation of ROS and consequently reduced oxidative stress during low-temperature storage, which alleviated CI symptoms. SA and SNP also protect the plant cell against the electrolyte leakage, therefore cell membrane integrity maintained during the low-temperature storage.

Electrolyte leakage: Electrolyte leakage is a measurement of loss of semi-permeability of cell membranes and it is used as an indicator of fruits and vegetables cell wall membrane integrity and used as an indicator of chilling injury. Irrespective of the treatments electrolyte leakage increased continuously up to the termination of storage (Table 1). Up to 3 days of storage, non-significant differences in electrolyte leakage were observed among the different treatments. However, 6-days onward control fruits showed a rapid increase in electrolyte leakage, compared to other treatments. After 12 days of storage, highest (34.71%) electrolyte leakage was observed in control sample, whereas it was lowest (22.47%) in summer squash fruits treated with SA (1.5 mM). At the end of storage, fruits treated with 1.5 mM SA and 1.0 mM SNP showed a 37.42% and 27.42% lower electrolyte leakage respectively, as compared to control.

Under the stress condition of the chilling injury, cell membrane lipids change from liquid-crystalline to a solid-gel state in plant tissues. Ultimately this phenomenon leads to the increase in cell membrane permeability and electrolyte leakage (Gómez-Galindo *et al.* 2004). Application of SA is effective in reducing CI in pomegranates by decreasing electrolyte leakage and phenylalanine ammonia-lyase (PAL) activity (Sayyari *et al.* 2009). Higher freshness in terms of less moisture loss could also have been the reason for lower electrolyte leakage incidence during storage.

Fruit firmness: Firmness of summer squash during storage decreased rapidly with the advancement of storage period (Fig 1). The insignificant difference in firmness among the treatments was observed up to 6th day of storage.

Table 1 Effect of salicylic acid and nitric oxide on physical and physiological parameters in summer squash fruit during cold storage

Treatment	Storage days	Chilling injury index	Electrolyte leakage (%)	Malondialdehyde content ($\mu\text{g/g}$ FW)	PME activity ($\Delta\text{A } 620 \text{ min/g /FW}$)
Control	0	0.00q	11.07m	0.016g	0.0013m
	3	0.45k	15.08k	0.082cdefg	0.0057j
	6	0.70g	22.99e	0.120cdefg	0.0076d
	9	1.12b	29.08b	0.203abc	0.0087b
	12	1.68a	34.71a	0.255ab	0.0101a
1.0 mM SA	0	0.00q	11.07m	0.016g	0.0013m
	3	0.16p	12.90l	0.034fg	0.0050k
	6	0.19o	17.27i	0.063defg	0.0061hi
	9	0.44k	21.03g	0.115cdefg	0.0071ef
	12	0.74f	24.84c	0.156abcdef	0.0080c
1.5 mM SA	0	0.00q	11.07m	0.016g	0.0013m
	3	0.19o	12.63l	0.052efg	0.0049kl
	6	0.26m	15.95j	0.079defg	0.0060ij
	9	0.54j	19.42h	0.127cdefg	0.0069f
	12	0.87d	22.47ef	0.165abcde	0.0078cd
1.0 mM SNP	0	0.00q	11.07m	0.016g	0.0013m
	3	0.17o	12.89l	0.027cd	0.0045l
	6	0.23n	17.20i	0.076defg	0.0057j
	9	0.58i	20.65g	0.133bcdefg	0.0067gf
	12	0.85e	23.74d	0.168abcde	0.0075de
1.25 mM SNP	0	0.00q	11.07m	0.016g	0.0013m
	3	0.23n	13.07l	0.058defg	0.0052k
	6	0.33l	17.89i	0.084cdefg	0.0064gh
	9	0.68h	21.95f	0.142bcdef	0.0075de
	12	0.91c	24.15cd	0.176abcd	0.0085b

*Means with the same superscript letters are not significantly different.

After that, a marked decrease in fruit firmness was observed in control. However, after 6 days of storage, firmness was recorded higher in fruits treated with SA and SNP. At the end of storage, highest firmness (7.07 N) was found in 1.0 mM SNP and then 1.0 mM SA (6.96 N) while it was lowest (6.22 N) in control. 1.0 mM SNP and SA treated summer squash fruits retained $\approx 14\%$ and $\approx 12\%$ higher firmness respectively over the control. The interaction effect of treatment (T) $\times$ storage days (D) was found to be significant ($P \leq 0.05$).

The higher fruit firmness in summer squash fruits could be due to lower activity of fruit softening enzymes like pectin methylesterase (PME) and polygalacturonase (PG) due to inhibition of ethylene biosynthesis (Khan *et al.* 2007). Higher firmness in salicylic acid-treated summer squash could be mainly due to reduced ethylene biosynthesis and lower activity of the cell wall and cell membrane degrading enzymes like PME, PG and Lipoxxygenase etc (Zhang *et al.* 2003).

Malondialdehyde content: MDA content is an oxidative product of lipid peroxidation, considered as an indicator of oxidative damages under stress condition. MDA content in 1.0 mM SA treated squash fruits were significantly lower

from 6th day onward over other treatments and the same trend continued during the entire storage period of 12 days (Table 1). At the end of 12 days storage period, minimum

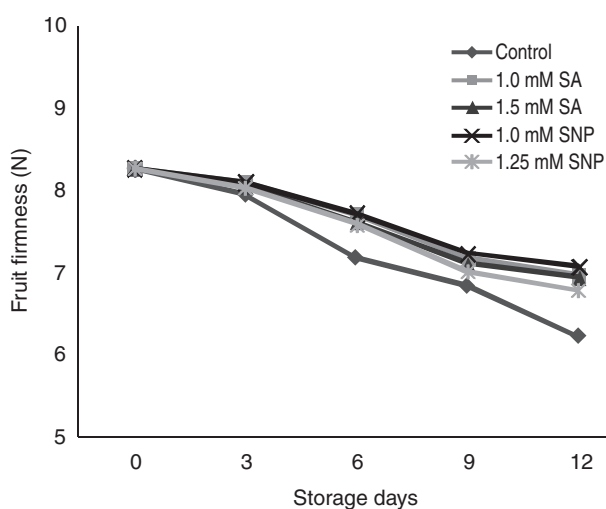


Fig 1 Effect of salicylic acid and nitric oxide on fruit firmness (N) of summer squash during storage.

MDA content (0.156 nmol/g FW) was observed in fruits treated with 1.0 mM SA followed by 1.5 mM SA (0.165 nmol/g FW), while it was highest (0.255 nmol/g FW) in control. Summer squash fruits treated with 1.0 and 1.5 mM SA showed a $\approx 39\%$ and $\approx 36\%$ lower MDA content respectively.

SA can diminish the injuries in cell membranes by enhancing the antioxidant potential of the plant under stress (Tasgin *et al.* 2006). Exogenous application of SA decreases the level of lipid peroxidation and electrolyte leakage from the plant tissues compared with untreated fruits (Kabiri *et al.* 2014). Least production of MDA content could be due to the application of nitric oxide which delayed senescence process and higher cell membrane integrity compared with untreated fruits (Manjunatha *et al.* 2010).

Pectin methylesterase activity: Fruits treated with SNP and SA showed significantly lower PME activity over control (Table 1). Up to 6th days of storage, nonsignificant differences were observed among the treatments. After that, the increase in PME activity was recorded in all the treatments, while this increase was much slower in SNP and SA treated fruits. Among the different treatments, SNP 1.0 mM and 1.25 mM treatments showed 26% and 16% lower PME activity while, in case of SA 1.0 mM and 1.5 mM this value was 21% and 23%, respectively. At the end of the experiment, the highest PME activity was recorded in control fruits, while it was lowest in SNP (1.0 mM) treated fruits.

Due to the occurrence of chilling injury, the physical state of cell membrane lipids changed, which ultimately leads to loss of membrane integrity and electrolyte leakage during low-temperature storage (Gomez-Galindo *et al.* 2004). Lower enzymatic activity in fruits treated with SA and SNP could also be due to the higher fruit firmness because of lower activity of cell wall degrading enzymes. Research findings are also supported by Barman and Asrey (2014), who reported that salicylic acid-treated mango fruits have significantly lower enzymatic activity than control.

Proline content: Proline accumulation in the stored summer squash fruit at low temperature was significantly affected by different treatment (Fig 2). Among different treatments of SA and SNP, 1.0 mM SA followed by 1.0 mM SNP was observed to be the best treatments over the entire storage duration. At the termination of the experiment; among the different treatments, highest proline content (333.87 $\mu\text{g/g}$ FW) was recorded in 1.0 mM SA followed by (310.28 $\mu\text{g/g}$ FW) in 1.0 mM SNP treated fruits while it was lowest (192.90 $\mu\text{g/g}$ FW) in control. At the end of storage, proline content was $\approx 73\%$ higher in 1.0 mM SA and $\approx 61\%$ in 1.0 mM SNP treated fruit compared to control.

Proline is a naturally occurring amino acid and whose synthesis is triggered under stress environment. Salicylic acid, which is considered to be a major component for signal transduction in plants, has been reported a positive correlation to proline synthesis (Pirasteh- Anosheh *et al.* 2014). Nitric oxide is known for anti-aging and antioxidants behavior in plants and other fresh fruits and vegetables. Therefore, it could be inferred that optimum doses of both

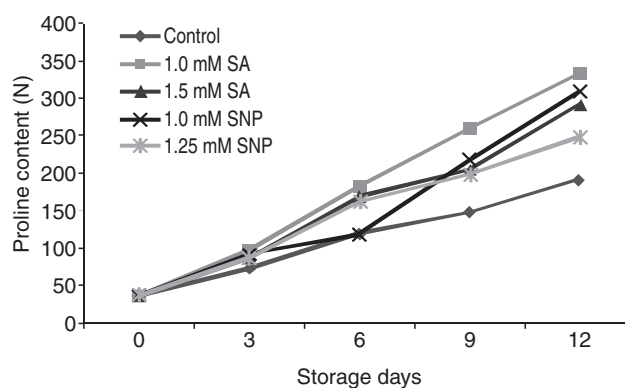


Fig 2 Effect of salicylic acid and nitric oxide on proline content ($\mu\text{g/g}$ FW) of summer squash during storage.

SA and SNP could have contributed to higher retention of proline by the virtue of their anticatabolic properties like suppression of degrading enzymes, respiration rate and ethylene evolution trait of the summer squash (Tasgin *et al.* 2006, Khan *et al.* 2013).

Among the used anti chilling molecules, salicylic acid @1.0 mM provide the best results for minimizing chilling injury, retaining higher proline (333.87 $\mu\text{g/g}$) and lower production of MDA content and a membrane lipid peroxidation indicator after 12 days cold storage. Fruits treated with 1.5 mM SA showed a 37.42% lower electrolyte leakage as compared to control. Application of 1.0 mM sodium nitroprusside had higher fruit firmness (7.07 N) retention even up to the end of 12 days cold storage. Results from the study confirmed that the medium dose (1.0 mM) of both anti-chilling molecules significantly inhibit and delayed the chilling injury symptoms and preserve the quality of summer squash fruits during cold storage at low temperature.

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