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Exogenous salicylic acid reduces decay and preserves bioactive compounds in bell pepper during cold storage

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ABSTRACT

Bell pepper is a valuable vegetable crop, but its postharvest quality is often limited by rapid deterioration and senescence. Salicylic acid (SA), a novel plant growth regulator, has gained significant attention for its ability to delay ripening and slow down senescence in various fruits and vegetables. This study evaluated the effect of SA treatment on the bioactive compounds, antioxidant activity and postharvest shelf life of bell peppers. The fruits were dipped in different SA concentrations (50, 100, 200 and 300 µM) for 15 min and then stored for 20 days at 10 ± 1°C temperature with 85-90% RH. Bell peppers treated with 200 µM SA showed a significant reduction in weight loss, respiration rate and malondialdehyde content while retaining higher firmness, total phenolics, flavonoids and ascorbic acid compared to untreated fruit. Additionally, it maintained higher DPPH radical scavenging activity, capsaicin and proline content throughout cold storage compared to the control. Notably, the 200 µM SA treatment extends the shelf-life of bell peppers up to 20 days without compromising sensory acceptability as against 10 days in control.

Key words: Firmness, ascorbic acid, capsaicin, proline, *Capsicum annuum* L.

INTRODUCTION

Bell pepper (*Capsicum annuum* L.), also known as capsicum or sweet pepper, belongs to the Solanaceae family and is widely grown across the globe. Its appeal lies in its unique combination of sweet and mildly pungent flavors, making it a versatile vegetable in many dishes (Cheema *et al.*, 5). In addition to its taste, bell pepper is rich in bioactive compounds like vitamins, phenolics and flavonoids, which offer numerous health benefits. These include immune-boosting properties, potent antioxidant effects and the potential to reduce cancer risk (Ahamad *et al.*, 2). Bell peppers are highly perishable due to their large surface area-to-volume ratio, which leads to significant water loss and shrinkage during storage (Prajapati *et al.*, 14). These fruits are particularly prone to spoilage and require careful handling to preserve their postharvest quality (Sharma *et al.*, 17). Their shelf life is limited typically 3-4 days at room temperature and about a week in cold storage due to rapid weight loss, shriveling, yellowing, loss of glossiness and increased susceptibility to decay (Ahamad *et al.*, 3). The quick senescence of bell peppers is driven by the production of reactive oxygen species (ROS), which cause oxidative damage to cellular structures (Cheema *et al.*, 5).

Salicylic acid (SA) is a phenolic compound and a natural plant growth regulator with great potential to reduce postharvest losses under cold storage conditions in various horticultural crops like tomato (Kumar *et al.,* 10), guava (Madhav *et al*., 12), bitter gourd (Prajapati *et al.,* 15), jujube (Yang *et al.*, 19) *etc.,* In addition to its agricultural benefits, dietary salicylates from fruits and vegetables are recognized as effective bioactive molecules with numerous health advantages and are classified as generally recognized as safe (GRAS) (Prajapati *et al.*, 15). Studies have shown that the external application of SA helps maintain the fruit quality (Jiang *et al.*, 8), slows down the ripening process and preserves antioxidant activity (Yang *et al.*, 19). However, to our knowledge, there is a scarcity of scientific research on the effects of SA in preserving the quality of bell peppers during storage. Therefore, this study aims to assess the potential effect of SA treatments in extending the postharvest shelf life and maintaining the quality of bell peppers during cold storage.

MATERIALS AND METHODS

Bell pepper (cv. Indam Laxmi) fruits were harvested at commercial maturity from the experimental farm of the Center for Protected Cultivation and Technology (CPCT), ICAR-IARI, New Delhi, India. Uniform,

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defect-free fruits were arranged into five groups and soaked in varying concentrations of SA solutions (50, 100, 200 and 300 µM) for 15 min. A control group (untreated) was dipped in distilled water for the same duration. All samples were stored at 10 ± 1°C temperature and 85-90% RH. The fruits were asessed in triplicate every 5 days over a 20 day period to assess physicochemical properties and sensory scores. Physiological loss in weight was determined by comparing initial and final weights at each 5 day interval and expressed as a percentage (%). Decay incidence was measured by counting fruits with dark spots, rotted fruit and presenting the results as a percentage (Menaka *et al.*, 13). Fruit firmness was assessed using a texture analyzer (TA+Di, Stable Micro Systems, UK) equipped with a 2 mm diameter probe, following the method outlined by Prajapati *et al.* (14). The respiration rate was determined using a static headspace technique (Kant and Arora, 9). Oxygen (O_2) and carbon dioxide (CO_2) levels in the headspace were measured with a gas analyzer (Checkmate 9900 model, PBI Dansensor, Denmark), and the results were expressed as mL CO_2 kg^{-1} h^{-1}. Capsaicin content was quantified using the method by Ahamad *et al.* (3), with minor adjustments. For this procedure, 2 g of fruit pulp was incubated in 100 mL of ethyl acetate for 24 h. After incubation, 1 mL of the extract was taken and diluted to 5 mL with ethyl acetate. Then, 0.5 mL of vanadium oxy-chloride solution (0.5% in ethyl acetate) was added just before measuring absorbance at 720 nm.

The total phenolic content (TPC) and total flavonoid content (TFC) of bell pepper were determined using the Folin-Ciocalteau reagent method (TPC) by Singleton *et al.* (18) and the aluminum chloride method (TFC) by Zhishen *et al.* (20). Results for TPC and TFC were expressed as mg of gallic acid equivalent (GAE) per 100 g of fresh weight (FW) and mg of catechin equivalent (CE) 100 g^{-1} FW, respectively. Proline content was estimated according to the method by Menaka *et al.* (13) using the sulphosalicylic acid procedure, with absorbance measured at 520 nm and results expressed as µM g^{-1} FW. Ascorbic acid levels were determined through a 2,6-dichlorophenol indophenols dye visual titration and results were presented as mg 100 g^{-1} FW (Ahamad *et al.*, 3). The DPPH inhibition percentage was measured by the method of Brand-Williams *et al.* (4) using a spectrophotometer at 517 nm. Malondialdehyde content in bell pepper was analyzed using the Thiobarbituric Acid colorimetric assay, with results expressed as µM g^{-1} FW (Ahamad *et al.*, 3). Sensory attributes such as color, texture, taste, flavousr, appearance and overall acceptability were evaluated by a panel of ten participants, who rated the bell peppers using a 9-point hedonic scale (Ahamad *et al.*, 2). A two-way ANOVA was performed on the data (Storage days × Treatments) for various parameters using a completely randomized design with the R software package. Differences between means were tested for significance using Duncan's multiple range test at a probability level ($p \leq 0.05$).

RESULTS AND DISCUSSION

Weight loss is a key indicator of postharvest freshness and the storage potential of bell pepper fruits, as it significantly impacts fruit quality and consumer appeal (Kant and Arora, 9). During storage, the PLW of bell peppers increased across all treatments. However, SA treatments played a significant role in reducing weight loss over the 20 day storage period. The highest weight loss (17.23%) occurred in the untreated control group, while the lowest (5%) was observed in fruits treated with 200 µM SA on the 20th day of storage (Fig. 1a). The reduced weight loss in fruits treated with SA is likely due to its ability to limit moisture loss by regulating water movement through the cuticle and plasma membrane, enhancing fruit integrity and decreasing respiration and transpiration during storage (Prajapati *et al.*, 15). At 20th day of storage, the lowest decay incidence (10%) was observed in fruits treated with 200 µM SA, while the control group exhibited the highest decay rate (90%) (Fig. 1b). This reduction in decay may be attributed to the upregulation of the antioxidant defense system, characterized by increased total antioxidant levels and proline content (Ahamad *et al.*, 3; Menaka *et al.*, 13). The respiration rate is a key indicator of fruit and vegetable quality during storage. Although respiration rates increased in all samples over time, SA treatments notably slowed this rise (Table 1). The highest respiration rate (63.25 mL CO_2 kg^{-1} h^{-1}) was recorded in the control fruits, whereas those treated with 200 µM SA had the lowest rate (32.05 mL CO_2 kg^{-1} h^{-1}) on the 20th day. Respiration is a cellular process where stored organic materials are broken down, using oxygen and releasing carbon dioxide. SA treatment helps preserve these stored materials by acting as an electron donor, ultimately delaying senescence and slowing respiration (Prajapati *et al.*, 15). There was a consistent decrease in the firmness of bell pepper fruits throughout the storage period (Table 1). Notably, the group treated with 200 µM SA maintained a higher firmness level (11.59 N) compared to the control group, which showed its lowest firmness (4.89 N) on the 20th day of storage ($p \leq 0.05$). The improved firmness in SA-treated fruits is likely due to reduced activity of softening enzymes, such as pectin methyl esterase and polygalacturonase, which helps

Table 1: Effect of salicylic acid on firmness, respiration rate, ascorbic acid (AsA), antioxidant capacity (DPPH), total phenols content (TPC) and total flavonoid content (TFC) of bell pepper during cold storage

Parameter	Treatment	Storage days (D)				
		0	5	10	15	20
Firmness (N)	CT	20.43±0.55f	17.54±0.29c	12.59±0.28e	7.52±0.36e	4.89±0.62e
	SA-50 µM	20.43±0.55f	18.81±0.68b	16.27±0.26c	11.32±0.11c	7.58±0.64c
	SA-100 µM	20.43±0.55f	19.28±0.73ab	17.1±0.37b	12.45±0.85b	8.65±0.24b
	SA-200 µM	20.43±0.55f	19.81±0.45b	18.44±0.42a	14.58±0.16a	11.59±0.17a
	SA-300 µM	20.43±0.55f	18.14±0.37c	15.4±0.55d	10.29±0.18d	6.32±0.39d
Respiration rate (mL CO_2 kg^{-1} h^{-1})	CT	15.49±0.12n	30.55±1.04hi	39.39±0.29de	50.84±0.54b	63.25±1.29a
	SA-50 µM	15.49±0.12n	25.25±0.36jk	32.02±1.08h	36.84±1.55ef	43.04±1.34c
	SA-100 µM	15.49±0.12n	22.59±0.49l	28.54±0.58ij	32.79±1.56gh	36.54±1.54ef
	SA-200 µM	15.49±0.12n	19.34±0.15m	23.24±0.96l	26.85±0.6jk	32.05±0.86h
	SA-300 µM	15.49±0.12n	27.02±0.82jk	35.05±1.49fg	42.25±0.62cd	49.54±0.95b
AsA (mg 100 g^{-1} FW)	CT	112.59±2.04a	100.06±1.9cd	82.77±1.07ef	65.53±1.25hi	45.33±0.42l
	SA-50 µM	112.59±1.93a	103.02±0.99bcd	89.18±0.18ef	71.78±2.75gh	57.42±2.48jk
	SA-100 µM	112.59±1.93a	106.07±0.51abc	97.27±1.41d	79.27±2.24f	62.87±1.13ij
	SA-200 µM	112.59±1.93a	109.17±1.88ab	102.75±0.4bcd	87.11±2.05e	77.19±1.21fg
	SA-300 µM	112.59±1.93a	100.89±0.3cd	85.9±0.34e	69.23±1.84hi	53.6±2.55k
DPPH (%)	CT	62.43±1.57a	56.1±1.79cd	45.03±1.26g	34.49±0.85hi	22.3±0.62l
	SA-50 µM	62.43±1.57a	58.13±0.68abc	50.6±0.25ef	38.24±1.23hi	29.32±0.19jk
	SA-100 µM	62.43±1.57a	60.1±0.12abc	52.02±2.49de	44.03±0.53g	33.23±0.21ij
	SA-200 µM	62.43±1.57a	61±0.65abc	58.96±0.4abc	52.12±0.87de	44.29±1.76g
	SA-300 µM	62.43±1.57a	57.54±2.29bc	47.3±2.14fg	36.09±1.46hi	27.52±1.35k
TPC (mg GAE 100 g^{-1} FW)	CT	168.16±1.26a	156.05±2.81bc	142.66±1.63ef	121.04±2.23gh	96.68±1.15j
	SA-50 µM	168.16±1.26a	158.15±2.79abc	148.39±1.26cde	132.2±0.13fg	106.89±1.17ij
	SA-100 µM	168.16±1.26a	160.04±2.7abc	151.05±2.27cde	139.66±1.66ef	116.91±2.45hi
	SA-200 µM	168.16±1.26a	163.48±1.46abc	157.47±2.81abc	148.39±0.14cde	132.67±2.89fg
	SA-300 µM	168.16±1.26a	155.56±1.61bcd	144.04±2.48def	124.89±1.67gh	100.1±2.65j
TFC (mg CE 100 g^{-1} FW)	CT	40.07±1.81a	34.85±1.73cd	28.84±0.1f	20.98±1.02h	12.46±0.53j
	SA-50 µM	40.07±1.81a	37.06±0.55ab	32.87±1.61de	25.18±1.01g	17.01±0.19i
	SA-100 µM	40.07±1.81a	38.46±0.35ab	34.7±0.69cd	28.69±0.9f	22.84±0.15gh
	SA-200 µM	40.07±1.81a	39.07±0.89ab	37.22±1.8abc	32.99±0.5de	29.68±0.51f
	SA-300 µM	40.07±1.81a	36.12±0.47bc	30.07±0.67ef	22.46±0.17gh	15.48±0.59ij

Note: The results are presented as the mean of three replicates ± standard deviation. Different letters in the same column specify significant differences ($p \leq 0.05$; DMRT)

preserve protopectin, calcium and pectin in the cell walls (Madhav *et al.*, 12; Yang *et al.*, 19).

The distinctive pungency of bell pepper is due to the presence of capsaicin. Throughout the storage period, capsaicin content decreased ($p \leq 0.05$) in all treatments (Fig. 3a). By the end of the storage life, the 200 µM SA treatment preserved a higher capsaicin content (0.26%) compared to other treatments as well as control fruits (0.11%). It is well established that capsaicin can undergo oxidation, facilitated by peroxidase in the presence of H_2O_2 during storage (Cheng *et al.*, 6). However, SA treatment helped mitigate this loss, likely by scavenging ROS and enhancing antioxidant enzyme activity. Similarly, Ahamad *et al.* (3) found that treating bell pepper fruits with brassinosteroids resulted in better retention of capsaicin content during cold storage. The total phenolic content (TPC) and total flavonoids content

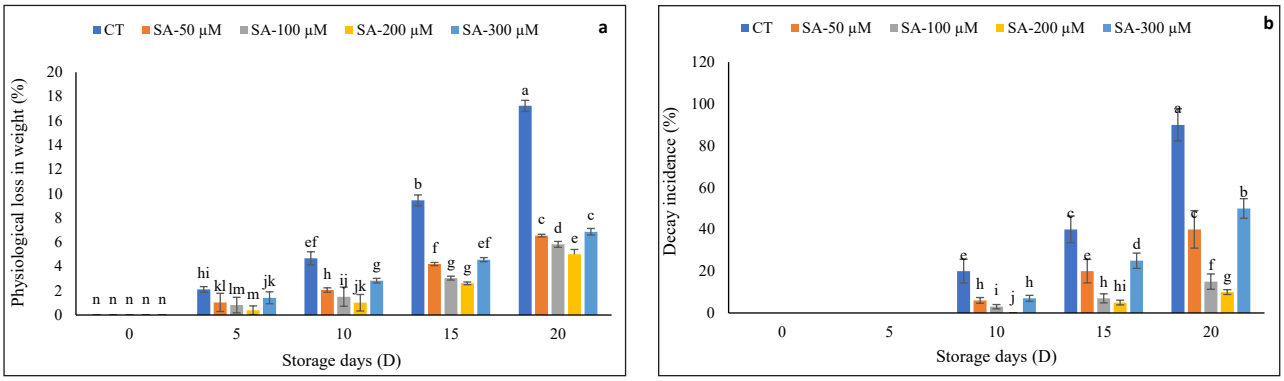

Fig. 1. Effect of salicylic acid on physiological loss in weight and decay incidence of bell pepper during cold storage.
(Error bar specify standard deviation and alphabetic letters specify significant difference at $p \leq 0.05$).

(TFC) gradually decreased as storage time increased, regardless of the treatments applied. However, throughout the storage period, fruits treated with 200 µM L^{-1} SA consistently exhibited higher TPC (132.67 mg GAE 100 g^{-1}) and TFC (29.68 mg CE 100 g^{-1}). In contrast, control fruits experienced the most significant reductions ($p \leq 0.05$), with TPC decreasing to 96.68 mg GAE 100 g^{-1} and TFC to 12.46 mg CE 100 g^{-1} by the 20th day (Table 1). Phenolic and flavonoid compounds, synthesized through the shikimate and phenylpropanoid pathways, demonstrate strong non-enzymatic antioxidant activity, helping to reduce membrane lipid peroxidation by scavenging ROS (Ahamad *et al.*, 3). The key enzymes involved in the phenylpropanoid pathway, including phenylalanine ammonia-lyase (PAL), cinnamate-4-hydroxylase (C4H) and 4-coumarate-CoA ligase (4CL), catalyze essential reactions for the synthesis of these compounds. In our study, we observed a significantly higher retention of TPC and TFC in bell peppers during storage. This effect may be linked to the increased activity of key enzymes, including shikimate dehydrogenase (SKDH), PAL. C4H, and 4CL in SA-treated fruits throughout the storage period (Gao *et al.*, 7). Additionally, SA treatment resulted in lower activity levels of polyphenol oxidase (PPO) compared to the control fruits. The ascorbic acid (AsA) content in bell pepper fruit decreased during storage, irrespective of the treatment applied (Table 1). However, the fruits treated with 200 µM SA significantly ($p \leq 0.05$) retained higher AsA content (77.19 mg 100 g^{-1}) compared to control fruits, which had the lowest levels (45.33 mg 100 g^{-1}) by the end of storage. AsA, a well-known water-soluble vitamin, has notable antioxidant properties that allow it to effectively neutralize free radicals. In this study, the observed loss of AsA content during the storage of bell pepper fruits may be attributed to the rapid conversion of L-AsA into dehydroascorbic acid by the enzyme AsA oxidase (Ahamad *et al.*, 2). Exogenous SA may stimulate the production of AsA and glutathione, or enhance the regulation of enzyme activity, resulting in greater retention of AsA (Yang *et al.*, 19). The ability to scavenge DPPH radicals decreased in both treated and untreated fruits throughout the storage period (Table 1). By the end of storage, bell peppers treated with 200 µM SA showed significantly higher DPPH activity (44.29%), whereas control fruits exhibited the lowest activity (22.30%) on the 20th day ($p \leq 0.05$). SA treatment increased the activity of enzymes in the phenylpropanoid pathway while reducing PPO enzyme activity, which contributed to the greater accumulation of bioactive compounds and enhanced total antioxidant capacity (Aghdam *et al.*, 1). These findings suggest that SA treatment actively promotes the induction of the antioxidant system, effectively alleviating the negative effects of oxidative damage in bell peppers during storage.

The malondialdehyde (MDA) content gradually increased during storage, regardless of the treatments applied (Fig. 2a). However, bell peppers treated with SA consistently showed significantly lower MDA levels compared to the control throughout the storage period. Specifically, fruits treated with 200 µM SA exhibited a much lower MDA accumulation (4.09 µM g^{-1}) compared to the control group (10.24 µM g^{-1}) on the 20th day of storage ($p \leq 0.05$). In this study, the increase in MDA content during cold storage was effectively reduced by SA treatment. Elevated MDA levels in postharvest broccoli indicate lipid peroxidation, which accelerates chlorophyll loss and contributes to quality deterioration and yellowing. SA treatment reduces the production of ROS, which can cause oxidative damage through lipid peroxidation, thus delaying MDA accumulation (Madhav *et al.*, 12; Rehman *et al.*, 16). Proline content exhibited an increasing trend from the beginning to the end of storage (Fig. 2b). Specifically, the 200

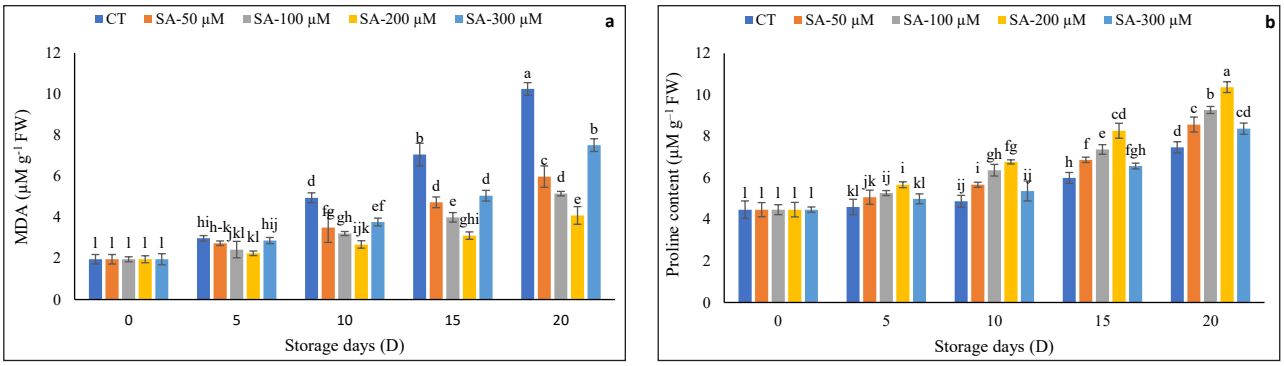

Fig. 2. Effect of salicylic acid on malondialdehyde (MDA) and proline content of bell pepper during cold storage.
(Error bar specify standard deviation and alphabetic letters specify significant difference $p \leq 0.05$).

µM SA treatment significantly increased proline levels (10.37 µM g^{-1}) compared to the control (7.47 µM g^{-1}). Proline acts as a powerful non-enzymatic antioxidant, functioning as a molecular chaperone, enhancing ROS-scavenging enzyme activity, and triggering alternative detoxification pathways (Liu *et al.*, 11). In our study, we found that an increase in storage duration was associated with elevated proline content. This effect is likely due to SA treatment, which regulates proline metabolic enzymes, resulting in higher synthesis and lower degradation rates. The overall appearance of SA-treated fruits was significantly better than that of the control fruits (Fig. 3b). Notably, after 20 days of storage, bell peppers treated with 200 µM SA received considerably higher sensory ratings for various attributes, including appearance (7.14), colour (7.25), flavour (7.44), texture (7.36), odour (7.45) and overall acceptability (7.45). These scores exceeded those of the other treatments and the control (Fig. 3b). Fruits treated with SA 200 µM maintained their glossiness, vibrant green colour and firm texture while experiencing less weight loss. Thus, SA has proven effective in preserving bioactive compounds and antioxidant activity in bell pepper fruits, preventing oxidative membrane damage and maintaining consumer acceptability for up to 20 days without a decline in quality.

The study demonstrated that applying 200 µM L SA is an effective and innovative approach to extending the shelf-life of bell peppers by enhancing bioactive compounds and antioxidant activity during cold storage. The findings indicate that SA treatment significantly reduces weight loss, decay incidence, respiration rate and capsaicin degradation, while also maintaining fruit firmness, thereby positively influencing overall fruit quality. Notably, SA treatment increased antioxidant potential, as evidenced by elevated levels of total phenolics, flavonoids, proline and ascorbic acid. Additionally, SA effectively inhibited the accumulation of malondialdehyde, thereby reducing oxidative stress, preventing membrane damage and preserving sensory attributes. This research underscores the potential of 200 µM SA, a green molecule, in extending the shelf-life of bell peppers for up to 20 days without compromising quality

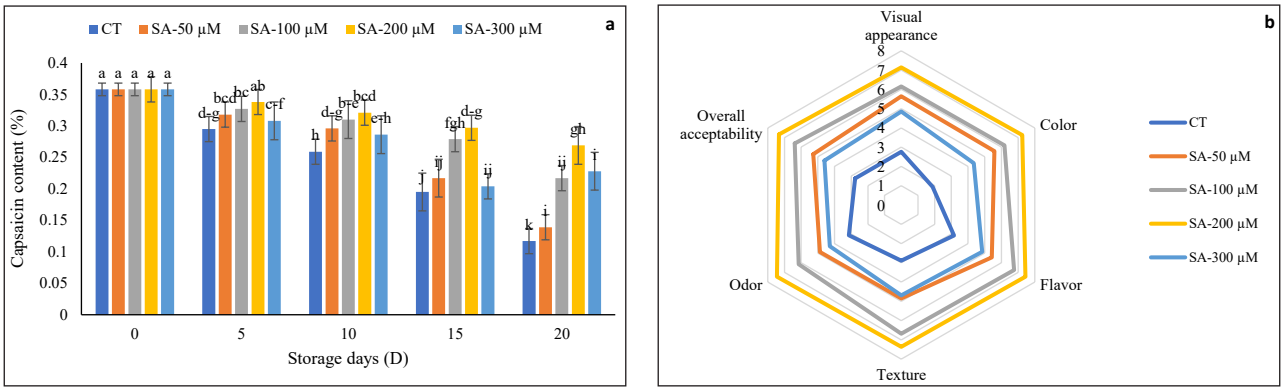

Fig. 3. Effect of salicylic acid on capsaicin content and sensory score of bell pepper during cold storage.
(Error bar specify standard deviation and alphabetic letters specify significant difference $p \leq 0.05$).

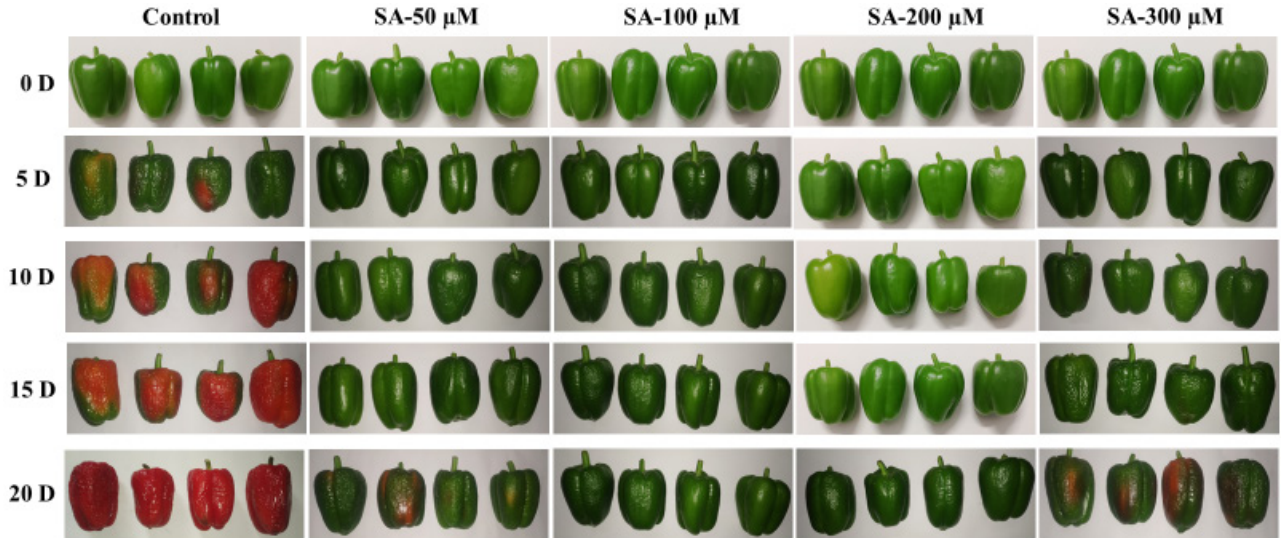

Fig. 4. Effect of salicylic acid on visual quality of green bell pepper during cold storage.

or consumer acceptability. Further investigations are needed on the effect of SA on genes responsible for the upregulation and downregulation of quality parameters during storage.

AUTHORS' CONTRIBUTION

Conceptualization of research (SA); Designing of the experiments (SA, VR); Contribution of experimental materials (SA, MM); Execution of lab experiments (SA, TPB); Analysis of data and interpretation (SA, DK); Preparation of manuscript (SA, MM).

DECLARATION

The authors declare that there is no conflict of interest.

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