



Exogenous spermine treatment modulates senescence and maintains postharvest quality of guava fruit

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ABSTRACT

Guava is one of the widely demanded commercial fruits due to its pleasing flavour and rich nutritional value. The fruit has short postharvest life due to rapid ripening, softening and susceptibility to spoilage. In this study, the guava fruits after harvest were treated with spermine (0.5 mM, 1.0 mM and 1.5 mM) and stored at room temperature for 12 days. The spermine 1.5 mM treatment was noted most effective in retaining desirable physico-chemical and functional quality of stored fruit. Fruit under this treatment showed about 44% lower weight loss, ~57% lower decay loss, and higher retention of chlorophyll (~59%), ascorbic acid (~25%), phenolics (~18%) and flavonoids (~28%) compared to control. Lower accumulation of carotenoids (~22%) and malondialdehyde (~41%) was also recorded in 1.5 mM spermine-treated fruits. The treated fruits also showed 14% higher antioxidant capacity and 37% higher radical scavenging activity over control. The study indicated that shelf-life of guava fruit can be extended up to 12 days, at ambient condition with desirable physico-chemical and functional quality attributes by postharvest treatment of 1.5mM spermine.

Keywords: *Psidium guajava* L., polyamine, shelf life, storage, antioxidants.

INTRODUCTION

Guava (*Psidium guajava* L.) is a commercially important fruit crop, cultivated in tropical and subtropical regions of the world. Due to its pleasant flavour and rich nutritional and digestive properties, it is popularly known as 'Apple of Tropics'. The fruit is a rich source of vitamin C, minerals, pectins and phenolic compounds. Nevertheless, climacteric nature of this fruit lead to rapid upsurge in respiration and ethylene production, causing very fast ripening after harvest. As a consequence, rapid skin yellowing together with softening take place which reduces acceptability of fruit, and make it prone to attack by decay causing microorganisms. The fruit cannot be stored for more than 3 – 4 days at ambient conditions (Kanwal *et al.*, 8). Further, development of chilling injury at the temperature below 10°C limits its storage at low temperature (Etemadipoor *et al.*, 5). Rapid postharvest ripening, high perishability and susceptibility to chilling injury are considered as the major limitations for shelf-life extension of guava, both at ambient and low temperature storage condition.

Polyamines, the natural ubiquitous molecules present in plants are involved in many biological processes (Sharma *et al.*, 11). The major polyamines present in plant are putrescine, spermidine and spermine. The antagonistic relationship of polyamines

with ethylene due to common precursor S-adenosyl methionine (SAM) shows suppression of ethylene production in response to exogenous polyamines treatment in several fruits. Application of polyamines delays ripening, color change, softening, decay symptoms and extends shelf-life in fruit (Sharma *et al.*, 11). Therefore, the present investigation aimed to study the response of pre-storage spermine treatment on quality retention of guava during storage at room temperature (20±3°C temperature and 73±5% relative humidity).

MATERIALS AND METHODS

Physiologically mature guava fruit of cv. Lucknow-49 (average total soluble solids 9.03%) were harvested from the trees of 10-year old in an orchard during January 2019 and immediately brought to the Postharvest Laboratory of the Department of Horticulture, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. Fruits were then sorted for uniform size, colour, maturity and free from blemishes. Treatments were given by immersing fruit in different spermine (0.5 mM, 1.0 mM and 1.5 mM) solutions at 20°C for 5 minutes, with addition of Tween-20 (2 g/L) as surfactant. Fruit dipped in distilled water for the same period were used as control. Then the fruit were dried to evaporate surface moisture and stored at room temperature (20±3°C). At the 3 days interval, the fruits were randomly selected and analyzed for different physico-chemical and functional

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quality attributes up to 12 days. The fruit weight loss was determined by measuring initial weight and weight on the sampling day. Total chlorophyll and carotenoids contents in the fruit skin were estimated to determine the change in fruit colour during storage. It was determined by spectrophotometric method following procedure of Arnon (2). Malondialdehyde (MDA) content was estimated to determine the senescence of fruit during storage as per the method of Zheng and Tian (15).

Functional quality attributes like ascorbic acid content were determined by titration method with 2,6-dichlorophenol indophenol dye and results were expressed as mg/100 g FW (Jones and Hughes, 7). Total phenolics content was estimated by Folin–Ciocalteu reagent at 760 nm in a spectrophotometer (Singleton *et al.*, 12). The results were expressed as gallic acid equivalent (GAE). The method of Zhishen *et al.* (16) was used for determination of total flavonoids content. Aluminium chloride method was used for its estimation at 510 nm and expressed as catechin equivalent (CE). Cupric ion reducing antioxidant capacity (CUPRAC) was followed for estimation of total antioxidant (AOX) capacity (Apak *et al.*, 1). It was expressed as $\mu\text{mol TE/g FW}$ using Trolox as standard. The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity (RSA) was determined at 515 nm in a spectrophotometer and expressed in per cent (Brand-Williams *et al.*, 4).

The experiment was performed using completely randomized design (CRD), with four replications in each treatment. The data of different parameters were expressed as the mean \pm standard error (SE). For estimation of significant difference ($P \leq 0.05$) between different treatments, HSD Tukey's test was used using SAS software (Version 9.2, SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

A gradual increase in weight loss in guava was noted with the storage period. Control fruit had maximum weight loss, reaching up to 22% at the final day (day 12) of storage (Fig. 1A). All the SPM treatments proved effective in minimizing weight loss, but the best result was noted in 1.5 mM SPM-treated fruit, which showed 31.8% lower weight loss than control. The reduction in fruit weight with the storage duration was primarily attributed to loss of moisture. SPM treatment probably maintained high membrane integrity by its conjugation with cell membrane phospholipids and carboxyl groups of pectic substances that altered the biophysical properties of fruit (Mishra *et al.*, 9). As a result, moisture loss from guava was reduced, causing lower weight loss in treated fruit than control. In addition,

the antisenescence property of SPM might have further contributed to reduced weight loss in guava fruit during storage.

In this study, decay symptom was observed in control, SPM 0.5 mM and 1.0 mM treated guava fruit after 6 days of storage while, it appeared after 9 days in 1.5 mM SPM-treated fruit, showing a delay of 3 days (Fig. 1B). Thereafter, rapid upsurge in decay loss was noted in control fruit reaching up to 36.7% after 12 days of storage. All the SPM treatments irrespective of their concentrations were found effective in reducing the disease incidence in guava significantly. However, fruit treated with 1.5 mM SPM showed ~57% lower decay loss than control, which was minimum among the treated fruit. Lower decay loss in SPM-treated fruit might be associated with increased accumulation of hydroxycinnamic acid amides, showing anti-pathogenic property. Moreover, increased hypersensitive reaction and accumulation of pathogenesis related (PR) proteins probably enhanced the disease resistance in fruits in response to SPM treatment (Seifi and Shelp, 10).

Malondialdehyde (MDA) content in fruit increased invariably among the treated fruits with the storage period. About 8.3-fold increase in MDA content was recorded in control fruit however, its accumulation was significantly lower (~7.7–4.9 fold) in SPM-treated fruit (Fig. 1C). After 12 days of storage, minimum MDA content was noted in 1.5 mM SPM-treated fruits, which was ~41% lower than control. MDA content in the fruit is an indication of senescence, as it is produced as a secondary product during membrane lipid peroxidation (Mishra *et al.*, 9). The rate of lipid peroxidation is dependent upon the balance between production of reactive oxygen species by respiration, and their consumption by antioxidant systems. Thus, reduced accumulation of MDA in response to SPM treatment indicates high membrane integrity and reduced membrane permeability. The polycationic SPM probably delayed lipid peroxidation by binding with negatively charged phospholipids present in membranes, maintaining higher membrane integrity and reduced permeability (Sharma *et al.*, 11).

Rapid loss of chlorophyll content in the fruit skin was noted irrespective of treatments. Control fruit showed ~78% decline in total chlorophyll within 12 days of storage. Treatment with 1.5 mM SPM had minimum loss (~46%) of chlorophyll, retaining ~2.5-fold higher content over control (Fig. 1D). On the contrary, the total carotenoids level in the control fruit showed ~7.68-fold increase up to final day of storage (Fig. 1E). The SPM treatment significantly delayed accumulation of carotenoids in fruit. Among SPM treatments, 1.0 mM and 1.5 mM SPM treatment showed ~13% and 23% lower accumulation of

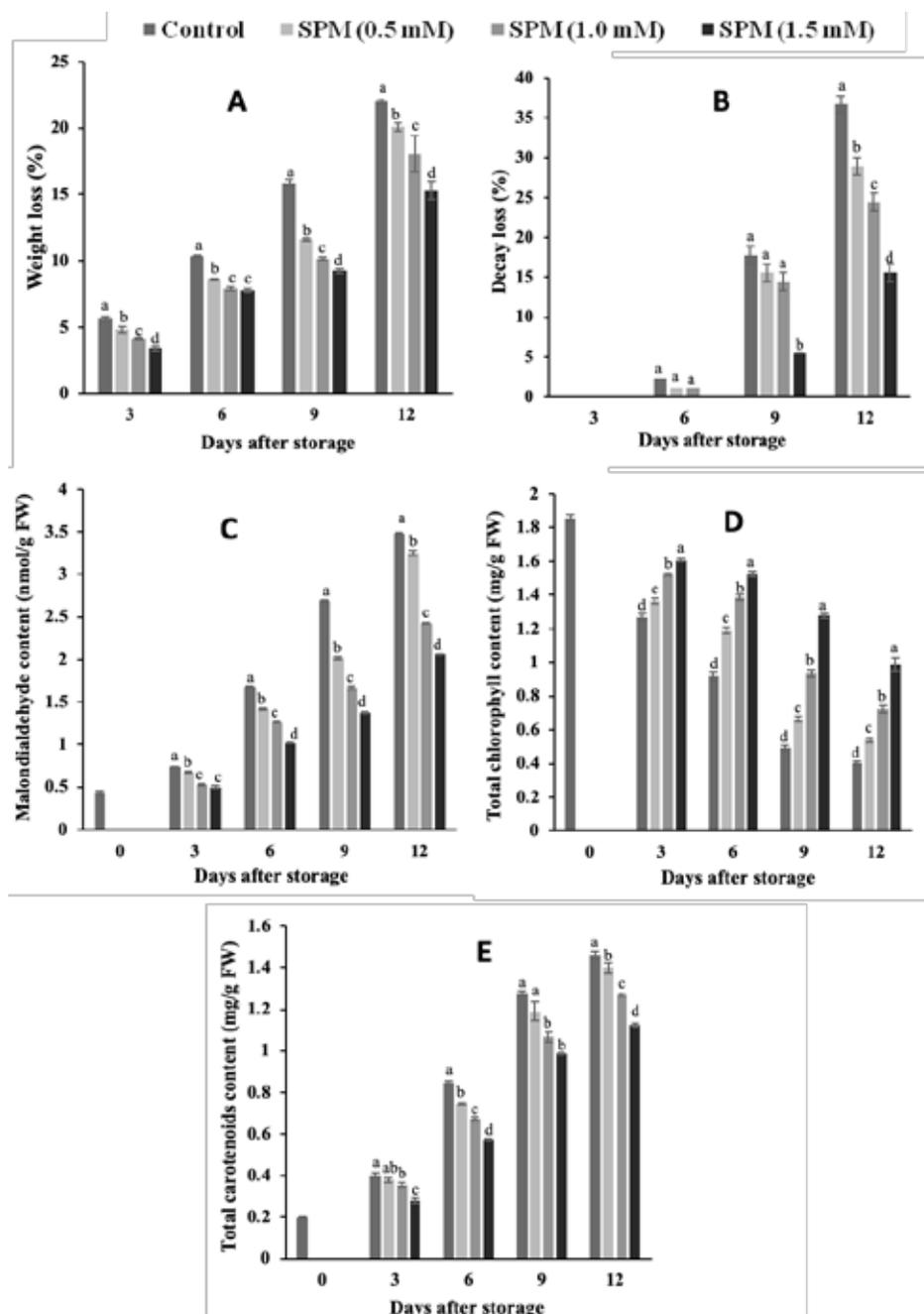


Fig. 1. Effect of spermine treatments on physico-chemical quality attributes of guava. Vertical bars represent standard error of means (n=4).

carotenoids, respectively over control. Chlorophyll pigment degrades with the onset of ripening and converts to pheophytin and pheophorbide. Change in cellular pH and activity of chlorophyllase enzyme also causes degradation of chlorophyll. In the present study, minimum loss of chlorophyll and delayed synthesis of carotenoids in SPM-treated fruit was due to delayed fruit ripening and senescence. SPM

treatment might have reduced ethylene production and delayed the onset of respiratory climacteric due to its competitive action with ethylene for the common precursor SAM (Barman *et al.*, 3). In addition, inhibition of hydrolytic activities on thylakoid membrane of chloroplast by SPM might be another reason behind better retention of chlorophyll in the fruit skin (Sharma *et al.*, 11).

Irrespective of treatments, ascorbic acid content declined with the storage period. Maximum loss (~38%) of ascorbic acid was noted in control fruit while, the loss was between 17-22% in 1.0 mM and 1.5 mM SPM-treated fruits (Fig. 2A). After 12 days of storage, highest ascorbic acid retention (~1.3-fold over control) was recorded in 1.5 mM SPM-treated fruits. Oxidation of ascorbic acid into dehydroascorbic acid by ascorbic acid oxidase, and depletion in level of polyamines is reported during ripening and senescence (Thapa *et al.*, 13). SPM treatment probably reduced activity of ascorbic acid oxidase and enhanced endogenous polyamine level that delayed the loss of ascorbic acid in fruit (Yahia *et al.*, 14). The antisenesescence property of SPM might also be another reason behind higher retention of ascorbic acid in guava.

The pulp and peel of guava fruit is a rich source of phenolic compounds. Both soluble and insoluble form of phenolic compounds are present in this fruit. In the present study, delayed loss of phenolics and flavonoids was noted with the increase in SPM concentration. Control fruits registered ~33% loss of phenolics (Fig. 2B) and ~43% loss of flavonoids

(Fig. 2C) during storage while, it was between 19 – 21% in 1.5 mM SPM-treated fruits. After 12 days, the maximum retention of phenolics and flavonoids were noted in 1.5 mM SPM-treated fruit, depicting ~1.2-fold and ~1.4-fold higher over control. The reduction in phenolics and flavonoids content with storage might be due to cell structure breakdown due to senescence (Thapa *et al.*, 13). The organic acids provide carbon skeletons for phenolic compounds biosynthesis. Thus, decline in phenolic compounds during storage may also be attributed to the decrease in organic acids due to ripening. Spermine treatment retained higher phenolics and flavonoids in fruit that might be associated with its antisenesescence property (Mishra *et al.*, 9).

The AOX capacity in fruit reduced gradually with the storage period irrespective of treatments. About 1.5-fold decline in AOX capacity was noted in control fruit during storage however, it was lower (~1.3 to 1.4-fold) in fruits treated with SPM 1.0 mM and SPM 1.5 mM. After 12 days, 1.5 mM SPM-treated fruit retained ~14% higher AOX capacity over control, which was maximum among the treated fruits (Fig. 3A). Likewise, ~54% reduction in RSA

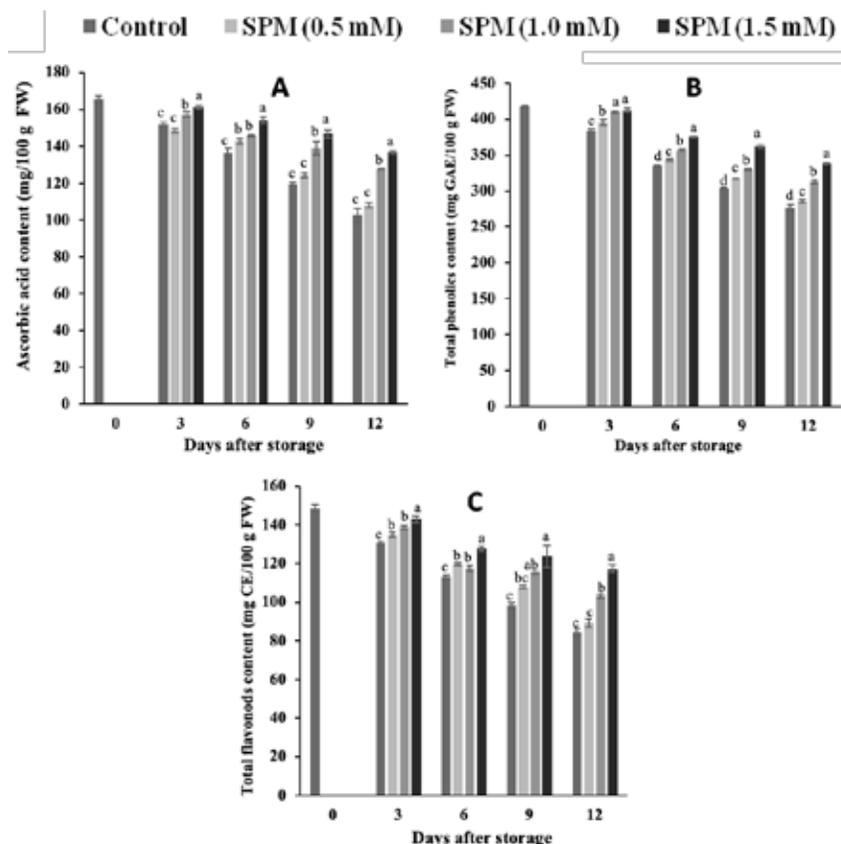


Fig. 2. Effect of spermine treatments on functional quality attributes of guava. Vertical bars represent standard error of means (n=4).

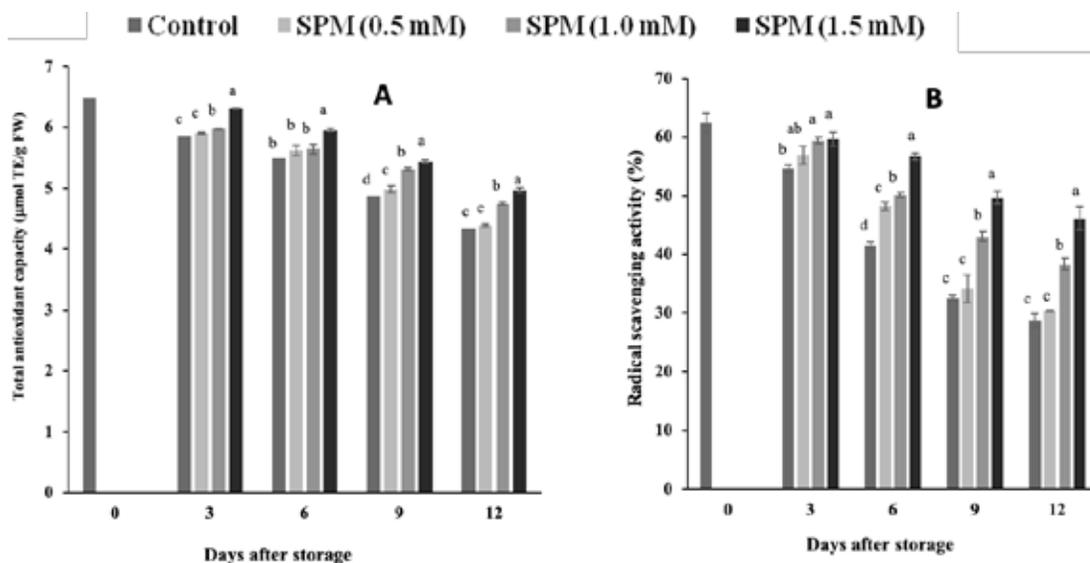


Fig. 3. Effect of spermine treatments on antioxidant capacity of guava. Vertical bars represent standard error of means (n=4).

was recorded in control fruit throughout the storage period but, SPM (1.5 mM) treatment proved most effective, and maintained maximum RSA, ~37.7% higher over control (Fig. 3B). After 12 days, AOX capacity and RSA in guava were statistically at par in control and 0.5 mM SPM-treated fruits. The levels of phenols, flavonoids and ascorbic acid in fruits are positively correlated with the AOX capacity and RSA. Compared to control, postharvest SPM treatment in guava retained higher level of these compounds, thereby maintained high AOX capacity and RSA (Thapa *et al.*, 13). Furthermore, binding of SPM with antioxidant enzymes like superoxide dismutase, catalase, ascorbate peroxidase and scavenging reactive oxygen species might be responsible for reducing oxidative stress and retaining higher AOX capacity (Jhalegar *et al.*, 6).

Exogenous application of 1.5 mM spermine in harvested guava fruit proved beneficial in maintaining desirable physico-chemical and functional fruit quality attributes. Minimum weight loss, decay incidence and delayed senescence facilitated storability of fruit up to 12 days at ambient condition. Better peel colour, ascorbic acid, phenolics, flavonoids and antioxidant capacity were retained in 1.5 mM spermine-treated fruit. The study may be useful to growers and traders for distant marketing and extending storage life of guava, thereby reducing its postharvest loss.

AUTHORS' CONTRIBUTION

Conceptualization of research (KB); Designing of the experiments (KB); Contribution of experimental

materials (KB and AKS); Execution of field/lab experiments and data collection (SKS); Analysis of data and interpretation (SKS and KB); Preparation of the manuscript (SKS, KB and AKS).

DECLARATION

The authors declare no conflict of interest.

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