Impact of salicylic acid, abscisic acid, and methyl jasmonate on postharvest quality and bioactive compounds of cultivated strawberry fruit

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Abstract

BACKGROUND: Strawberry is one of the most highly consumed fruits worldwide. However, it is highly perishable fruit postharvest.

OBJECTIVE: To assess the effect of dipping strawberry fruits after harvest in plant growth regulators to maintain postharvest quality.

METHODS: Treatments tested were: 2 and 4 mM salicylic acid (SA), 0.25 and 0.50 mM abscisic acid (ABA) and methyl jasmonate at 0.25 and 0.50 mM (MeJA). Bioactive compounds and fungal growth were assessed over 12 days of storage at 4 °C.

RESULTS: Both concentrations of SA and MeJA significantly suppressed weight loss, decay and respiration rate and 0.50 mM ABA also reduced decay. Both concentrations of SA retarded color development, and total soluble solids content was enhanced by 0.50 mM ABA and MeJA treatments. The most effective treatments for preserving firmness were 0.25 mM MeJA and 4 mM SA. Reduction in loss of ascorbic acid and bioactive compounds during

storage was achieved using the highest concentrations of SA, ABA, and MeJA. Fungal growth was suppressed by all treatments but the best treatment was MeJA at both concentrations.

CONCLUSIONS: All three plant growth regulators reduce postharvest changes in strawberry but effects differ amongst the treatments.

Keywords*: Fragaria ananassa*, quality, postharvest storage, bioactive compounds.

1. Introduction

Strawberry fruit is considered one of the most popular horticultural crops world-wide and is a rich source of important minerals, vitamins (vitamin C), and phytochemicals (anthocyanins carotenoids and polyphenols), that play a significant role in human health [1]. However, strawberry, a non-climacteric fruit, is highly perishable with limited shelf-life due to its high water content, respiration rate, susceptibility to mechanical injury, and to microbial attack (especially by *Botrytis cinerea*) during storage [2]. Strawberry fruit deteriorates rapidly after harvest with loss of economic and nutritional value, and it needs to be harvested at a precise stage of maturity in order to obtain maximum postharvest quality. Hence, there is a demand not only from the producers but also from the consumers to extend shelf-life and reduce decay of strawberry fruit.

Recently, many postharvest techniques have been applied for reducing decay of strawberry fruit such as edible coating with *Aloe vera* and ascorbic acid [3], dipping in essential oils [4], melatonin treatment [5], controlled atmosphere storage [6], γ-irradiation [7], hot air and hot water dipping [2, 8], Nano-ZnO treatment [9], pulsed light [10], and ethylene action inhibitor (1-MCP) treatment [11]. However, some of these treatments are not realistic due to low customer acceptance or high treatment price. Therefore, it is important to develop novel effective methods to reduce senescence and enhance quality of strawberry fruit. One of the

postharvest treatments for reducing senescence of fruits is application of exogenous plant growth regulators. However, relatively few previous studies have compared the effects of different plant growth regulators on postharvest decay and quality of strawberry fruits.

Salicylic acid (SA) is a natural compound and is responsible for suppressing ethylene production and fungal growth such as that of *B. cinerea*. It was reported that SA concentrations of 1 and 2 mmol L^{-1} were the most effective for reducing ethylene production, microbial load and retaining overall quality of strawberry fruits [12]. Moreover, postharvest treatment with SA enhanced total antioxidant content in strawberry fruit [13]. It also reduced weight loss, decay and redness, maintained firmness, and increased hue angle [14].

Abscisic acid (ABA) is one of the most important plant hormones, acting as an inhibitor of growth and metabolism. Previous studies indicated that ABA plays an important role in fruit ripening and senescence not only in climacteric fruits such as tomatoes [15] but also in non-climacteric fruits such as strawberry [16]. Previous reports indicated also that ABA might increase postharvest quality of some fruits such as tomato by enhancing suberin accumulation [17], and increasing soluble sugar concentrations [18]. In strawberry postharvest treatment with 1, 10 or 100 mM ABA resulted in increased accumulation anthocyanin and softening mediated by an increase in PAL activity [19, 20, 21].

Methyl jasmonate **(**MeJA) is found naturally in higher plants and plays a key role in plant defense against pathogen infection. For example, application of exogenous MeJA reduced postharvest decay of peppers by enhancing tissue resistance to *Botrytis cinerea* [22] and reduced decay development in strawberry fruit [23, 24, 25]. Previous studies indicated that crop quality traits were also improved following exogenous MeJA treatment. For example, treatment of *Fragaria chiloensis* with MeJA also maintained fruit firmness and anthocyanin levels [23]. Indeed MeJA treatment was also shown to enhance strawberry aroma while retaining nutritionally important compounds [26].

To our knowledge, no previous studies have been performed, however, to compare the effects of SA, ABA, and MeJA on storability, physico-chemical and sensory quality parameters of strawberry fruits. Thus, the aim of the current study was to evaluate comparatively the effects of postharvest treatment with SA, ABA, and MeJA on retarding senescence, reducing decay, and improving quality traits of strawberry fruit during storage at 4 °C for 12 days.

2. Materials and methods:

2.1. Plant materials and treatments:

Strawberry (*Fragaria* × *ananassa*) cv. 'Festival' fruits were harvested at commercial ripeness stage (¾ of fruit surface showing red colour) from the Faculty of Agriculture, Cairo University experimental station and transported to the postharvest laboratory within 2 h. The fruits were selected for uniformity of size and being free from any visual defects, and they were randomly divided into seven groups (about 100 fruits per group). The strawberry fruit groups were immersed in the following six solutions for 5 min at room temperature (20 °C). The six solutions for treatments were prepared in distilled water as follows: SA-2 (2 mmol L^{-1} salicylic acid), SA-4 (4 mmol L^{-1} salicylic acid), MeJA-0.25 (0.25 mmol L^{-1} methyl jasmonate), MeJA-0.50 (0.50 mmol L^{-1} methyl jasmonate), ABA-0.25 (0.25 mmol L^{-1} abscisic acid), and ABA-0.50 (0.50 mmol L^{-1} abscisic acid). The concentrations used were selected based on preliminary experiment and previous work [SA (14), MeJA (23), and ABA (16)]. The seventh group was the control (CON) and was dipped in distilled water. After immersion, the fruit were recovered using autoclaved forceps and allowed to dry in a laminar air flow hood at room temperature for 60 min. After drying, the fruit for each treatment were packed in clamshells (each containing about 200 g of fruit) and stored at 4 °C and 90% RH

for 12 d. Clamshells for each treatment were divided into two groups. The first group was stored continuously throughout the experimental storage period to determine weight loss and decay. The second group was used to determine fruit quality parameters (firmness, total soluble solids, respiration rate, and colour intensity), chemical parameters (pH, titratable acidity, vitamin C, anthocyanin, total phenolic content and antioxidants capacity), and fungal counts. All the measurements were performed at time intervals of 0, 4, 8 and 12 days after the treatments and each treatment was replicated three times. All physical and chemical analyses were performed on fresh fruits on the day of assay. The experiment was repeated twice.

2.2. Chemicals

Salicylic acid, methyl jasmonate, abscisic acid, ethyl alcohol, ACS spectrophotometric grade, 95.0%, methyl alcohol, gallic acid and Folin & Ciocalteu's phenol reagent were purchased from Sigma-Aldrich (USA). Potato dextrose Agar**,** sodium carbonate, potassium acetate and hydrochloric acid were purchased from Al Gomhoria CO, Cairo, Egypt.

2.3. Weight loss

Weight loss percentage was determined by weighing strawberry fruits using digital scales on each sampling day during storage and calculated using the following equation:

Weight loss (%) = (Initial weight - Final weight/ Initial weight) × 100.

2.4. Decay percentages

The decay percentage was determined at each sampling point and calculated according to the following equation:

Decay percentage (%) = (Number of decayed fruits / Total number of fruits) × 100.

2.5. Firmness (N)

Ten random strawberry fruits from each treatment were used for determining firmness at two points. The two points tested were located in the central zone on opposite sides of fruits. Firmness was measured using a FT011 penetrometer (Wagner Instruments, Italy) and values are presented as Newtons (N).

2.6. Soluble solids content *(SSC)*

Five strawberry fruits were selected for measuring SSC from each treatment (with three replicates). The fruits were mixed in a blender for 2 mins and SSC was determined using a digital refractometer (model PR101, Co. Ltd., Japan) at room temperature (25°C). Readings were taken as % of total soluble solids in the fruit. The same juice was used for determining titratable acidity and pH.

2.7. *Titratable acidity (TA) and pH*

TA of strawberry juice was measured using a digital burette and determined by titrating 5 g (diluted with 50 mL distilled water) of strawberry juice sample with 0.1 mol L^{-1} sodium hydroxide to an end point of pH 8.1 and expressed as percent of citric acid in the fruit juice.

151 The pH of the juice was determined using a pH-meter (EuTech, Instruments, pH 510,
152 Singapore).
153
154 *2.8. Skin fruit colour*
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156 Skin colour of strawberry fruit was measured with a Minolta colorimeter (Model CR-400,
157 KonicaMinolta, INC, Tokyo, Japan) on five fruit per replicate. *L**, *a**, *b**, chroma (*C**) and
158 hue angle (*h*°) were determined. Each measurement was taken at three locations for each
159 individual fruit. A standard white calibration plate was used to calibrate the colorimeter.
160
161 *2.9. Respiration rate*
162
163 Five separate single fruits were placed in separate gas-tight jars (200 ml) at 5°C for 2 h. After
164 2 h, 1 mL of air sample was removed from the headspace and was analyzed using an O_2/CO_2
165 gas analyzer (model 902D, MA, USA). Respiration rate was expressed as mmol CO_2
166 $kg^{-1}FWh^{-1}$.
167
168 *2.10. Ascorbic acid and total anthocyanin content*
169
170 Ascorbic acid (AA) content was determined using a titrimetric method with 2, 6-
171 dichlorophenol indophenol [27]. The results of AA content are expressed as mg/100 g fresh
172 weight.
173 Five strawberry fruits were selected randomly from each replicate and homogenized in a
174 laboratory blender (Heidalph DGH Rundfunk- Fernsehen, Typ-DR 22054, Germany) at high
175 speed to determine anthocyanin and total phenolic compounds. Anthocyanin content was

176 determined using the pH-differential method described by Tonu et al. [28]. Briefly, 4 g of
177 strawberry puree was extracted with 40 ml of solvent, ethanol: 0.1 M HCl (85:15%, V:V).
178 The mixture was centrifuged at 6.000 × g for 20 min and then the supernatant was filtered
179 using Whatman No.1 filter paper; the supernatant was collected and used for anthocyanin
180 determination. Extractions were done in triplicate. Extracts (3 ml) were diluted in 5 ml of two
181 different buffers; pH = 1.0 and pH = 4.5. After 30 minutes of incubation at room temperature,
182 absorption (A) was measured at 510 nm and at 700 nm. The absorbance values of the diluted
183 samples (A) were calculated as follows:
184 $A = (A_{510} – A_{700})\ pH_{1.0} - (A_{510} – A_{700})\ pH_{4.5}$
185 Total anthocyanin content was calculated as follows:
186 $TAC = A \times MW \times df \times 1000/(\varepsilon \times \lambda \times m)$
187 Total anthocyanin content was calculated as mg cyanidin-3-glucoside equivalent per kg dry
188 extract (mg C3GE/kg) by using (A) the difference of absorbance between pH 1 and pH 4.5
189 solutions, a dilution factor (df), conversion factor to kg (1000), a molar absorptivity (**ε**) of
190 24,825 M^{-1} cm^{-1} (at 510 nm), a molecular weight (MW) of 484.82, cuvette optical path length
191 (λ)(1 cm), and weight of the sample (m)(g).
192
193 *2.11. Total phenolic content*
194
195 The total phenolic content (TPC) was determined according to Aaby et al. [29] using Folin-
196 Ciocalteau reagent with gallic acid as standard. Aliquots of strawberry puree were centrifuged
197 at 8000 × g for 20 min at room temperature. The resulting homogenate was filtered through
198 filter paper to obtain a clear juice. One mL of collected clear juice was mixed with 5 mL of a
199 1/10 dilution of Folin-Ciocalteau reagent and 4 mL sodium bicarbonate (7.5% w/v), and the
200 mixture was diluted to 100 mL with distilled water. The solution was kept in the dark at room

temperature for 2 h; the absorbance was then measured at 765 nm with a spectrophotometer (model UV-2401 PC, Shimadzu, Milano, Italia). TPC was expressed as gallic acid equivalents in mg per 100 g fresh weight (mg GAE/100 g FW) using a gallic acid standard curve.

2.12. Antioxidant capacity

The effect of different treatments on strawberry fruit antioxidant capacity was determined according to the method of Yen and Chen [30]. Strawberry samples (10 g) were homogenized in 200 mL of distilled water, and then filtered using Whatman No.1 filter paper and 5 mL of filtrate was diluted into 25 mL of distilled water. Strawberry extract (1 mL) was added to 3 mL of methanol and 1 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (0.012 g DPPH in 100 mL^{-1} of methanol). The mixture was shaken in the dark at room temperature for 10 min. The absorbance was measured at 517 nm. The antioxidant capacity was expressed as % of inhibition according to the formula:

Inhibition (%) = $(A_{control} - A_{sample}/A_{control}) \times 100$

where *A* control and *A* sample are the absorbance of the control and sample, respectively [31].

2.13. Microbiological evaluation

Fruit samples (10 g) were crushed and diluted (1:10 w/v) in 0.1% buffered peptone water, homogenized by hand massaging for 5 min and serially diluted with buffered peptone water. The homogenate (0.1 ml) was plated on potato dextrose agar in duplicate. Fungal counts (log CFU/g) were determined after incubation at 25-28 °C for 5 days [32].

2.14. Statistical analysis

The whole experiment was repeated twice and the data were pooled. Data were subjected to analysis of variance (ANOVA) with SPSS software. Sources of variation were storage period (days) and treatments. A Duncan test at $p < 0.05$ was used to compare means among treatments.

3. Results

3.1. Weight loss, respiration rate, and decay were reduced by postharvest treatments

The effect of dipping strawberry cv. 'Festival' fruit in different concentrations of SA, ABA, and MeJA on weight loss, respiration rate, and decay percentage during 12 days of storage at 4°C is shown in Fig. 1. Weight loss n increased during the storage period following all treatments (Fig. 1A). After 8 days of storage, all treatments significantly ($p < 0.05$) reduced weight loss compared to the control except the two concentrations of ABA. However, at the end of the storage period (12 days), only treatment with 0.25 mM MeJA significantly reduced weight loss compared to the control.

The 2 and 4 mM SA treated fruits showed significant ($p < 0.05$) reduction of respiration rate at all storage period time points compared to the control fruits (Fig. 1B). After 4 and 8 days of storage, there were no differences in respiration rate between fruit treated with 0.25 and 0.50 mM ABA and the control. However, after 12 days of storage, treatment of strawberry fruit with 0.50 mM ABA resulted in greater respiration rate than the control or the other treatments. MeJA at 0.50 mM significantly reduced respiration rate at all storage period time points.

No decay was observed on the surface of strawberry fruit after 4 days of storage after any of the treatments, however, strawberry fruit treated with 2 and 4 mM SA showed greater

resistance against decay when compared with the control and the other treatments after both 8 and 12 days of storage (Fig. 1C). Treatment with the higher concentration of SA resulted in less surface decay than the lower concentration after 8 days of storage, however, the difference between two concentrations of SA was not significant after 12 days of storage. Treatment with ABA showed the same trend of results as SA but after 12 days, treatment with 0.25 mM ABA was not effective at reducing decay compared to the control. Treatments with MeJA (0.25 and 0.50 mM) significantly delayed the development of decay compared to control fruits throughout the storage periods. However, no significant difference was observed in decay between 0.25 and 0.50 mM MeJA treated fruit.

3.2. Changes in colour

The effect of dipping strawberry cv. 'Festival' fruit in different concentrations of SA, ABA, and MeJA on L*, a*, and ascorbic acid content during 12 days of storage at 4°C is shown in Fig. 2. The L* value is an indicator for brightness of the fruit surface: high values indicate less pigment accumulation and less ripening while the lower values indicate more intense colour and more ripening. Results shown in Supplementary Table (1) indicate that L* values of fruit surfaces generally decreased during storage. After 4 days of storage, the L* values of fruit subjected to all treatments were significantly higher (lighter colour) than those of the control except for the 0.25 mM MeJA treatment. After 8 days only the SA treated fruit and after 12 days the SA treated and the 0.25 mM JA treated fruit, had higher L* values than the control. ($p < 0.05$).

A positive (+) a* value is an indicator for redness while, negative (-) values are a sign of greenness. Thus a positive a* value is correlated with anthocyanin concentration in strawberry fruits [17]. Here, a* values increased with storage duration from 4 to 8 days following all

treatments (Supplementary Table 1). Both SA treatments resulted in significantly lower a* values than the control at all time points (Fig. 2B). There were no differences in a* values between the two concentrations of ABA or the lower concentration of MeJA treatment and the control after 8 storage. However, no differences were recorded between control and either concentration of ABA and MeJA after 12 days of storage.

3.3. Strawberry fruit quality is affected by treatments

Firmness, SSC, pH, and titrable acidity (TA) were determined as indicators of strawberry fruit ripening and quality as well as their metabolic activity (Table 1). SSC was significantly ($p < 0.05$) influenced by treatments. SSC at harvest was 10.87±0.13 Brix, and decreased significantly after 4 d of storage in both control and most treated fruits (Supplementary Table 1). Treatment with both ABA concentrations reduced SSC loss compared to the control while the other treatments had no effect after 4 days of storage. After 8 and 12 d of storage, both treatments with ABA (0.25 and 0.50 mM) and MeJA (0.25 and 0.50 mM) showed significantly higher SSC values compared to control.

Fruit firmness was 4.71±0.03 N at harvest time and decreased during storage after all treatments (Supplementary Table 1). No significant difference was observed between treated fruit and the control after 4 days of storage (Table 1). After 8 and 12 days of storage, fruit firmness was found significantly ($p < 0.05$) higher in all treated fruits when compared with control. Among all treatments, MeJA at 0.25 mM and SA at 4 mM showed highest fruit firmness at both time points during the storage period.

In the controls, pH rose from dya0 to day 8 and then fell back, while TA contents rose from day 0 to day 8 and thereafter remained constant (Supplementary Table 1). However,

299 postharvest treatments with SA, ABA, and MeJA had no clear effect on either character
300 during storage.
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302 *3.4. Effect of treatments on bioactive compounds and antioxidant* capacity
303
304 *3.4.1. Changes in ascorbic acid*
305 Ascorbic acid decreased with increasing storage time (Fig. 2C). No significant difference was
306 observed in AA amongst all treated fruit and the control after 4 days of storage. However after
307 8 and 12 days of refrigerated storage, treatment of fruit with 4 mM SA, 0.50 mM ABA, and
308 0.25 mM MeJA significantly reduced ($p < 0.05$) the loss of AA compared to the control.
309 Neither the lower concentration of SA or ABA was able to reduce AA loss after 8 or 12 days
310 of storage. Both concentrations of MeJA also reduced loss of AA after 12 days and when used
311 at 0.25 mM a loss reduction was also seen after 8 days of storage.
312
313 *3.4.2. Changes in antioxidant* capacity
314
315 Antioxidant capacity was 78.32±2.27 % at the beginning of the storage period and decreased
316 with increasing storage periods at 4°C in all treated fruits (Supplementary Table 1). However,
317 all treated strawberry fruit retained more antioxidant capacity compared to the control
318 treatment at each time point (Fig. 3A). Furthermore, strawberries treated with the higher
319 concentration of SA, ABA, and MeJA showed higher values of antioxidant capacity at each
320 time point compared to the lower concentrations, although the difference was not significant
321 for ABA at day 8 of storage.
322
323 *3.4.3. Changes in TPC*

A slight increase in TPC was observed after 4 days of storage at 4°C in strawberry fruits with most of the plant hormone treatments, which ranged from (211- 224) mg GAE/100g FW, compared to the untreated control (206 GAE/100g FW) (Fig. 3B). After 4 days of storage, there was a decrease in TPC in all treated fruit and control (Supplementary Table 1). However, the fruit treated with the higher concentrations of all three hormones retained significantly higher TPC after both 8 and 12 days of storage compared to the control.

3.4.3. Changes in total anthocyanin content

Total anthocyanin content was significantly affected by treatment with SA, ABA, and MeJA (Fig. 3C). Anthocyanin content increased slightly between 4 and 8 days of storage following treatment with the lower concentrations of SA, ABA, and MeJA, but by day 12, it had decreased (Supplementary Table 1). However, anthocyanin content of all treated fruits was significantly greater at 8 and 12 days of storage compared to the control. Strawberries treated with the lower concentration of ABA showed the highest anthocyanin content at the end of storage period followed by the strawberries treated with 2 mM SA and 4 mM SA.

3.5. Fungal count (log CFU/g) was affected by postharvest treatments

The effect of dipping strawberry cv. 'Festival' fruit in different concentrations of SA, ABA, and MeJA on fungal counts (log CFU/g) during storage for 12 days at 4°C is presented in Table 2. The principal decay fungi detected were *Botrytis cinerea* and *Rhizopus stolonifer*. No fungal growth was detected from fruit treated with SA (4 mM), ABA (0.50 mM), and MeJA (0.25 and 0.50 mM) at day 0. At all storage time points (4, 8 and 12 days) fungal growth in all

treated fruits was either absent or significantly lower than in the control ($p > 0.05$). After 8 days of storage, fruit treated with the higher concentration of SA and both concentrations of MeJA recorded significantly lower fungal counts than the other treatments or control and ranged from 2.5 – 2.6 log CFU/g. At the end of the storage period, the control sample reached 4.2 log CFU/g followed by the SA and ABA treated fruit (3.9 log CFU/g). The most effective treatments for controlling fungal growth were the two concentrations of MeJA without any significant difference between them.

4. Discussion

4.1. Weight loss, respiration rate, and colour changes

Our results showed that the most effective treatments for reduction of weight loss were SA and MeJA. Application of SA has been found to reduce water loss during refrigerated storage of various crops including strawberry [14]. The positive effects of SA for reducing weight loss are related to its overall effects in maintaining fruit quality [13, 14]. This in turn is likely due to the effect of SA treatments in reducing respiration rate and ethylene production [12,13]. In this study respiration rate was significantly reduced by the SA treatments.

The effect of MeJA in reducing weight loss is likely related to its effect in reducing loss of firmness (Table 1). This in turn may be due to effects on total antioxidants [33] that result in increasing lignin content as observed in previous work on *Fragaria chiloensis* fruit [23]. Treatment with 0.50 mM MeJA also significantly reduced the respiration rate during storage of the strawberry fruit, presumably related to the maintenance of firmness and fruit quality. This is in agreement with previous work [34] showing that whereas in unripe fruit MeJA increased respiration, at later stages of ripening MeJA treatment had the opposite effect. The increased of respiration rate induced by 0.5 mM ABA could be due to enhanced ethylene

374 production elicited by ABA treatment which was previously reported in strawberry cv.
375 'Everest' [20].
376 Here treatment with 4 mM SA resulted in a shinier/lighter skin colour (higher L* values)
377 compared to the control throughout storage at 4 ^{o}C. Effects of post-harvest treatments on
378 strawberry colour appear to vary [14]. For example, 2 mM SA treatment of *F. ananassa* cv
379 'Camarosa' fruit did not affect lightness (L*) [14]. The difference to the results presented here
380 may be due to the cultivar, or the different SA concentration used. In other fruit, lower
381 moisture loss leads to higher brightness (higher L* values) [35]. This would fit here with the
382 reduction in weight loss, but would need further verification. No difference was observed in
383 L* with ABA treatment at later storage time points. In previous work exogenous application
384 of 0.1 mM ABA to strawberry fruit, accelerated colour development by increasing
385 anthocyanin content and phenylalanine ammonia-lyase (PAL) activity [21]. The difference
386 with our results could be explained by the difference in the fruit maturity stage used, or the
387 method of ABA application, which in the case of Chen et al. [21] was via the peduncle.
388 In this study, the lowest a* values were obtained by both SA treatments during all storage
389 periods. This could be due to the reduction in weight loss (Fig 1.A) and respiration rate (Fig
390 1.B) leading to a delay in the accumulation of anthocyanin. Again this contrasts with results
391 reported by Shafiee et al. [14], who did not find any changes in a* values when strawberry cv.
392 'Camarosa' fruit were dipped in SA. The different cultivar used in this study could again
393 explain the differences with our results. Compared to the control, treatment with ABA had no
394 significant effect on a* value. However, Li et al. [19] reported a significant increase in a*
395 value of strawberry fruits using a 1 mM ABA treatment compared to controls. The difference
396 with our results could be due to the use of a higher concentration of ABA (1 mM) compared
397 to our study (0.50 mM).
398

399 *4.2. SSC, Firmness, pH, and TA*

400

401 SA treatment did not significantly affect SSC content, in agreement with Shafiee et al. [14].
402 However, in our study, ABA treatments did significantly increase SSC content. This is in
403 agreement with previous work [19] where ABA treatment also increased colour formation,
404 and anthocyanin accumulation while decreasing firmness. This combined effect was ascribed
405 to an overall acceleration of ripening. Here firmness was actually increased by ABA
406 treatment. The difference to the previous study may relate to the stage of maturity used for the
407 studies: in this study fruit were treated at commercial ripeness while in the previous study the
408 fruit were at the large green stage of maturity.
409 Firmness is a key factor for strawberry fruit quality. In this study, compared with controls,
410 strawberry fruit treated with 4 mM SA showed higher firmness (Table 1), in agreement with a
411 previous report showing that strawberry cv. 'Camarosa' fruits treated with SA had higher
412 firmness than controls [14]. This result might be related to the effects of SA in reducing the
413 activity of the main cell wall degrading enzymes (pectin methylesterase, cellulase,
414 polygalacturonase) and reducing the activity of enzymes such as lipoxygenase which leads to
415 higher firmness of fruits [13]. Here MeJA also increased firmness, in agreement with a
416 previous study that tested pre-harvest applications of MeJA on postharvest qualities of *F.*
417 *chiloensis*. In contrast Concha et al [36] found that application of MeJA decreased firmness,
418 however in their study fruit were treated at a less mature stage which may account for the
419 difference.
420 Our results show that SA treatment resulted in a higher TA than the control after 8 and 12
421 days of storage. This result does not agree with Shafiee et al. [14] or Ayub et al [37] who
422 found that postharvest treatment with SA did not significantly affect TA, however these
423 studies different in the treatment combinations used [14] and length of treatment [37] making

424 a full comparison difficult. Our results do however support the hypothesis that SA conserves
425 acidity in fruits via a reduction in respiration rate [13, 37].
426
427 *4.3. Ascorbic acid, Total Phenolics, anthocyanin and antioxidant* capacity

428 The positive effect of SA in reducing loss of AA is in agreement with previous studies [13,
429 33] and is likely due to its stimulation of the biosynthesis of ROS scavenging enzymes [13].
430 Treatment with 0.50 mM ABA also significantly increased AA retention. This result is in
431 agreement with Li et al. [19] who found an increase in antioxidant capacity in the first few
432 days post-harvest but not at later time points. On the other hand, Ayub et al. [37] did not find
433 any changes in AA related to ABA treatment. This could be due to the method of ABA
434 application, which was performed by injecting 100µL of 1 mM ABA diluted in 2% ethanol
435 solution into the fruit receptacles. In our study, MeJA also reduced AA loss in strawberry
436 fruits after longer term storage. This is in agreement with, Lolaei et al. [38] who reported a
437 significant increase in AA by treating strawberry cvs. ‘Selva’ and ‘Queen Elisa’ fruits with
438 0.50 and 1 mM MeJA.

439 The antioxidant capacity of all treated fruits was already higher than the control after only 4
440 days of storage. Moreover effects were most pronounced with the highest concentration of
441 each of the growth regulators. This contrasts with the ascorbic acid levels which were not
442 affected by the treatments at day 4 and suggests that the antioxidant effects of the treatments
443 were not mediated by changes in ascorbic acid. Phenolics are an important class of
444 antioxidant compounds in berries [9, 29]. In accordance with Ayala-Zavala et al., [40] MeJA
445 significantly increased retention of TPC at all time points.
446 The pattern of the effects of treatments on TPC do follow quite closely the pattern of
447 antioxidant capacity changes, although at day 4 the effects of all the treatments on antioxidant
448 capacity seemed to be more pronounced compared to the trend of TPC change, suggesting

449 that other antioxidant pathways may also be stimulated by the treatments. The retention of
450 TPC by treatments with high concentrations of SA and MeJA are consistent with previous
451 studies indicating that both growth regulators enhance the efficiency of antioxidant systems in
452 plants [40, 13].
453 Anthocyanin is one of the major compounds present in strawberries. In our study we observed
454 few differences in total anthocyanin content after 4 days of storage with the treatments tested.
455 However, at later time points all the treatments significantly improved anthocyanin retention.
456 This result is in agreement with the study by Ayala-Zavala et al., [40], where strawberries
457 treated with MeJA showed the highest values of anthocyanin after 12 days of storage at
458 7.5°C. Moreover, Yueming and Daryl [41] reported that treatment with ABA stimulated
459 accumulation of anthocyanin and increased ethylene production, and suggested that this may
460 be due to the effects of ABA in enhancing PAL activity.
461 The antioxidant capacity of anthocyanins may be one of their most significant biological
462 properties [42], however in our study the pattern of effects of the treatments on anthocyanin
463 content did not match antioxidant activity closely, indicating that other antioxidants are also
464 affected by the treatments
465
466 *4.5. Decay and fungal count (log CFU/g)*
467 Results presented here show that treatment of fruits with SA, ABA, or MeJA reduced decay
468 development during storage at 4°C. Plants use several mechanisms to protect themselves from
469 pathogenic attack; one of them is accumulation of SA [13, 43]. *Botrytis cinerea* and *Rhizopus*
470 *stolonifer* were the main decay fungi detected in our study. Our results are in agreement with
471 those previously reported [12] showing that postharvest treatment with SA reduced fungal
472 decay of strawberry cv. 'Selva' fruits caused by *Botrytis cinerea*. The role of SA in
473 controlling postharvest spoilage is likely due to its role in increasing hydrogen peroxide

(H_2O_2) in plants which acts as a signal molecule to activate plant resistance systems against pathogen attack [13]. To our knowledge, no previous work has studied the effect of exogenous ABA postharvest treatment on the decay development of strawberry fruit. Our results indicate that 0.50 mM ABA retards decay during cold storage. This result could be due to induced activity of defence enzymes by the ABA such as phenylalanine ammonia-lyase (PAL) [20]. Our results also showed that MeJA at the two tested concentrations could delay the development of decay in strawberry fruits, in agreement with previous studies [24, 25, 44]. The action of MeJA here is likely due to its activation of defence pathways [45].

5. Conclusions

In summary, a comparison of our results with the literature clearly indicates the need for comparative studies using fruit of the same maturity and equivalent application methods. Our results confirm and expand on previous studies showing that application of SA, ABA and MeJA are potentially useful postharvest treatments to enhance strawberry shelf life. However, the direct comparison of their effects provided in this study, indicates subtly different responses that are worthy of further investigation to understand underlying mechanisms and potential synergies.

Conflict of interest

The authors have declared no conflict of interest.

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Tables

Table 1: Effect of salicylic acid (2 and 4 mM), abscisic acid (0.25 and 0.50 mM), and methyl jasmonate (0.25 and 0.50 mM) on TSS, firmness, pH, and acidity of strawberry fruits stored for 12 d at 4 °C. Data are mean of three replicates ± standard errors. Different letters indicate significant differences amongst treatments at each time point (Duncan test, p <0.05%).

Time	**Treatment**	**SSC**	**Firmness**	**pH**	**TA**
(day)	(mM)	Brix	N		% citric acid
0		10.87±0.63	4.71±0.03	3.54±0.06	0.98±0.06
4	2 SA	9.23±0.88 bc	4.11±0.05 a	3.62±0.03 bc	0.92±0.01 ab
4	4 SA	9.23±0.06 bc	4.16±0.03 a	3.57±0.01 c	0.97±0.01 a
4	0.25 ABA	9.76±0.16 ab	4.03±0.37 a	3.57±0.02 c	0.91±0.00 ab
4	0.50 ABA	10.43±0.23 a	4.13±0.08 a	3.66±0.00 b	0.89±0.01 b
4	0.25 MeJA	8.63±0.12 c	4.20±0.05 a	3.62±0.01 bc	0.95±0.01 ab
4	0.50 MeJA	9.46±0.08 bc	4.16±0.12 a	3.74±0.01 a	0.93±0.01 ab
4	Control	8.63±0.63 c	4.13±0.14 a	3.64±0.02 b	0.89±0.03 b
8	2 SA	9.46±0.09 bc	3.66±0.03 bc	3.69±0.00 ab	0.92±0.01 ab
8	4 SA	9.46±0.17 bc	3.78±0.04 ab	3.58±0.01 c	0.96±0.01 a
8	0.25 ABA	9.61±0.19 b	3.50±0.06 c	3.67±0.03 ab	0.91±0.03 bc
8	0.50 ABA	10.56±0.19 a	3.60±0.06 bc	3.69±0.00 ab	0.87±0.01 cd
8	0.25 MeJA	9.96±0.30 ab	3.90±0.10 a	3.65±0.03 b	0.90±0.00 bc
8	0.50 MeJA	10.03±0.49 ab	3.70±0.06 b	3.72±0.01 a	0.88±0.01 cd
8	Control	8.68±0.09 c	3.28±0.04 d	3.71±0.02 a	0.84±0.01 d
12	2 SA	9.80±0.06 cd	3.50±0.16 bc	3.57±0.01 a	0.89±0.02 a
12	4 SA	9.70±0.10 cd	3.70±0.06 a	3.50±0.01 a	0.89±0.01 a
12	0.25 ABA	10.11±0.07 bc	3.34±0.03 d	3.60±0.07 a	0.86±0.01 ab
12	0.50 ABA	11.23±0.35 a	3.43±0.03 cd	3.56±0.03 a	0.87±0.00 ab
12	0.25 MeJA	10.76±0.46 ab	3.60±0.06 ab	3.59±0.07 a	0.89±0.03 a
12	0.50 MeJA	10.18±0.09 bc	3.48±0.04 bcd	3.64±0.05 a	0.88±0.01 ab
12	Control	9.16±0.44 d	3.13±0.03 e	3.50±0.01 a	0.84±0.00 b

Table 2: Effect of salicylic acid (2 and 4 mM), abscisic acid (0.25 and 0.50 mM), and methyl jasmonate (0.25 and 0.50 mM) on mold and yeast (log CFU/g) of strawberry fruits stored for 12 d at 4 °C. Data are mean of 3 replicates ± stander errors. Different letters indicate significant differences (Duncan test, P<0.05%).

Treatments	Storage period (Days)			
	0	4	8	12
2 SA	2.60± 0.05 b	2.6 ± 0.05 c	3.70 ± 0.06 b	3.93 ± 0.03 b
4 SA	ND*	ND	2.53 ± 0.03 d	3.86 ± 0.03 b
0.25 ABA	2.53 ± 0.03 b	2.7 ± 0.03 b	3.53 ± 0.03 c	3.86 ± 0.03 b
0.50 ABA	ND	2.6 ± 0.05 c	3.53 ± 0.03 c	3.86 ± 0.03 b
0.25 MeJA	ND	ND	2.43 ± 0.03 d	3.63 ± 0.03 c
0.50 MeJA	ND	ND	2.53 ± 0.03 d	3.66 ± 0.03 c
Control	2.9 ± 0.03 a	3.1 ± 0.05 a	4.00 ± 0.06 a	4.23 ± 0.03 a

***ND : mean (not detected) there is no fungal growth found.**

Figures

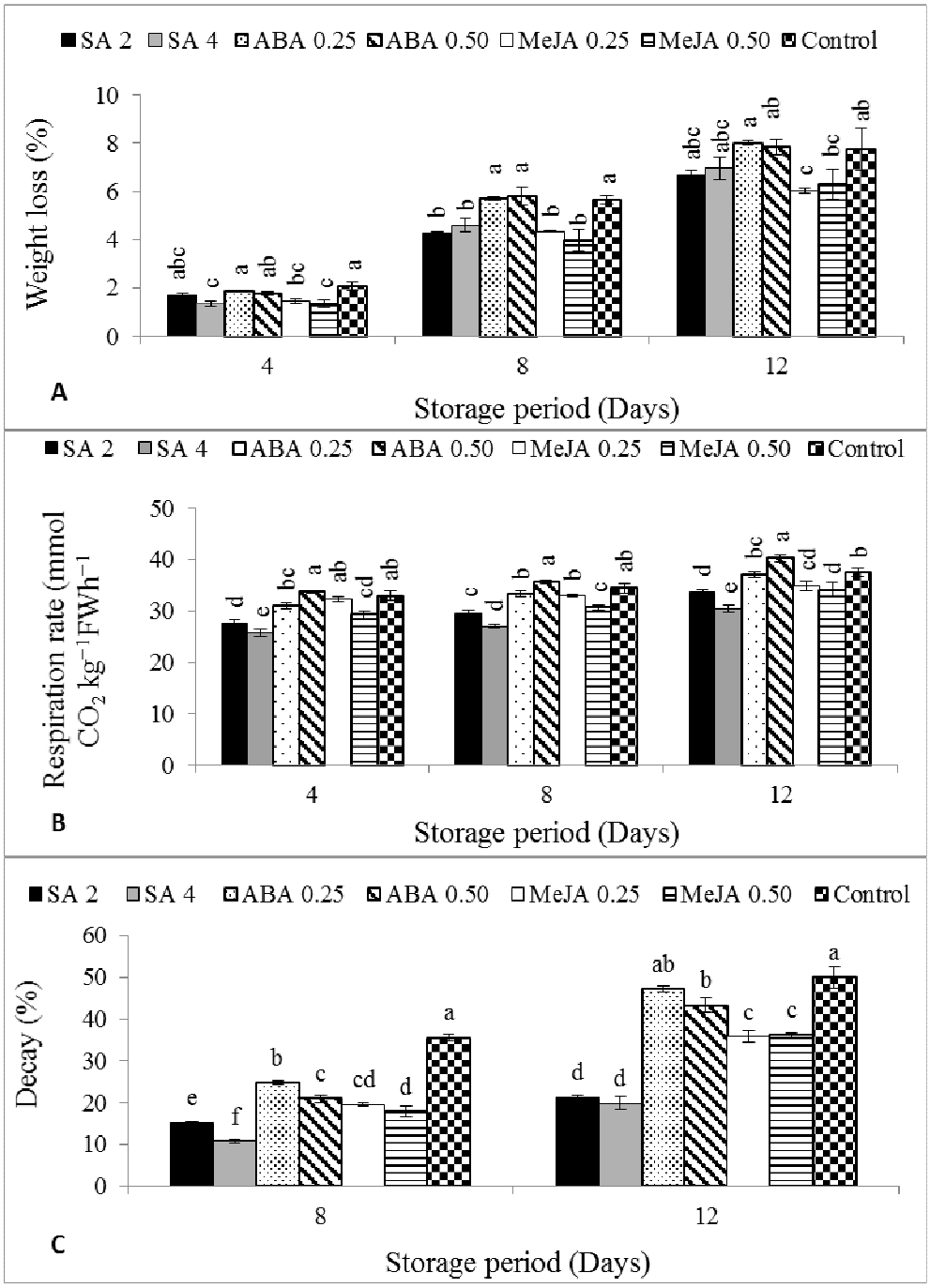

Figure 1: Effect of salicylic acid (SA) (2 and 4 mM), abscisic acid (ABA) (0.25 and 0.50 mM), and methyl jasmonate (MeJA) (0.25 and 0.50 mM) on (A) weight loss (%), (B) respiration rate (mmol CO_2 $kg^{-1}FWh^{-1}$), and (C) decay % of strawberry fruits stored for 12 d at 4 °C. Respiration rate at start of the storage was 24.75±0.32 mmol CO_2 $kg^{-1}FWh^{-1}$. No decay was observed at 4 d from start of the storage, hus, panel (C) shows just 8 and 12 d. Data are mean of three replicates. Different letters for every storage point indicate significant differences (Duncan test, $p < 0.05$).

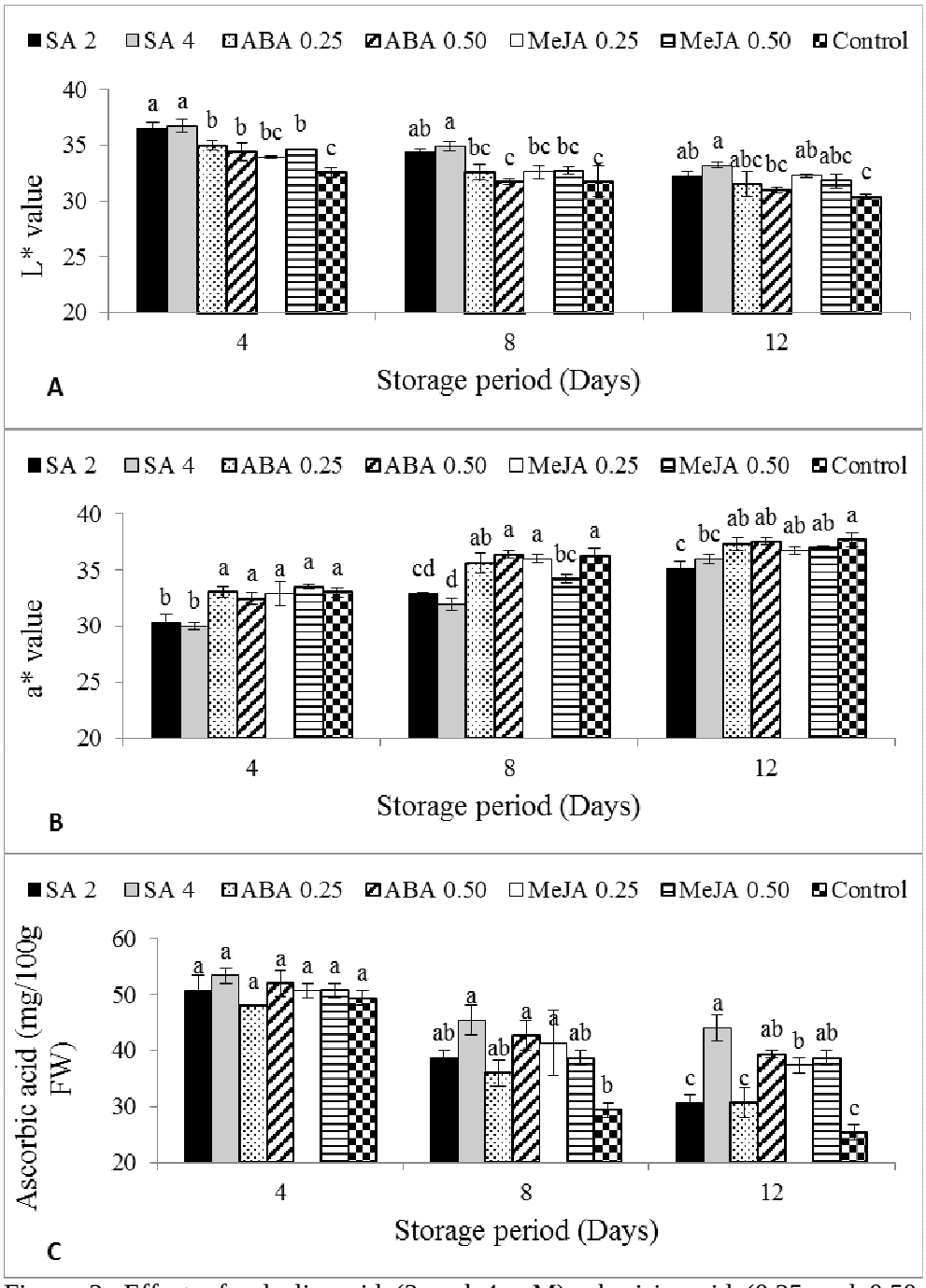

Figure 2: Effect of salsylic acid (2 and 4 mM), abscisic acid (0.25 and 0.50 mM), and methyl jasmonate (0.25 and 0.50 mM) on (A) *L** value, (B) *a** vlaue, and (C) ascorbic acid (mg/100g FW) of strawberry fruits stored for 12 d at 4 °C. *L** value, *a** value, and ascorbic acid value at start of the storage were 34.52±0.23, 33.60± 0.20, and 54.66±1.33 (mg/100g FW), respectively. Data are mean of 3 replicates. Different letters for every storage point indicate significant differences (Duncan test, P<0.05%).

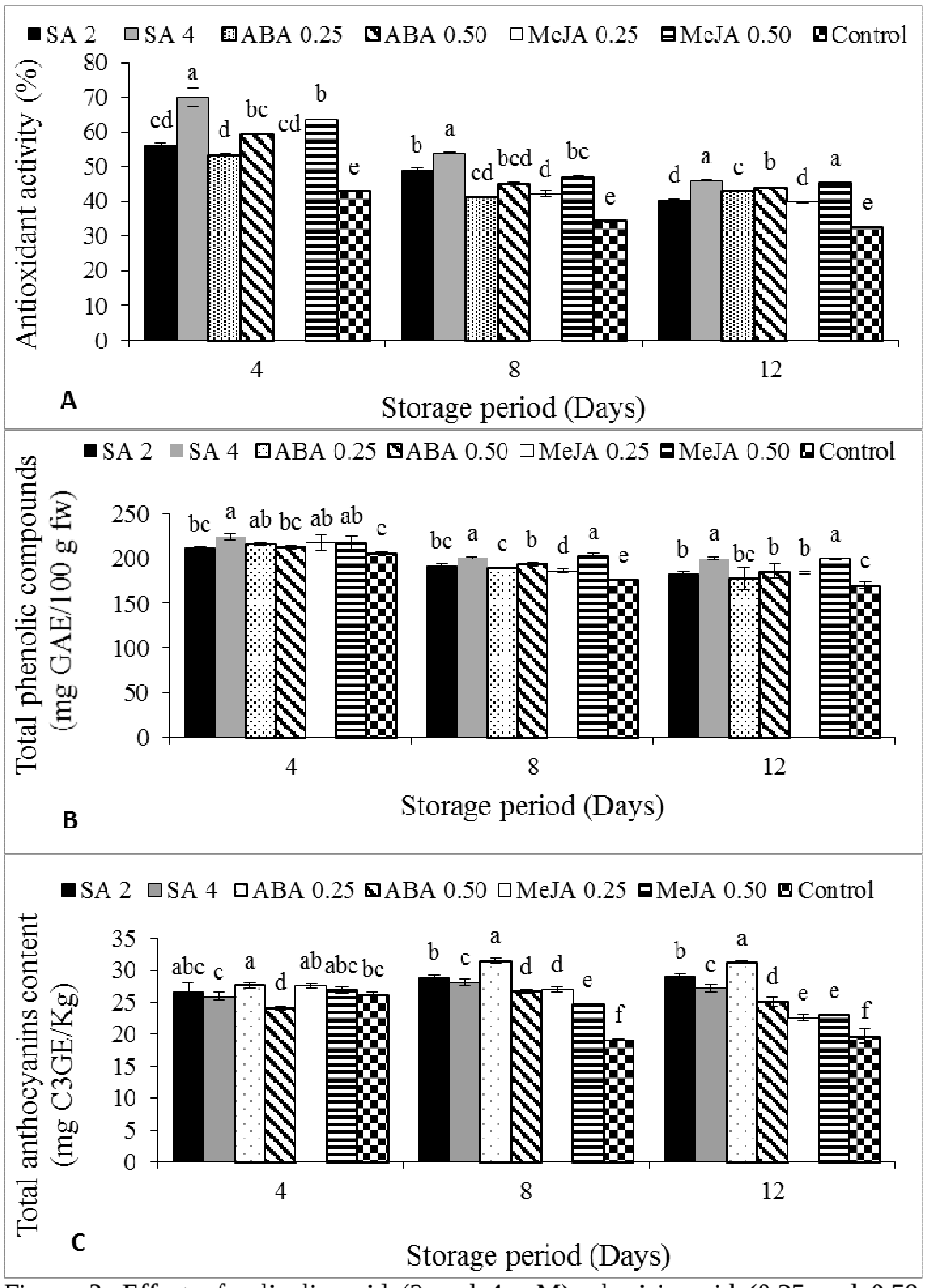

Figure 3: Effect of salicylic acid (2 and 4 mM), abscisic acid (0.25 and 0.50 mM), and methyl jasmonate (0.25 and 0.50 mM) on (A) antioxidant activity %, (B) total phenolic compunds (mg GAE/100 g fw), and (C) total anthocyanin content (mg C3GE/Kg) of strawberry fruits stored for 12 d at 4 °C. Antioxidant activity, total phenolic compunds, and total anthocyanin content at start of the storage were 78.32±2.27 %, 210.33±2.72 (mg GAE/100 g fw), and 26.22±0.91 (mg C3GE/Kg), respectively. Data are means of three replicates. Different letters indicate significant differences within each time point (Duncan test, P<0.05%).