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RESEARCH PAPER

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PHYSIOLOGICAL RESPONSES OF GREEN BEANS TO POSTHARVEST SALICYLIC ACID TREATMENTS UNDER COLD STORAGE CONDITIONS

Saide Güler[1], Melek Demirel[2], Yusuf Güvenaltın[3], Mehmet Ufuk Kasım[4], Rezzan Kasım[5]

ABSTRACT

This study evaluated the effects of different salicylic acid (SA) concentrations (0.5, 1, and 2 mM) on the postharvest quality of green beans during cold storage (4 ±1 °C). Physiological parameters (respiration rate, electrolyte leakage, weight loss, and cutting resistance), biochemical traits (total soluble solids, TSS), and colorimetric properties (L*, h°, C*, and browning index) were assessed to determine treatment efficacy. Multivariate analyses, including principal component analysis (PCA), correlation analysis, and hierarchical heatmap clustering, were applied to characterize multidimensional treatment responses. Salicylic acid exhibited dose-dependent and parameter-specific effects. The 0.5 mM SA treatment improved visual quality by maintaining higher chroma values and a more stable hue angle, while also enhancing cutting resistance. The 1 mM dose increased respiration rate and electrolyte leakage, indicating elevated metabolic activity and membrane stress. In contrast, 2 mM SA provided a more balanced preservation of overall quality by reducing weight loss and maintaining moderate color stability. Regardless of treatment, storage duration significantly affected color parameters (L*, h°, and browning index), reflecting natural chlorophyll degradation and senescence processes. PCA revealed that PC1 (43.0% of total variance) was primarily associated with visual quality attributes (L*, C*, TSS, and browning index), PC2 (19.9%) with stress-related variables (respiration rate and electrolyte leakage), and PC3 (12.1%) with cutting resistance. Heatmap clustering and correlation analysis supported these relationships by grouping color and biochemical attributes separately from stress indicators. Overall, the results demonstrate that SA dose optimization is critical for maintaining postharvest quality of green beans under cold storage conditions. These findings highlight the usefulness of multivariate approaches in integrating physiological and visual quality attributes and support the practical application of optimized salicylic acid doses for extending the postharvest life of green beans.

Keywords: *Phaseolus vulgaris* L., postharvest quality, low-temperature storage, color attributes, electrolyte leakage, dose optimization

INTRODUCTION

Green bean (*Phaseolus vulgaris* L.) is an economically and nutritionally important vegetable crop belonging to the Fabaceae family and is widely consumed as immature pods in many regions of the world. The genus *Phaseolus* comprises more than 50 species, among which *P. vulgaris*, *P. lunatus*, *P. coccineus*, *P. acutifolius*, and *P. polyanthus*

[1] Department of Horticulture, Graduate School of Natural and Applied Sciences, Kocaeli University, Kocaeli, Türkiye, https://orcid.org/0009-0004-7973-5938
[2] Department of Horticulture, Faculty of Agriculture, Kocaeli University, Kocaeli, Türkiye, https://orcid.org/0000-0002-8477-1122
[3] Department of Horticulture, Graduate School of Natural and Applied Sciences, Kocaeli University, Kocaeli, Türkiye, https://orcid.org/0000-0002-4311-8450
[4] Department of Horticulture, Faculty of Agriculture, Kocaeli University, Kocaeli, Türkiye, https://orcid.org/0000-0003-2976-7320
[5] Department of Horticulture, Faculty of Agriculture, Kocaeli University, Kocaeli, Türkiye, https://orcid.org/0000-0002-2279-4767; corresponding author: rkasim@kocaeli.edu.tr

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are domesticated [USDA 2025]. Green beans are valued for their high dietary fiber and protein content, as well as essential micronutrients such as vitamins A, C, and K, and minerals including calcium, potassium, iron, and magnesium [Brigide et al. 2014]. Owing to these nutritional attributes, green beans contribute significantly to balanced diets and food security. Globally, green bean production reached 1.36 million tons in 2023 [FAO 2023]. The United States, Morocco, the Philippines, and Mexico are among the leading producers, while Türkiye ranks fifth with an annual production of 70,021 tons [TÜİK 2023]. Although production levels fluctuate annually, Türkiye has shown nearly a 15% increase over the past three decades, highlighting the growing agricultural and economic importance of this crop. Despite its importance, green beans are highly perishable due to its high moisture content (≈90%) and intense metabolic activity after harvest. Because pods are harvested at an immature stage, they exhibit a high respiration rate and are therefore prone to rapid senescence and quality deterioration [Ogumo et al. 2018]. Postharvest losses are commonly associated with water loss, textural softening, browning, decay, and reduced visual and nutritional quality [El-Mogy and Kitinoja 2019]. Consequently, reducing respiration rate and maintaining membrane integrity are critical for preserving postharvest quality. Cold storage is the most widely used method to slow metabolic activity and extend the shelf life of green beans. Storage at 5–7.5 °C and 95–100% relative humidity allows green beans to maintain acceptable quality for 8–12 days, and up to 21 days under optimal handling conditions [Gross et al. 2016, El-Mogy and Kitinoja 2019]. However, exposure to low temperatures may cause physiological stress on the pods, potentially leading to quality deterioration and reduced marketability if not properly managed [Zong et al. 1992, Lv et al. 2024]. Therefore, complementary postharvest treatments are often required to enhance physiological tolerance and maintain quality during cold storage. Various physical and chemical approaches have been investigated to delay senescence and preserve quality in perishable vegetables, including gibberellic acid [Ding et al. 2015], intermittent warming [Liu et al. 2015], biodegradable coatings [Germano et al. 2019], 1-methylcyclopropene, methyl jasmonate [Rehman et al. 2021], nitric oxide [Wang et al. 2015], chitosan-based coatings [Elbagoury et al. 2022], and salicylate-related compounds [Zhang et al. 2015]. Among these treatments, salicylic acid (SA) has received increasing attention due to its role as an endogenous signaling molecule involved in plant stress responses. Salicylic acid is an aromatic phenolic compound known to delay postharvest senescence by enhancing membrane stability, stimulating antioxidant defense systems, and modulating ethylene biosynthesis through the inhibition of ACC synthesis and conversion [Leslie and Romani 1988, Özeker 2005, Erbaş and Koyuncu 2019]. Previous studies have demonstrated that SA application can improve postharvest performance and quality retention of several horticultural commodities under cold storage conditions. Nevertheless, information on the dose-dependent effects of SA on the physiological performance and quality attributes of green beans during low-temperature storage remains limited. Therefore, the objective of this study was to evaluate the effects of postharvest salicylic acid treatments at different concentrations (0, 0.5, 1, and 2 mM) on the physiological responses and major quality attributes of green beans (*Phaseolus vulgaris* L.) during cold storage. This study aims to provide insight into the potential of SA as a practical and safe postharvest treatment for maintaining green bean quality under low-temperature storage conditions.

MATERIALS AND METHODS

Plant material. Green bean pods (*Phaseolus vulgaris* L., cv. Ayşe Kadın) were obtained from a commercial grower in Antalya, Türkiye, during the 2024 spring growing season. The pods were transported to the Postharvest Physiology Laboratory (Department of Horticulture, Faculty of Agriculture) under cooled conditions. Upon arrival, visibly defective pods (broken, bruised, diseased, or mechanically damaged) were removed. Marketable pods were washed under running tap water to eliminate dust and field residues, then air-dried prior to treatment.

Salicylic acid treatments, packaging and storage. The green beans were subjected to four postharvest treatments. In the control treatment, the pods were immersed in tap water. In the 0.5 mM SA treatment, the beans were immersed in a 0.5 mM salicylic acid solution. For the 1 mM SA treatment, the pods were immersed in a 1 mM salicylic acid solution, and in the 2 mM SA treatment, they were immersed in a 2 mM salicylic acid solution. All immersions were carried out for 5 minutes, after which the pods were surface-dried prior to storage. After drying, pods were packed into lidded PET containers (10 pods per container) for storage and subsequent analyses. Packaged pods were stored for 18 days at 4 ±1 °C and 85–90% RH. Measurements were conducted on days 0, 3, 6, 9, 12, 15, and 18.

Respiration rate. The respiration rate (RR) was determined using an O_2/CO_2 gas analyzer (Systech Illinois Gaspace 6000). Three PET packages per treatment were used. CO_2 accumulation inside each container was recorded, and RR was calculated using following formula, correcting for daily CO_2 leakage from the PET material.

$$\mathrm{RR} = \frac{(C_t - C_0) \times V}{w \times t}$$

where:
RR = respiration rate (mL CO_2 kg^{-1} h^{-1}),
C_t = CO_2 concentration at time t,
C_0 = initial CO_2 concentration,
V = package volume (L),
w = sample weight (kg),
t = elapsed time (h).

Electrolyte leakage (EL). Electrolyte leakage was quantified following Kasım and Kasım [2015], with minor modifications. Three pods per replicate were cut into ~1 cm pieces, rinsed twice in distilled water, then immersed in 50 mL distilled water for 2 h at room temperature. Initial conductivity (EC_1) was measured. Samples were then frozen (–18 °C), thawed, and final conductivity (EC_2) was recorded. EL was calculated using:

$$\mathrm{EL}\ (\%) = \frac{\mathrm{EC}_1}{\mathrm{EC}_2} \times 100$$

Weight loss. Weight loss was determined gravimetrically using the same PET containers throughout storage. Weight loss (%) was calculated using following formula:

$$\text{Weight loss}\ (\%) = \frac{W_0 - W_t}{W_0} \times 100$$

where:
W_0 = initial weight (g),
W_t = weight (g) at each sampling day.

Cutting resistance. Cutting resistance was measured using a Shimadzu EZ-LX texture analyzer fitted with a Warner–Bratzler shear blade. Three pods per replicate were evaluated. Results were expressed in Newtons (N).

Total soluble solids. TSS (%) was measured using a digital refractometer (Atago Co. Ltd., Japan). Juice extracted from pods was filtered and analyzed directly. Calibration was performed using distilled water.

Color measurement. Color values (L*, *a**, *b**) were recorded using a Minolta CR-400 colorimeter. Five pods per replicate were randomly selected, and readings were taken from the midsection of each pod. Calibration was performed using a standard white tile (L = 97.52, a = –5.06, b = 3.57). The following indices were calculated:

$$\mathrm{Hue} = \tan^{-1}\left(\frac{b^*}{a^*}\right), \mathrm{BI} = \frac{100(x-0.31)}{0.17},\ \mathrm{C} = \sqrt{a^2 + b^2}$$

Statistical analysis. The experiment was arranged in a completely randomized design (CRD) with three biological replications, each replication consisting of 10 pods. The study followed a factorial structure with two fixed factors: salicylic acid (SA) concentration (0, 0.5, 1, and 2 mM) and storage duration (0, 3, 6, 9, 12, 15, and 18 days). All statistical analyses were performed using replicate-level data rather than averaged values. Data were subjected to two-way analysis of variance (ANOVA) to evaluate the main effects of SA concentration, storage duration, and their interaction. When significant differences were detected, mean separations were performed using Duncan's Multiple Range Test (DMRT) at a significance level of $p < 0.05$. Results are presented as mean ± standard error (SE) based on three replications (n = 3). Multivariate analyses, including principal component analysis (PCA), Pearson correlation matrix, and hierarchical clustering heatmap, were conducted to explore relationships among quality parameters and to visualize treatment-dependent patterns. These multivariate analyses were performed using the Python programming language, employing standard scientific libraries for data analysis and visualization.

RESULTS AND DISCUSSION

Analysis of variance (ANOVA)

The analysis of variance (ANOVA) revealed that salicylic acid (SA) treatments significantly affected several postharvest quality attributes of green beans (*Phaseolus vulgaris* L.) during cold storage (Table 1). Specifically, respiration rate, electrolyte leakage, weight loss, cutting resistance, hue angle, chroma, and browning index were all influenced by the treatment factor ($p < 0.05$). In contrast, SA treatments had no significant effect on soluble solids content (SSC) or L* values ($p > 0.05$). Storage duration exhibited a strong and highly significant effect ($p < 0.001$) on all measured parameters, confirming that cold storage time is a major determinant of physicochemical and physiological changes in green beans. The interaction between treatment and storage duration was not significant for most variables, with the exception of cutting resistance, indicating that texture responded differently to SA doses across storage intervals.

Table 1. Analysis of variance (*F* and *p* values) for the effects of salicylic acid treatments and storage duration on the quality parameters of green beans (*Phaseolus vulgaris* L.)

Parameter	Treatment	Storage duration	Treatment × Storage duration
Respiration rate	2.214*	483.881***	0.363 ns
Electrolyte leakage	2.657*	115.085***	1.827 ns
Weight loss (%)	15.399***	461.048***	0.617 ns
Cutting resistance (CR, N)	9.240**	7.787***	3.686***
Soluble solids content (SSC)	0.464 ns	29.030***	0.554 ns
L* value	0.408 ns	9.869***	0.553 ns
Hue angle (h°)	5.523*	52.265***	0.792 ns
Chroma (C)	2.601*	8.818***	1.304 ns
Browning index (BI)	1.703*	9.541***	0.818 ns

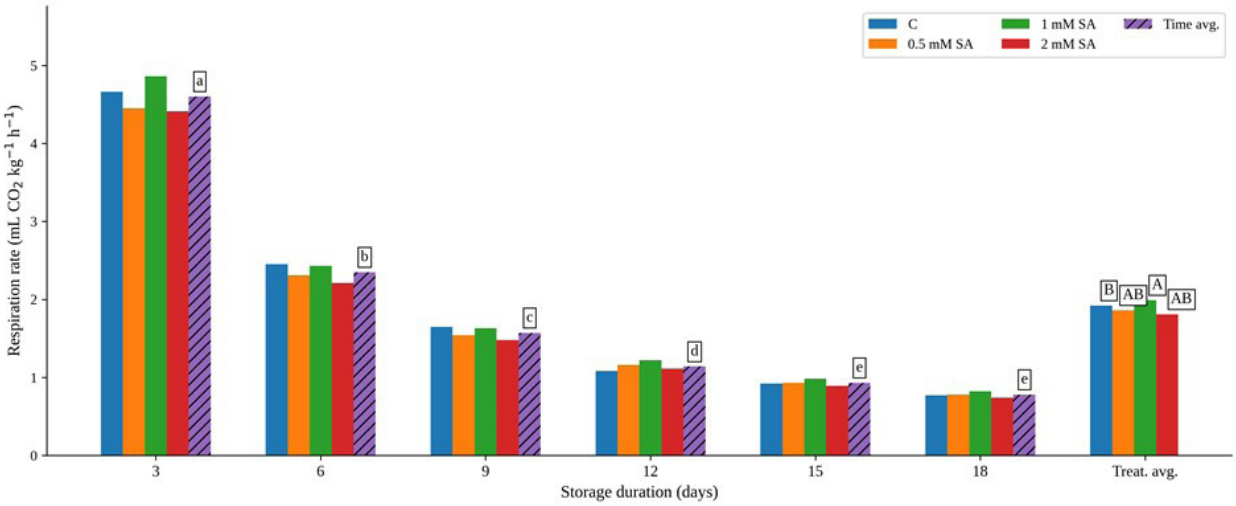
* Significance levels: ***$p < 0.001$, **$p < 0.01$, $p < 0.05$, ns = not significant. Treatments: 0 (Control), 0.5, 1, and 2 mM SA. Sampling days: 0, 3, 6, 9, 12, 15, and 18

Respiration rate

Figure 1 shows the changes in respiration rate (RR, mL CO_2 kg^{-1} h^{-1}) of green beans during cold storage. A gradual and significant decrease in RR was observed as storage progressed ($p < 0.001$). The highest respiration rate occurred on day 3 (4.60 mL CO_2 kg^{-1} h^{-1}), indicating high initial metabolic activity, whereas the lowest value was recorded on day 18 (0.78 mL CO_2 kg^{-1} h^{-1}). This trend reflects a natural decline in metabolic intensity under prolonged low-temperature conditions. Similar reductions in RR during cold storage have been reported for other legumes; for example, Kaur et al. [2022] demonstrated that respiration significantly decreased in cold-stored green pea pods, consistent with the present findings.

Salicylic acid (SA) treatments also significantly affected respiration rate ($p < 0.05$). The 1 mM SA treatment resulted in the highest RR among all treatments, whereas 2 mM SA resulted in a noticeable reduction compared with the control. The difference between these two doses was statistically significant, indicating a dose-dependent, bidirectional response. The stimulatory effect of 1 mM SA suggests that this concentration may enhance respiratory enzyme activity or energy metabolism, thereby increasing CO_2 production. In contrast, 2 mM SA appears to exert a suppressive influence, potentially moderating metabolic activity under cold stress. These observations align with mechanistic studies showing that the effect of SA on respiration is strongly dependent on concentration and tissue type. Shugaev et al. [2014] reported that SA up to 1 mM acted as a mitochondrial uncoupler in sugar beet roots, stimulating oxygen uptake in the absence of ADP but exhibiting little effect when ADP was present. The authors also noted that in *Lupinus albus* cotyledons, oxygen uptake increased only slightly in response to SA, suggesting that plant mitochondrial responses vary with both dose and exposure duration. Together, these findings support the present observation that moderate SA concentrations (1 mM) may transiently activate metabolic pathways, whereas higher concentrations (2 mM) may limit respiration through inhibitory or protective mechanisms.

Figure 1. Changes in respiration rate of green beans during cold storage as affected by salicylic acid (SA) treatments



Respiration rate (mL CO_2 kg^{-1} h^{-1}) of green beans treated with different SA concentrations (0, 0.5, 1, and 2 mM) over 18 days of storage at 4 ±1 °C. Different lowercase letters (a–e) indicate significant differences among storage days, while uppercase letters (A–B) indicate significant differences among treatments according to Duncan's multiple range test ($p < 0.05$). Bars represent mean ± SE (n = 3)

Table 2. Electrolyte leakage (%) of green beans treated with different concentrations of salicylic acid (SA) during cold storage

Parameter	Storage duration (days)							Treatment average
	0	3	6	9	12	15	18	
Control	7.2 ±0.6 ns	18.8 ±1.6 ns	7.4±1.8 ns	12.5 ±1.6 ns	20.7 ±1.8 ab	13 ±0.7 ab	12.9 ±0.8 ns	13.2 B
SA 0.5 mM	7.2 ±0.6 ns	18.7 ±1.8 ns	8.6±0.6 ns	12.6 ±0.8 ns	18.7 ±0.5 b	15.3 ±1.3 a	15.4 ±2.3 ns	13.8 AB
SA 1 mM	7.2 ±0.6 ns	18.4 ±1.3 ns	9.3±0.2 ns	13.6 ±2.0 ns	23.5 ±3.2 a	11.9 ±2.4 b	15.6 ±3.0 ns	14.2 A
SA 2 mM	7.2 ±0.6 ns	17.3 ±0.7 ns	8.8±0.6 ns	12.7 ±2.1 ns	18.4 ±0.9 b	13.1 ±1.4 ab	13.9 ±1.2 ns	13.1 B
Time average	7.2 f	18.3 b	12.8 e	12.8 d	20.3 a	13.3 cd	14.5 c	–

Mean electrolyte leakage (EL) values (%) of green beans subjected to salicylic acid treatments (0, 0.5, 1, and 2 mM) across storage days (0–18). Values represent means ± SE (n = 3). Different lowercase letters indicate significant differences among storage days, and uppercase letters indicate differences among SA treatments according to Duncan's multiple range test ($p < 0.05$)

Electrolyte leakage

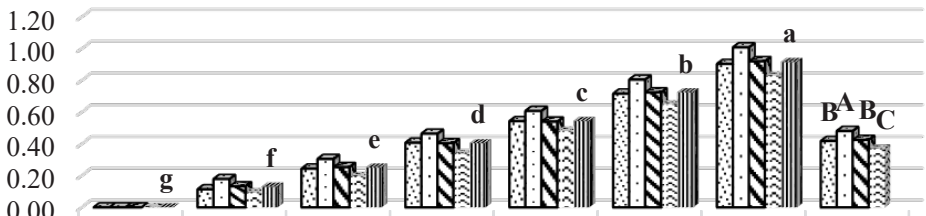
Electrolyte leakage (EL) is a key physiological indicator reflecting the integrity of cellular membranes, and therefore provides a reliable measure of membrane stability and sensitivity to chilling stress. In this study, EL values increased progressively across all treatments as storage time advanced (Table 2), demonstrating the cumulative impact of cold stress on membrane functionality. Analysis of treatment means showed that the lowest EL value occurred in the 2 mM SA treatment (13.1%), whereas the highest EL value was recorded in the 1 mM SA treatment (14.2%). These results indicate that 1 mM SA did not confer membrane protection in green beans; rather, under cold-storage conditions, this concentration may have induced a mild stress stimulus that enhanced membrane permeability. By contrast, 0.5 mM SA and 2 mM SA treatments fell within the same statistical group as the control, suggesting that these concentrations maintained membrane stability at levels comparable to untreated pods. The dose-dependent nature of SA responses observed here aligns with previous reports highlighting the dual, sometimes contrasting, roles of SA depending on concentration, tissue type, and environmental conditions. The general rise in EL during storage is characteristic of chilling-induced physiological disruption, including membrane lipid phase transitions, reactive oxygen species (ROS) accumulation, and lipid peroxidation – processes that collectively weaken membrane barrier properties [Wang et al. 2018]. The elevated EL values under the 1 mM SA treatment suggest that this dose may have failed to adequately stimulate antioxidant defenses or may have transiently activated oxidative pathways, thereby increasing membrane leakage. Previous studies have similarly noted variable EL responses depending on SA concentration. For example, Zhang et al. [2015] reported that a chitosan

(0.5%) + SA (0.1%) coating effectively reduced EL in cucumber, whereas Khademi et al. [2019] demonstrated that 1 mM SA decreased EL in bananas by strengthening membrane structure during cold storage. In contrast, the present findings show that 2 mM SA reduced EL more effectively than the lower doses. This suggests that higher SA concentrations may have more strongly activated antioxidant enzyme systems, improving membrane resistance to chilling-related oxidative damage. Overall, the EL results confirm that SA exhibits a concentration-dependent effect on membrane stability in green beans. While 0.5 mM and 2 mM SA provided partial membrane protection, 1 mM SA unexpectedly increased membrane permeability, indicating overstimulation of stress-related metabolic processes rather than mitigation.

Weight loss

Weight loss is a major quality determinant in green vegetables, primarily resulting from moisture loss through transpiration as well as respiratory mass loss. In the present study, green bean pods exhibited a significant increase in weight loss ($p < 0.001$) as storage progressed. This progressive rise can be attributed to the inherently high moisture content and broad surface area of green bean pods, which render them highly prone to dehydration even under optimal low-temperature storage conditions. Supporting this observation, Manzoor et al. [2019] reported that green beans, with moisture levels approaching 90% and ongoing metabolic activity at harvest, deteriorate rapidly and maintain relatively high respiration rates despite cold storage. Among the salicylic acid treatments, 2 mM SA demonstrated the greatest effectiveness in minimizing weight loss, displaying significantly lower values than all other treatments ($p < 0.001$). Although statistical differences among treatments were evident, overall weight loss remained low, with final values ranging between 0.83% and 1.01%, indicating that dehydration did not reach levels that would negatively affect visual quality. The dose-dependent trend observed in this study highlights the superior ability of 2 mM SA to limit water loss, likely through enhanced stomatal regulation, reduced epidermal transpiration, and improved membrane stability. Similar mechanisms were previously. Jahan [2019]

Figure 2. Weight loss (%) of green beans subjected to different salicylic acid (SA) treatments during cold storage. Lowercase letters (a–g) indicate significant differences among storage days, while uppercase letters (A–C) show significant differences among SA treatments according to Duncan's multiple range test ($p < 0.05$)



	0	3	6	9	12	15	18	Treat avg.
	Storage duration (days)							
C	0.00	0.11	0.24	0.41	0.54	0.72	0.90	0.42
SA 0.5 mM	0.00	0.18	0.30	0.46	0.61	0.81	1.01	0.48
SA 1 mM	0.00	0.13	0.25	0.40	0.54	0.72	0.92	0.43
SA 2 mM	0.00	0.09	0.20	0.34	0.48	0.65	0.83	0.37
Time avg.	0.00	0.13	0.25	0.40	0.54	0.72	0.92	–

reported that SA mitigated oxidative damage in heat-stressed tomatoes by reducing reactive oxygen species and preserving membrane integrity, while El-Beltagi et al. [2025] showed that SA strengthened antioxidant defenses and improved anatomical structures associated with water retention. In contrast, the 0.5 mM SA treatment resulted in higher weight loss, suggesting that lower concentrations may be insufficient to activate protective pathways, while the 1 mM SA treatment behaved similarly to the control, indicating minimal regulatory influence. Collectively, these findings align with the hormetic dose–response behavior of salicylic acid, wherein low doses are ineffective, moderate doses stimulate stress responses, and higher doses exert protective effects depending on plant species and tissue type [Miura and Tada 2014, Brito et al. 2018, Li et al. 2022].

Cutting resistance

Cutting resistance (CR) values of green beans exhibited clear variations throughout storage, and both storage duration ($p < 0.001$) and SA treatments ($p < 0.01$) significantly influenced this parameter (Table 3). As one of the primary indicators of textural quality, CR reflects changes in tissue firmness, elasticity, and turgor pressure during postharvest storage. At the beginning of storage (day 0), beans exhibited a CR value of 49.9 N, which increased sharply to 57.4 N by day 3. This peak likely represents an early-stage physiological adjustment to cold conditions, during which transient moisture redistribution or slight epidermal hardening may temporarily enhance tissue firmness. Thereafter, CR values gradually declined and reached 50.3 N on day 18, indicating progressive softening of the pod tissues as senescence advanced – a typical pattern reported for cold-stored legumes and other non-climacteric vegetables. Based on treatment means, the 0.5 mM SA treatment resulted in the highest CR value (55.1 N), significantly exceeding all other treatments. This suggests that low SA concentration was insufficient to stabilize membranes or mitigate cold-induced stress, potentially allowing passive water loss and leading to increased tissue rigidity. Similar observations have been reported in other horticultural commodities, where suboptimal SA doses induced mild oxidative stress that promoted cell wall stiffening rather than protection [Liu et al. 2022]. Conversely, the lowest CR values were recorded in the 1 mM SA treatment, although this group did not differ statistically from the control or 2 mM SA treatments. The reduced CR in 1 mM SA–treated pods indicates a crisper and more hydrated tissue structure, suggesting that this concentration helped maintain membrane stability and regulate oxidative stress, thereby preserving turgor pressure. These findings are consistent with the dose-dependent nature of SA responses, in which moderate concentrations often exhibit the most beneficial effects on tissue quality. Supporting evidence from García-García et al. [2022] confirms that CR measurements reliably detect texture alterations in bean pods during storage, while Wang et al. [2022] demonstrated that SA can enhance firmness and mitigate water loss, although its efficacy varies with concentration. Collectively, the results indicate that SA exerts a biphasic, dose-dependent effect on textural stability. The 0.5 mM SA dose promoted tissue stiffening – likely through insufficient stress mitigation – whereas the 1 mM dose maintained crispness by better preserving cellular integrity. The 2 mM SA dose showed intermediate behavior, providing moderate stabilization without marked textural enhancement. These findings highlight that optimizing SA concentration is crucial for maintaining desirable texture attributes in green beans during cold storage.

Table 3. Effect of different salicylic acid (SA) treatments on cutting resistance (N) of green green bean pods during cold storage (4 ±1 °C)

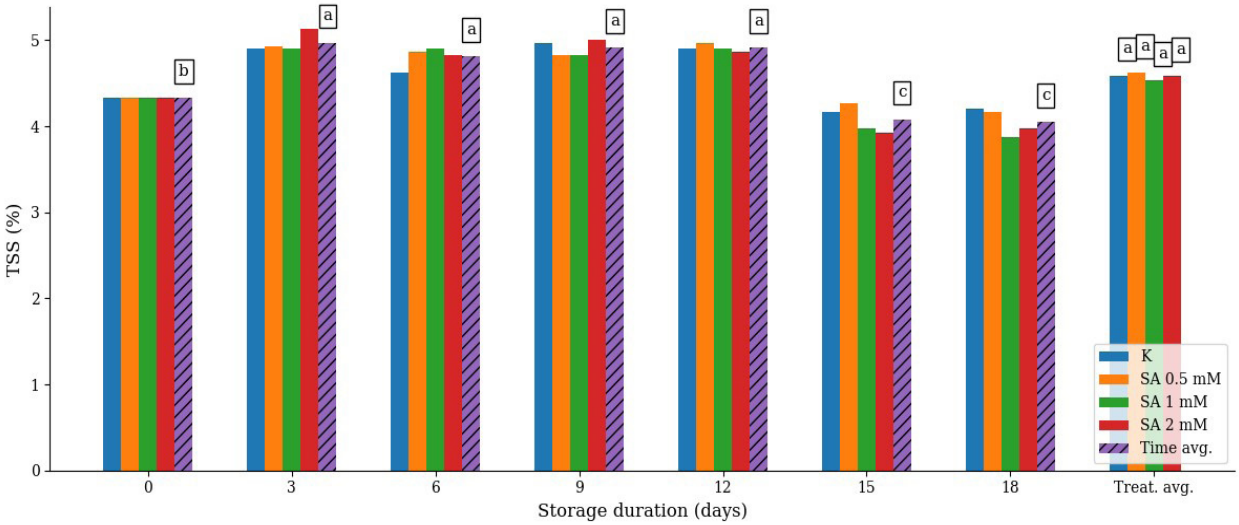
Parameter	Storage duration (days)							Treatment average
	0	3	6	9	12	15	18	
Control	49.7 $^{\pm 0.4}$ ns	58.7 $^{\pm 0.7}$ a	54.3 $^{\pm 4.8}$ ab	55.6 $^{\pm 1.0}$ ns	45.7 $^{\pm 1.0}$ b	53.7 $^{\pm 1.3}$ a	47.1 $^{\pm 2.4}$ b	52.1 B
SA 0.5 mM	50.4 $^{\pm 0.4}$ ns	60.5 $^{\pm 0.6}$ a	56.6 $^{\pm 5.9}$ a	52.3 $^{\pm 3.4}$ ns	55.8 $^{\pm 4.9}$ a	53.1 $^{\pm 1.5}$ a	57.4 $^{\pm 2.2}$ a	55.1 A
SA 1 mM	49.7 $^{\pm 0.5}$ ns	54.0 $^{\pm 1.3}$ c	53.4 $^{\pm 2.8}$ ab	51.4 $^{\pm 1.2}$ ns	47.5 $^{\pm 1.5}$ b	48.6 $^{\pm 0.1}$ b	47.7 $^{\pm 1.0}$ b	50.3 B
SA 2 mM	49.7 $^{\pm 1.0}$ ns	56.3 $^{\pm 1.6}$ b	46.6 $^{\pm 1.8}$ b	50.8 $^{\pm 9.6}$ ns	58.2 $^{\pm 4.8}$ a	51.9 $^{\pm 3.1}$ ab	49.1 $^{\pm 1.8}$ b	51.8 B
Time average	49.9 b	57.4 a	52.7 b	52.5 ab	51.8 ab	51.8 ab	50.3 ab	–

Values represent mean ± standard deviation (n = 3). Different lowercase letters within each row indicate significant differences among storage days according to Duncan's multiple range test ($p < 0.05$). Different uppercase letters in the "Treatment average" column indicate significant differences among treatments. "ns" denotes non-significant differences. Cutting resistance is expressed in Newtons (N); higher values indicate firmer and more elastic pods, whereas lower values represent crisper and less rigid tissue with higher moisture content

Total soluble solids

The effect of salicylic acid (SA) treatments on the total soluble solids (TSS) content of green beans was statistically non-significant ($p > 0.05$), indicating that none of the applied SA concentrations produced a measurable change in soluble solids during storage (Figure 3). In contrast, storage duration exerted a highly significant influence on TSS ($p < 0.001$). The fluctuations observed in TSS throughout the storage period were independent of treatment effects and are likely attributable to physiological processes such as carbohydrate utilization during respiration, concentration changes associated with moisture loss, progressive textural modifications, and minor developmental differences among pods despite simultaneous harvest.

Figure 3. Effects of different salicylic acid (SA) treatments on the total soluble solids (TSS, %) content of green beans during cold storage



Values represent the mean of three replicates (n = 3). Different lowercase letters (a–c) denote significant differences among storage days according to Duncan's multiple range test ($p < 0.05$). Treatment effects were not significant (ns)

The similarity among treatment means demonstrates that SA at 0.5, 1, or 2 mM does not markedly affect sugar metabolism or the accumulation of soluble solids in green beans. This outcome is consistent with previous research indicating that SA primarily influences stress-related physiological responses rather than carbohydrate metabolism. For example, El-Beltagi et al. [2022] reported that SA did not significantly alter TSS in broccoli florets, although it improved tissue firmness and pigment stability. Similarly, Hanaei et al. [2022] observed no substantial changes in TSS in SA-treated bell peppers; however, SA effectively enhanced stress tolerance-related parameters such as reduced weight loss and suppressed respiration. Collectively, these findings suggest that the postharvest role of SA is more closely associated with maintaining membrane integrity, modulating oxidative responses, and stabilizing tissue structure rather than altering soluble solids content, which is largely governed by inherent physiological and biochemical processes during storage.

Color values (L, h°, C, BI)

Table 4 presents the effects of different salicylic acid (SA) treatments on the L*, hue (h°), chroma (C*), and browning index (BI) values of green beans during storage. According to the ANOVA results (Table 1), SA treatments had no significant effect on L* ($p > 0.05$), whereas hue, chroma, and BI were significantly affected by the treatment factor ($p < 0.05$). Storage duration was highly significant for all color parameters, including BI ($p < 0.001$), indicating substantial color evolution during the storage period.

L* Values. SA treatments caused no meaningful changes in L*, and the mean values (65.3–65.8) remained statistically similar. L* increased slightly during early storage (days 9–12), indicating a transition toward lighter green tones. This trend is consistent with previous findings reporting increased L* values during cold storage as a result of cellular and structural changes affecting light reflectance [Yılmaz 2022]. By the end of storage, L* values slightly declined, reflecting the onset of tissue senescence. Differences reported between intact and green-cut beans in earlier studies [Kasım and Kasım 2015] highlight the influence of tissue integrity on L* evolution.

Hue (h°). Hue values decreased progressively throughout storage, demonstrating a gradual shift from vivid green toward more yellowish coloration. SA treatments modulated hue values to a limited extent: the lowest hue was observed in the 2 mM SA treatment (≈115.1°), suggesting slightly more advanced chlorophyll degradation compared with the control and 0.5 mM SA treatments. This decline reflects the well-documented degradation of chlorophyll and the increasing prominence of carotenoids during postharvest senescence [El-Mogy and Kitinoja 2019, Zhang et al. 2021].

Chroma (C*). Chroma values, which indicate color saturation, were significantly influenced by SA dose. Notably, 0.5 mM SA treatment resulted in higher chroma values than the control, suggesting better preservation of color vividness. This observation agrees with previous reports showing that SA-based treatments can delay yel-

lowing and help maintain color intensity by reducing oxidative stress and slowing pigment degradation [El-Beltagi et al. 2022, Kibar et al. 2024]. Chroma increased during mid-storage (days 9–12), reflecting a brighter color appearance, but declined toward the end of storage as senescence progressed.

Table 4. Effects of salicylic acid (SA) treatments on the color attributes (L, hue, chroma) and browning index (BI) of green beans during cold storage

Parameter		Storage duration (days)							Treatment average
		0	3	6	9	12	15	18	
L values	Control	64.49 ±2.6	61.71 ±1.1	65.12 ±1.4	66.52 ±1.0	68.33 ±1.2	65.99 ±0.9	66.81 ±0.6	65.57 ns
	SA 0.5 mM	64.49 ±2.6	61.61 ±1.6	64.84 ±0.8	67.23 ±1.2	66.65 ±1.8	67.05 ±1.1	68.99 ±0.3	65.84 ns
	SA 1 mM	64.49 ±2.6	62.56 ±0.9	65.62 ±0.8	64.47 ±0.1	67.16 ±1.9	65.84 ±1.2	66.69 ±1.7	65.26 ns
	SA 2 mM	64.49 ±2.6	63.34 ±0.7	65.57 ±0.5	66.72 ±1.2	67.73 ±1.1	66.96 ±0.7	66.07 ±1.2	65.84 ns
	Time average	64.49 c	62.3 d	65.29 bc	66.23 ab	67.47 a	66.46 ab	67.14 a	–
Hue	Control	117.0 ±1.0	117 ±0.3	116.9 ±0.2	116.4 ±0.2	115.7 ±0.4	114.5 ±0.5	113.4 ±1.0	115.9 a
	SA 0.5 mM	117.0 ±1.0	117.9 ±0.4	117.2 ±0.5	116.3 ±0.4	115.7 ±0.8	114.0 ±0.3	113.7 ±1.0	116.0 a
	SA 1 mM	117.0 ±1.0	116.8 ±0.6	116.6 ±0.4	116.3 ±0.8	115.6 ±0.7	113.4 ±0.8	112.8 ±0.2	115.5 ab
	SA 2 mM	117.0 ±1.0	116.1 ±0.7	116.5 ±0.6	115.9 ±0.9	114.6 ±1.2	112.3 ±1.6	113.1 ±0.7	115.1 b
	Time average	117.0 a	116.9 a	116.8 ab	116.2 b	115.4 c	113.6 d	113.2 d	–
Chroma	Control	32.86 ±1.0	31.67 ±1.37	33.17 ±0.28	34.98 ±0.38	33.23 ±3.93	34.71 ±0.42	35.38 ±0.40	33.71 b
	SA 0.5 mM	32.86 ±1.0	33.93 ±0.52	34.24 ±0.66	35.38 ±0.23	35.61 ±0.36	34.80 ±1.07	34.32 ±0.44	34.53 a
	SA 1 mM	32.86 ±1.0	33.64 ±0.26	33.72 ±0.62	35.55 ±0.46	35.34 ±0.13	34.91 ±0.57	34.2 ±0.48	34.32 ab
	SA 2 mM	32.86 ±1.0	33.79 ±0.71	33.58 ±0.47	35.3 ±0.45	34.24 ±1.14	33.29 ±1.24	33.67 ±0.81	33.96 ab
	Time average	32.86 d	33.26 d	33.68 bc	35.30 a	34.85 a	34.43 ab	34.54 ab	–
BI	Control	38.51 ±2.23	39.07 ±3.70	38.51 ±0.3	41.07 ±0.57	38.17 ±2.60	43.73 ±2.22	45.76 ±1.08	40.69 b
	SA 0.5 mM	38.51 ±2.23	41.57 ±1.27	40.15 ±0.69	41.24 ±0.21	43.00 ±1.30	42.69 ±2.87	42.54 ±1.47	41.53 ab
	SA 1 mM	38.51 ±2.23	41.82 ±1.41	39.45 ±1.75	44.06 ±0.77	42.25 ±2.78	45.78 ±1.31	44.59 ±1.07	42.35 a
	SA 2 mM	38.51 ±2.23	42.35 ±2.33	39.35 ±0.64	42.08 ±0.84	42.92 ±1.52	43.35 ±4.80	43.67 ±1.32	41.75 ab
	Time average	38.51 c	41.20 b	39.37 c	42.11 b	41.58 b	44.13 a	44.14 a	–

Values represent mean ± standard error (n = 3). Different lowercase letters within rows indicate significant differences among storage days ($p < 0.05$), while different uppercase letters within columns indicate significant differences among SA treatments ($p < 0.05$) according to Duncan's multiple range test. "ns" denotes non-significant differences ($p > 0.05$). SA: salicylic acid; BI: browning index

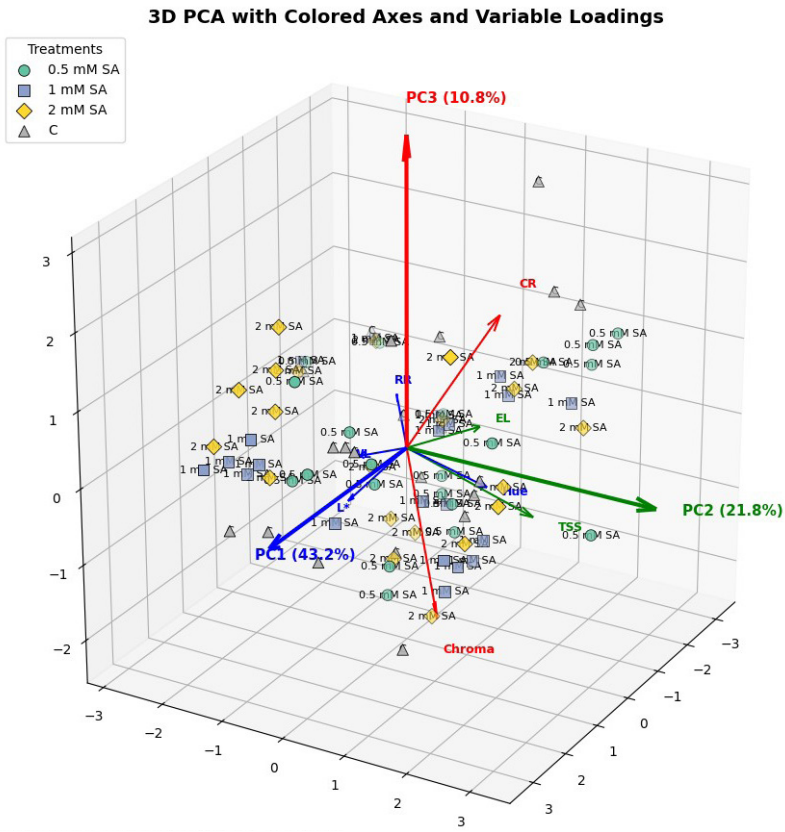
Browning index (BI). BI values provide an integrative measure of enzymatic and non-enzymatic browning, reflecting phenolic oxidation and pigment degradation. SA treatments significantly affected BI means ($p < 0.05$). The highest BI was observed in the 1 mM SA group (42.35), whereas the lowest BI belonged to 0.5 mM SA (41.53), followed closely by 2 mM SA and the control. These results indicate that 0.5 mM SA most effectively mitigated browning, whereas 1 mM SA slightly promoted it, possibly due to dose-dependent shifts between antioxidant activation and pro-oxidative stimulation – an effect previously described for SA in postharvest tissues. Storage duration strongly increased BI values, rising from approximately 38.5 at day 0 to 44.1–44.7 at day 18, reflecting progressive browning during senescence. This increase can be attributed to chlorophyll loss, phenolic oxidation, and membrane degradation – processes commonly associated with postharvest tissue aging. Similar browning progression has been documented in green beans and other vegetables during cold storage [El-Mogy and Kitinoja 2019, Zhang et al. 2021]. The multivariate PCA analysis (Figure 4) further supports these findings. The positive loading of BI on PC1 – together with L*, chroma, and TSS, suggests that browning progression is closely aligned with general color and compositional changes. The separation of the 0.5 mM SA treatment from higher BI regions indicates its stronger ability to preserve visual quality, whereas clustering of the 1 mM SA treatment near higher BI loadings, aligns with the observed increase in browning and decreased membrane stability. These multivariate relationships confirm that SA exhibits dose-dependent and parameter-specific effects, with 0.5 mM SA providing the most balanced preservation of appearance, color saturation, and browning resistance. Overall, storage duration was the dominant factor shaping color transitions, including lightness, hue, saturation, and browning. SA treatments exerted moderate but dose-dependent effects: 0.5 mM SA best preserved chroma and minimized browning, 2 mM SA slightly accelerated hue shift, 1 mM SA increased browning and was associated with less fa-

vorable color stability. Collectively, these findings indicate that SA modulates color attributes mainly through its influence on stress physiology and oxidative balance rather than direct effects on pigments, and that 0.5 mM SA emerges as the most effective dose for retaining visual quality in green beans during cold storage.

PCA, correlation matrix and heatmap findings

The multivariate evaluation obtained through the three-dimensional PCA, correlation matrix analysis, and hierarchical cluster-based heatmap (Figure 4) provided an integrated interpretation of how salicylic acid (SA) doses influenced the quality attributes of green beans. Overall, the treatments exhibited dose-dependent and parameter-specific effects.

Figure 4. 3D principal component analysis (PCA) of quality attributes in green beans treated with salicylic acid



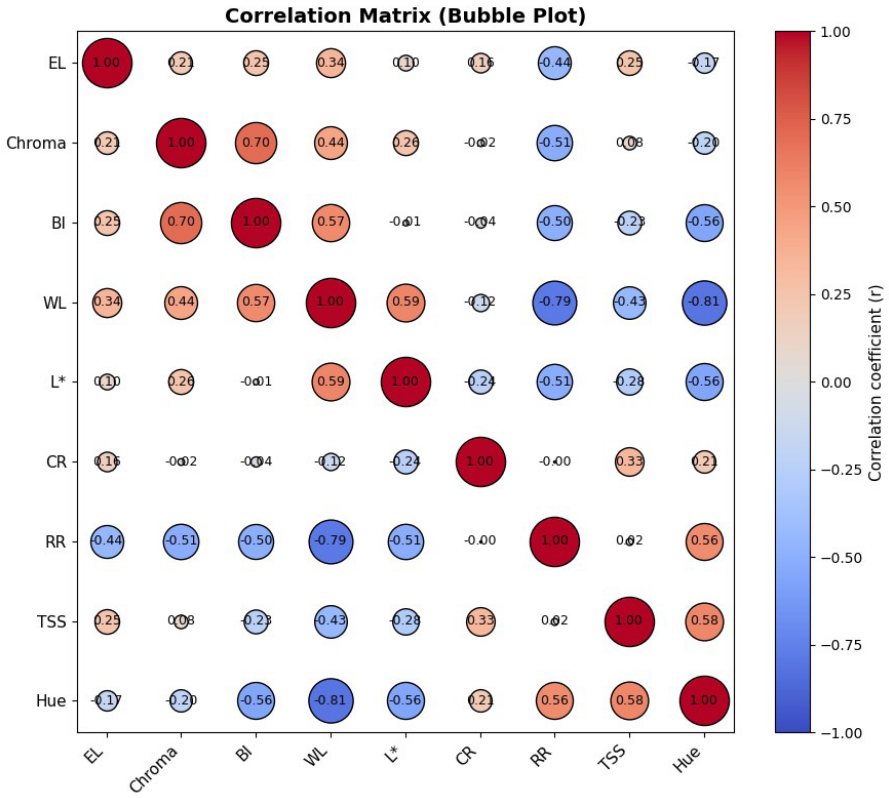
Total explained variance: 75.87% (PC1 = 43.2%, PC2 = 21.8%, PC3 = 10.8%)

PCA. According to the PCA results, the first three principal components explained 75.04% of the total variance, distributed as PC1 (43.0%), PC2 (19.9%), and PC3 (12.1%). PC1 was primarily associated with L*, chroma, hue, TSS, and to some extent, BI, indicating that this axis represented the overall appearance quality, including color brightness and saturation. The close alignment of the L* and chroma vectors suggested that pods with brighter and more saturated color tended to exhibit slightly lower BI values. The clustering of the 0.5 mM SA treatment along PC1 demonstrated that this dose supported higher color brightness, greater color saturation, and relatively elevated TSS values, consistent with Table 4 showing increased chroma under 0.5 mM SA. Therefore, this dose appeared to best preserve visual quality. PC2 was mainly associated with respiration rate (RR) and electrolyte leakage (EL), both indicators of stress physiology. The positive relationship between RR and EL on this axis indicated that higher respiration was accompanied by increased membrane permeability, reflecting reduced membrane stability. The distinct separation of the 1 mM SA treatment along PC2 confirmed that this dose enhanced metabolic activity and accelerated membrane deterioration, consistent with previously observed increases in RR and EL. PC3 was dominated by cutting resistance (CR), indicating that textural firmness represented an independent determinant

of quality. The positioning of the 0.5 mM SA treatment distant from other treatments along PC3 confirmed that this dose produced the highest cutting resistance, consistent with Table 3.

The correlation matrix further supported the multivariate structure revealed by the PCA analysis (Figure 5). A moderate positive correlation was observed between L* and hue (r ≈ 0.56), indicating that brighter pods tended to retain greener color tones during storage. This relationship reflects the coordinated progression of color lightness and chlorophyll preservation. In addition, chroma exhibited a strong positive correlation with the browning index (BI) (r ≈ 0.70), suggesting that increased color saturation was accompanied by intensified browning, likely due to parallel biochemical changes associated with senescence and pigment transformation. Respiration rate (RR) showed a strong negative correlation with weight loss – WL (r ≈ –0.79), indicating that higher respiratory activity was not directly associated with greater water loss. This suggests that WL is more strongly governed by physical properties of the cuticle and epidermal structure rather than by metabolic intensity alone. Moreover, the positive correlation between RR and electrolyte leakage – EL (r ≈ 0.44) confirmed that elevated metabolic activity was closely linked to membrane destabilization and increased cellular permeability. A moderate positive association between total soluble solids (TSS) and hue (r ≈ 0.58) further indicated that pods with higher soluble solids tended to maintain greener coloration.

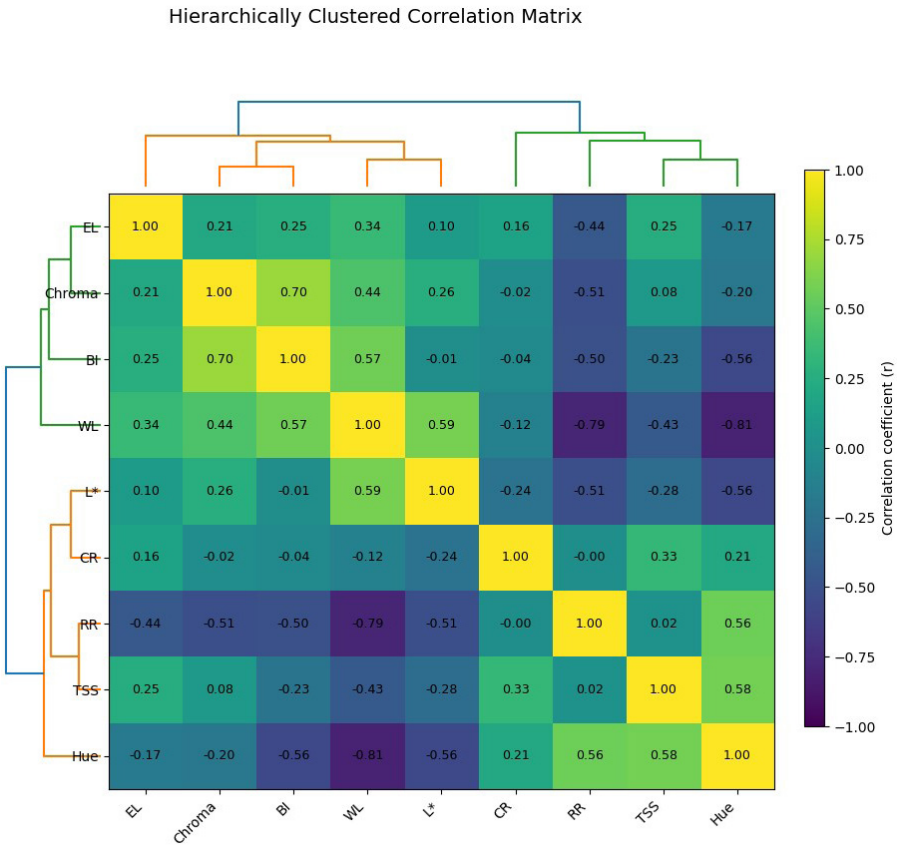
Figure 5. Correlation matrix of quality parameters in green beans treated with salicylic acid



Red circles indicate positive correlations, blue circles negative correlations, and circle size reflects the strength of each correlation

Heatmap and hierarchical clustering. The hierarchically clustered heatmap provided a clear visualization of these relationships and revealed three distinct parameter clusters (Figure 6). The first cluster consisted of chroma, BI, WL, and L*, representing visual and color-related quality attributes. This cluster closely corresponded to PC1 in the PCA, confirming that these parameters co-varied and jointly described overall visual quality changes. The second cluster included RR and EL, reflecting stress-related physiological responses; their close association indicates that increased respiration accelerated membrane degradation, in agreement with the stress axis represented by PC2. The third cluster comprised TSS and Hue, indicating a distinct subgroup related to compositional and pigment-related attributes, partially overlapping with visual quality but exhibiting independent behavior.

Figure 6. Hierarchically clustered correlation heatmap of quality attributes in green beans



The heatmap displays pairwise Pearson correlations among quality variables (WL, L*, EL, chroma, BI, RR, hue, CR and TSS) under different SA treatments. Positive correlations are shown in warm colors and negative correlations in cool colors, with dendrograms illustrating variable clustering patterns

Collectively, the PCA, correlation matrix, and heatmap analyses consistently demonstrated that salicylic acid (SA) applications exerted multidimensional and dose-dependent effects on postharvest quality of green beans. The 0.5 mM SA treatment emerged as the most advantageous dose by enhancing visual quality attributes (brightness and saturation) and supporting tissue firmness. In contrast, 1 mM SA intensified metabolic stress, as reflected by elevated respiration rate and electrolyte leakage, thereby limiting overall quality. The 2 mM SA treatment provided a more balanced and stable quality profile, without pronounced positive or negative deviations. These integrated findings emphasize that optimizing SA concentration is critical for preserving both physiological integrity and visual quality of green beans, and highlight the value of multivariate analytical approaches for interpreting complex postharvest responses.

CONCLUSION

This study demonstrates that salicylic acid exerts a clear dose-dependent influence on the postharvest physiology and visual quality of green beans. Among the tested concentrations, 0.5 mM SA was the most effective in maintaining color vibrancy, delaying browning, and enhancing tissue firmness – attributes strongly linked to consumer preference and market value. In contrast, 1 mM SA treatment induced higher respiration and electrolyte leakage, indicating elevated metabolic stress and reduced membrane stability. The 2 mM dose provided relatively stable quality preservation, particularly through reduced weight loss, but did not match the visual quality improvements observed at 0.5 mM. Multivariate analyses (PCA, correlation matrix, heatmap) clearly distinguished treatment responses and confirmed that color-related parameters co-varied closely with biochemical traits, whereas stress indicators (RR, EL) formed a separate cluster. PCA further revealed that quality variation was primarily explained by

visual attributes (PC1), followed by stress physiology (PC2) and texture firmness (PC3). Overall, the integrated findings indicate that 0.5 mM SA represents an optimal concentration for preserving postharvest quality by balancing physiological stability with attractive visual characteristics. These results highlight the importance of SA dose optimization for extending shelf life, delaying senescence, and improving the postharvest performance of green beans. Future research should explore combined treatments (e.g., SA + $CaCl_2$, SA + MAP) and molecular-level approaches to further elucidate underlying mechanisms. The multivariate analyses strengthened these conclusions by clearly separating visual quality attributes from stress-related physiological parameters, thereby confirming the dose-dependent responses observed in the univariate analyses. This integrated analytical approach provides a comprehensive understanding of postharvest quality dynamics and supports the selection of optimal salicylic acid concentrations for commercial cold storage applications.

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AUTHORS' CONTRIBUTIONS

R.K.: conceptualization, methodology, software, validation, formal analysis, investigation, data curation, writing – original draft, writing – review & editing, visualization. M.U.K.: conceptualization, methodology, software, writing – review & editing, visualization. S.G, M.D. and YG: formal analysis, investigation, visualization.

DATA AVAILABILITY

Data will be made available on request.

CONFLICT OF INTERESTS

The authors declare that they have no competing financial or non-financial interests that could have influenced the research, authorship, or publication of this article.

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