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Short communication

Effects of preharvest applications of methyl jasmonate and chitosan on postharvest decay, quality and chemical attributes of *Fragaria chiloensis* fruit

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ABSTRACT

Fragaria chiloensis fruit has a short postharvest life mainly due to its rapid softening. In order to improve its postharvest life, preharvest applications of methyl jasmonate (MeJA) and chitosan were evaluated during postharvest storage at room temperature. The quality and chemical parameters, and protection against decay were evaluated at 0, 24, 48 and 72 h of storage from fruits of two subsequent picks (termed as first harvest and second harvest). In general, fruits treated with MeJA and chitosan maintained higher levels of fruit firmness, anthocyanin, and showed significant delays in decay incidence compared to control fruit. MeJA-treated fruits exhibited a greater lignin content and SSC/TA ratio, and delayed decay incidences. Instead, chitosan-treated fruits presented higher antioxidant capacity and total phenol content. In short, both the elicitors were able to increase the shelf life of fruits as evidenced by the increased levels of lignin and anthocyanin, especially of the second harvest.

1. Introduction

At present, consumers demand more natural, environmentally friendly food production, with high quality and an extended shelf life, and without any chemical preservatives (Gol, Patel, & Rao, 2013). *Fragaria chiloensis* (L.) Mill., commonly known as Chilean strawberry, is noted for its good fruit quality characters, presenting a particular white color and an intense aroma, but it exhibits a high softening rate during ripening, which can negatively impact its postharvest life (Figueroa et al., 2010). Being a non-climacteric fruit, strawberries do not ripen during postharvest and therefore must be harvested at the nearly full-ripe stage (Cherian, Figueroa, & Nair, 2014). On the other hand, strawberries are found to be highly perishable due to fungal attack during their storage. Traditionally strawberries are treated with different fungicides to control postharvest decay, but they leave residues that have potential risks to humans and the environment (Li & Yu, 2001). Thus, the development of new and effective methods to increase postharvest shelf life and control on postharvest decay of strawberry fruits is warranted.

Chitosan, a high molecular polymer, nontoxic, bioactive agent has become a useful choice due to its fungicidal effects and elicitation of defense mechanisms in plants. It is able to induce host defense responses, including the accumulation of antifungal hydrolysates and phytoalexin (Li & Yu, 2001). Like chitosan, the application of preharvest and postharvest plant growth regulators also have been associated with improvement and preservation of fruit quality (Figueroa et al., 2012; Karaman, Ozturk, Genc, & Celik, 2012). In this sense, the effects of methyl jasmonate (MeJA) applications in fruits are reflected in physical changes such as color, weight, firmness and the amount of bioactive compounds (phenolic content, antioxidants) (Concha et al., 2013; Karaman et al., 2012; Rudell, Fellmann, & Mattheis, 2005).

Therefore, the objective of the present study was to establish the effects of preharvest applications of chitosan and MeJA on quality, chemical attributes and decay incidence of *F. chiloensis* fruit during postharvest storage.

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2. Materials and methods

2.1. Plant material and treatments

The experiment was conducted in a commercial orchard of Chilean strawberry located at Purén, Araucanía Region, Chile (latitude 38° 04′ S; longitude 73° 14′ W). Treatments consisted in preharvest applications of 0.25 mM MeJA (Sigma–Aldrich, USA) and 1.5% (w/v) chitosan (IONA Ltd., Chile) at pH 4.3 using 0.05% (w/v) Tween-20 as a surfactant in both solutions. Distilled water was used as a control. The MeJA concentration was selected as the minimum effective concentration according to previous field experiment on strawberry (Yilmaz, Yildiz, & Muradoglu, 2003). Chitosan concentration was selected according to the previously reported study of El Ghaouth, Ponnampalam, Castaigne, and Arul (1992).

One hundred and thirty plants were used per treatment under the same agronomic management. Applications were sprayed 3 times in different developmental stages of the crop during 2012 growing season: the first at 80% flowering (80% full bloom and 20% flower buds with absence of fruits, on October 26); the second at flowering (100% full bloom) and turning fruit stage (C3 stage according to Figueroa et al., 2008, on November 22); and the third at full ripe fruit stage (C4 stage according to Figueroa et al., 2008, on November 27). To check the long-term effect of both elicitors, postharvest evaluations were made in two batches: with the fruits of the first harvest (fruits were picked immediately after the third application) and with fruits of the second harvest (fruits picked 9 days after the third application). The picked fruits were immediately transported to the laboratory in refrigerated conditions. During postharvest analysis, fruits of each treatment was divided at random in 2 groups and stored at room temperature (22 °C) with 60% of relative humidity. The first group was analyzed for fruit quality (firmness, SSC/TA ratio, color) and chemical (phenolics, antioxidants, anthocyanin and lignin) parameters. The second group was evaluated for fungal decay by means of visual inspection (mycelial growth). All the analyses were performed at time intervals of 0, 24, 48 and 72 h of postharvest storage. For storage one layer of fruits was packaged in perforated plastic clamshell containers (12.5 cm width × 12.5 cm depth × 4.5 cm height). For the different analyses in each sampling time point per treatment 12 fruits were employed.

2.2. Fruit quality assessments

Fruits from each treatment were weighed, and the firmness and color of the fruits were recorded. Skin color was measured using a colorimeter (model CR-400, Konica-Minolta) and expressed as CIELAB scale (L^*, a^*, b^*) along with the dimensions of color chroma (C^*) and hue angle (h°). Firmness was measured using a fruit hardness tester (model A6510030, Veto, Santiago, Chile) expressing the results in Newton (N). For color and firmness determination, two measurements on each equatorial side of four fruits of each of the three replicates were performed. After the firmness measurements were performed, the fruits were cut into pieces, frozen in liquid nitrogen and stored at −80 °C until use. A bulk tissue sample was prepared from each replicate for other analyses.

For the analysis of soluble solids content (SSC) and titratable acidity (TA), three replicates of 2 g of frozen tissue from each treatment was ground in liquid nitrogen, homogenized with 5 ml distilled water and filtered using a miracloth. SSC was determined in the juice at 20 °C using a digital refractometer (Atago, Tokyo, Japan). TA was determined by diluting the remaining juice with distilled water (1/10, v/v), and then titrating to pH 8.2 using 0.05 N NaOH. The results were expressed as SSC/TA ratio.

2.3. Chemical assessments

2.3.1. Antioxidant capacity and total phenolic content determination

For the determination of total phenolic content and antioxidant capacity, frozen tissue (1.5 g) was ground in liquid nitrogen, homogenized with 10 ml of methanol and centrifuged for 20 min at 5000 rpm. The extracts were then filtered through a miracloth and stored at −20 °C until use. Total phenolic content was measured using the Folin–Ciocalteau reagent according to the method of Singleton and Rossi (1965), using gallic acid as the standard. Folin–Ciocalteau reagent (250 µl) was gently mixed with 3.75 ml of distilled water containing 500 µl of the extract, and 500 µl of 10% (w/v) Na_2CO_3. The samples were incubated at room temperature in darkness for 1 h and then measured at 765 nm. The results of three replicates were expressed as micrograms gallic acid equivalents (GAE), in the methanol extract, per gram of fresh weight (FW).

Determination of antioxidant capacity was performed according to Benzie and Strain (1996), considering the ferric reducing ability of plasma (FRAP) as a measure of antioxidant capacity. Three ml of FRAP reagent were mixed with 100 µl of fruit extract previously diluted in methanol (1/5, v/v). The samples were incubated at room temperature for 6 min and then measured at 593 nm. The results of three replicates were expressed as nmol $FeSO_4{\cdot}7H_2O$ equivalent per gram of FW.

2.3.2. Anthocyanin and lignin determination

Anthocyanin quantification was performed according to Lee, Durst, and Wrolstad (2005) with some modifications. Fruit skin without achenes (0.2 g) was ground with liquid nitrogen, homogenized in 1.5 ml of methanol/HCl (99/1, v/v) and centrifuged for 10 min at 12,000 rpm at 4 °C. The samples were then diluted (1/3, v/v) in methanol/HCl (99/1, v/v) and then measured at 515 nm. The results of three replicates were expressed as micrograms cyanidin 3-glucoside equivalent per gram of fruit skin.

The method of Yeh, Huang, Hoffmann, Mayershofer, and Schwab (2014) was used for lignin quantification. Briefly, at first a cell wall from 250 mg of frozen powder (without achenes) was prepared according to the method of Meyer, Shirley, Cusumano, Bell-Lelong, and Chapple (1998), and Franke et al. (2002). Afterwards, the lignin content was determined by thioglycolic-acid assay. Each sample was mixed in 750 µl of distilled water, 250 µl of 37% HCl, and 100 µl of thioglycolic acid (Sigma–Aldrich) and incubated at 80 °C for 3 h. Subsequent steps were described by Campbell and Ellis (1992). Finally, the insoluble lignin was dissolved in 1 ml of 1 M NaOH and the absorbance was noted at 280 nm. The amount of lignin was calculated from a linear calibration curve (0–20 µg) with hydrolytic lignin (Sigma–Aldrich). The results of three replicates were expressed as micrograms lignin per gram of FW.

2.4. Fruit decay

The fruit decay was visually evaluated during the storage time. Strawberries that showed any sign of surface mycelia development were considered decayed. Decay incidence was expressed as a percentage of infected strawberries as previously reported by Wang and Gao (2013).

2.5. Statistical analysis

The entire experiment was conducted using a complete randomized factorial design, with the main factors being the treatment (control, MeJA and chitosan) and sampling hours (0, 24, 48 and 72 h). Data was analyzed by ANOVA using SAS/STAT (version

9.2) software and significant differences were determined at $P \leqslant 0.05$ (LSD test).

3. Results and discussion

3.1. Effect of MeJA and chitosan on fruit quality parameters

Loss of texture is one of the main factors limiting quality and the postharvest shelf life in the Chilean strawberry fruit (Figueroa et al., 2012). In general, the postharvest fruit firmness increased considerably in both chitosan- and MeJA-treated fruits when compared to control (Fig. 1A and B). In the case of fruits from the first harvest, chitosan seems to have a more pronounced effect than MeJA except at 48 h of postharvest storage (Fig. 1A). However, considering the fruits of second harvest, the MeJA-treated fruits showed higher fruit firmness than chitosan, especially at 24 and 48 h of postharvest storage (Fig. 1B). In agreement with our results, Rudell et al. (2005) reported that the fruit firmness increased with MeJA treatment in apple. Changes in fruit firmness can be the result of changes in cell wall properties. In this respect, it should be noted that the increment in lignin content observed in MeJA-treated fruits (Fig. 3C and D) could also be related to increase in fruit firmness. On the other hand, a down regulation of genes coding for pectinases (polygalacturonase and pectin methylesterase) was observed under MeJA treatment during an *in vitro* ripening assay in *F. chiloensis* (Concha et al., 2013). Following the same pattern as that of MeJA, chitosan has also been reported as a beneficial compound for the strawberry fruit firmness. Hernández-Muñoz, Almenar, Ocio, and Gavara (2006) found that chitosan coatings markedly slowed the fruit ripening by their retention of firmness proposing that the high percentage of water loss by uncoated versus chitosan samples could contribute to firmness differences.

It has been reported that color changes during ripening are not so pronounced in Chilean strawberry fruit (Figueroa et al., 2008, 2010). However, there are cases, where the MeJA treatment accelerated the acquisition of red color during an *in vitro* ripening assay in *F. chiloensis* fruit (Concha et al., 2013). No significant differences were found when surface color of treated and control fruits were evaluated during the storage time periods of the present study, except for some specific points in MeJA-treated fruits at the start of the storage time (0 h), when they presented a higher value of a^* than control (data not shown). In the case of chitosan, there were no differences in any of the storage time periods and harvests (data not shown). Vargas, Albors, Chiralt, and González-Martínez (2006) also found no significant differences in external color changes by the application of chitosan and oleic acid on cold stored-strawberries.

In terms of SSC/TA ratio, MeJA-treated fruits showed highest value at 24 h of storage in the first harvest, however, under chitosan treatment, SSC/TA ratio decreased except at 48 h when compared to control (Fig. 1C). In the case of second harvest, both treatments did not exhibit any differences in SSC/TA ratio when compared to control (Fig. 1D). The effects of preharvest application of MeJA on blackberry showed a higher SSC/TA ratio than untreated-control fruits (Wang, Bowman, & Ding, 2008). Similarly, Concha et al. (2013) reported that MeJA accelerated fruit ripening of *F. chiloensis* fruit by means of a transitory increase in the SSC/TA ratio in an *in vitro* ripening assay. Gol et al. (2013) reported that the addition of chitosan to the coating formulations had a strong effect in maintaining lower SSC values compared with control at the end of the storage period. The authors ascribed the

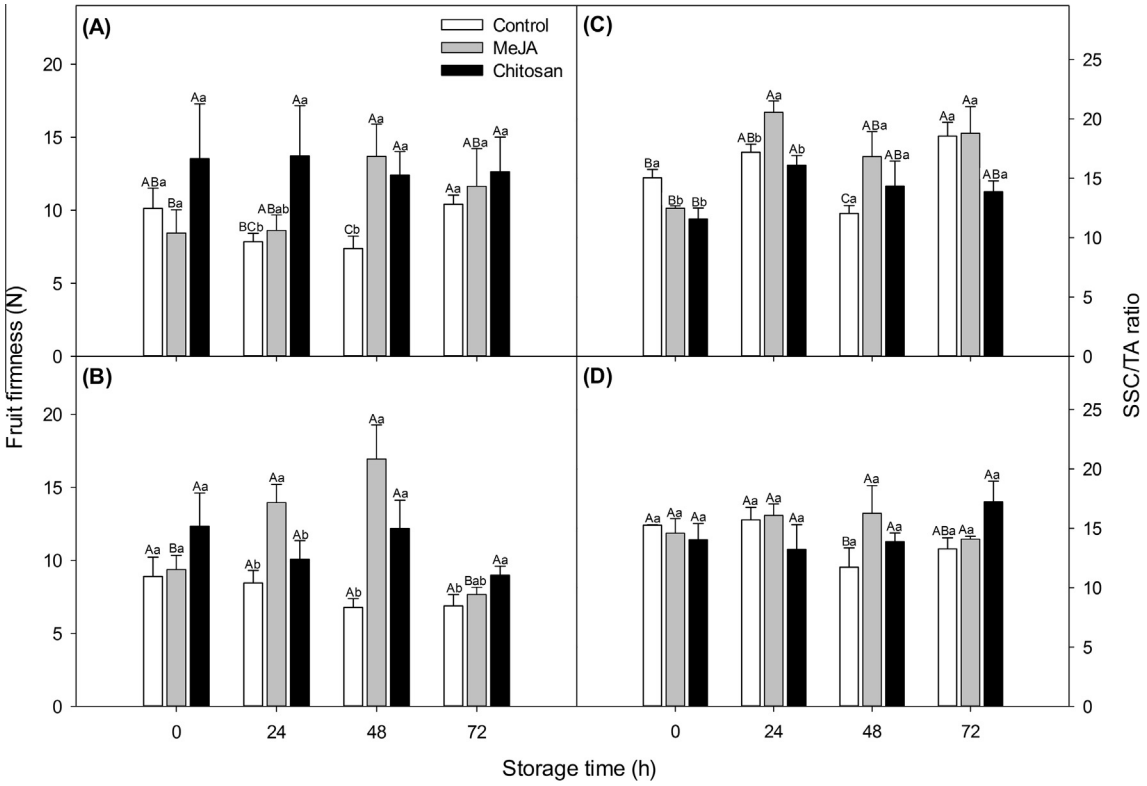

Fig. 1. Changes in firmness (A, B) and SCC/TA ratio (C, D) of *F. chiloensis* fruits treated with MeJA, chitosan, and water (control) during preharvest. Fruits were picked and analyzed immediately after the third application (first harvest, A and C) and 9 days after the third application (second harvest, B and D) during 72 h of postharvest storage. Treatments and analyses details are described in Section 2. For each treatment, different capital letters indicate significant differences between the storage time points ($P \leqslant 0.05$). For each storage time point, different lower-case letters indicate differences between the treatments ($P \leqslant 0.05$). Data correspond to the mean ± SE of three independent replicates.

probable reasons for the low levels of SSC accumulation in the chitosan-coated fruits to the slowing down of the respiration and metabolic activity, and hence retarding the ripening process. Application of chitosan in liquid form adopted in the present study had a smaller effect in retarding the strawberry fruit ripening and senescence processes than the direct chitosan coatings.

3.2. Effect of MeJA and chitosan on the chemical properties of fruit

3.2.1. Total phenolic content and antioxidant capacity

Changes in total phenolic content and antioxidant capacity of treated fruits were shown in Fig. 2. Significantly higher total phenolic contents were not observed in both treatments during the first and second harvest, except for chitosan-treated fruits at 24 h of storage in the first harvest (Fig. 2A and B). Concomitant with our results, González-Aguilar, Tiznado-Hernández, Zavaleta-Gatica, and Martínez-Téllez (2004) reported that total phenols were not affected by the MeJA treatment in guava fruit. In second harvest, it is remarkable to note that while the GAE values decreased in the control over time, they increased and remained stable in chitosan- and MeJA-treated fruits, respectively (Fig. 2B). The incorporation of chitosan to tomatoes and strawberries promoted higher contents of total phenols in the fruits (Badawy & Rabea, 2009; Gol et al., 2013; Wang & Gao, 2013).

The antioxidant capacity of fruits treated with chitosan and MeJA showed a decreasing trend up to 24 h and 48 h, respectively, and then showed a significant increase at 72 h in the case of postharvest storage of fruits of first harvest (Fig. 2C). Specifically, chitosan-treated fruits showed significantly higher antioxidant capacity than the control at 72 h of storage in the first harvest. In the case of fruits of second harvest, MeJA treatment could enhance the antioxidant capacity at 48 and 72 h (Fig. 2D). It has been reported that loquat fruits treated with MeJA, showed a significantly higher superoxide radical scavenging activity during storage compared to the control fruit (Cao, Zheng, Yang, Wang, & Rui, 2009). In this particular study, the harvested fruits were kept in contact with MeJA solution chamber. Probably field applications of MeJA, as in our present study, are less effective for an increased antioxidant capacity of strawberry fruits during postharvest storage.

3.2.2. Anthocyanin and lignin content

The effect of both treatments on anthocyanin content did not show a steady pattern during the first harvest period (Fig. 3A). Regarding the second harvest, both treatments significantly enhanced the total anthocyanin content at all the time points tested in comparison to the control (Fig. 3B). In *F. ×ananassa* (cv. Camarosa), a similar effect had been found with MeJA treatment during *in vitro* ripening, where the total anthocyanin content was significantly higher in treated than non-treated fruits (Pérez, Sanz, Olías, & Olías, 1997). Similarly, Concha et al. (2013) reported a clear increase in anthocyanin accumulation and its related gene expression under 100 μM MeJA treatment in *F. chiloensis* fruit. In the case of chitosan, previous reports indicated that this polymer could enhance the anthocyanin accumulation. Gol et al. (2013) demonstrated that during 8 days of storage coated-strawberries had a higher accumulation of anthocyanins than uncoated ones.

A constant increment of lignin content was observed in MeJA-treated fruits from 24 to 72 h of storage time (Fig. 3C) probably through the up-regulation of lignin biosynthesis genes as has been recently described (Concha et al., 2013). Both MeJA and chitosan treatments significantly increased the lignin content of strawberry fruits at time points from 24 to 48 h compared to the control in the second harvest (Fig. 3D), suggesting a long-term

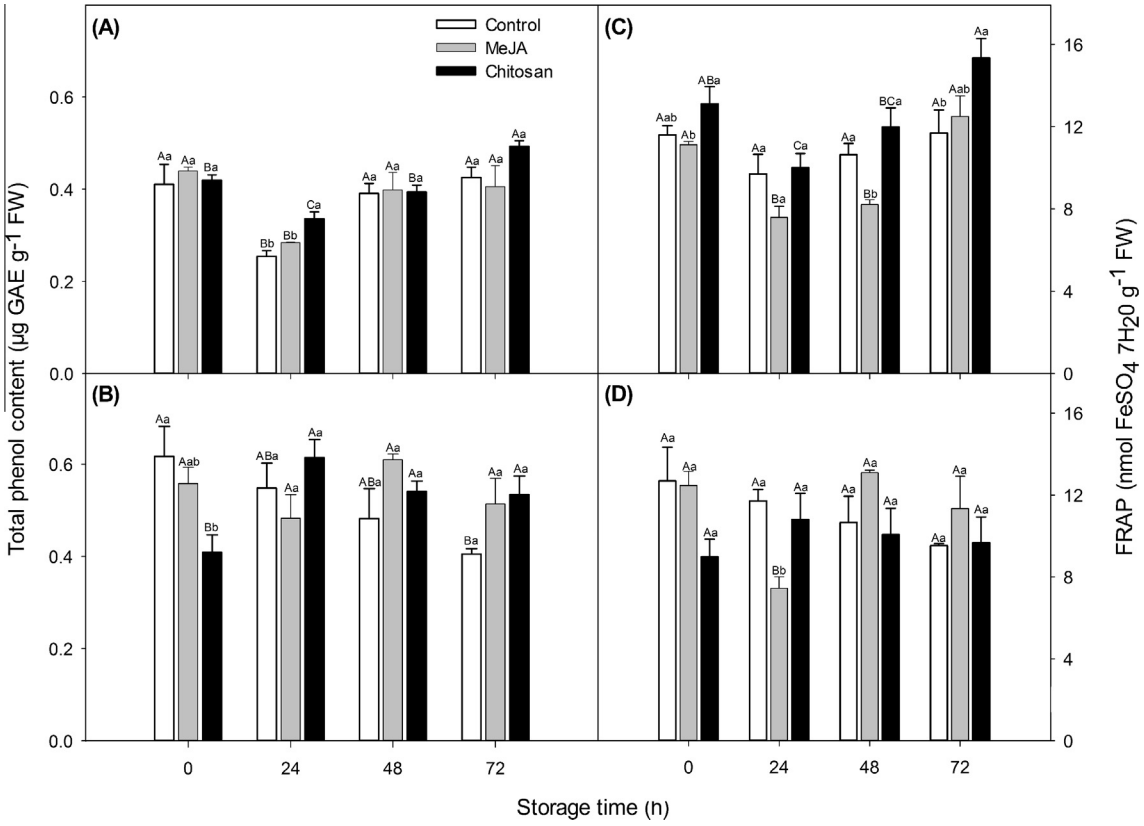

Fig. 2. Changes in total phenol content (A, B) and antioxidant capacity (C, D) of *F. chiloensis* fruit treated with MeJA, chitosan, and water (control) during preharvest. Legend as in Fig. 1.

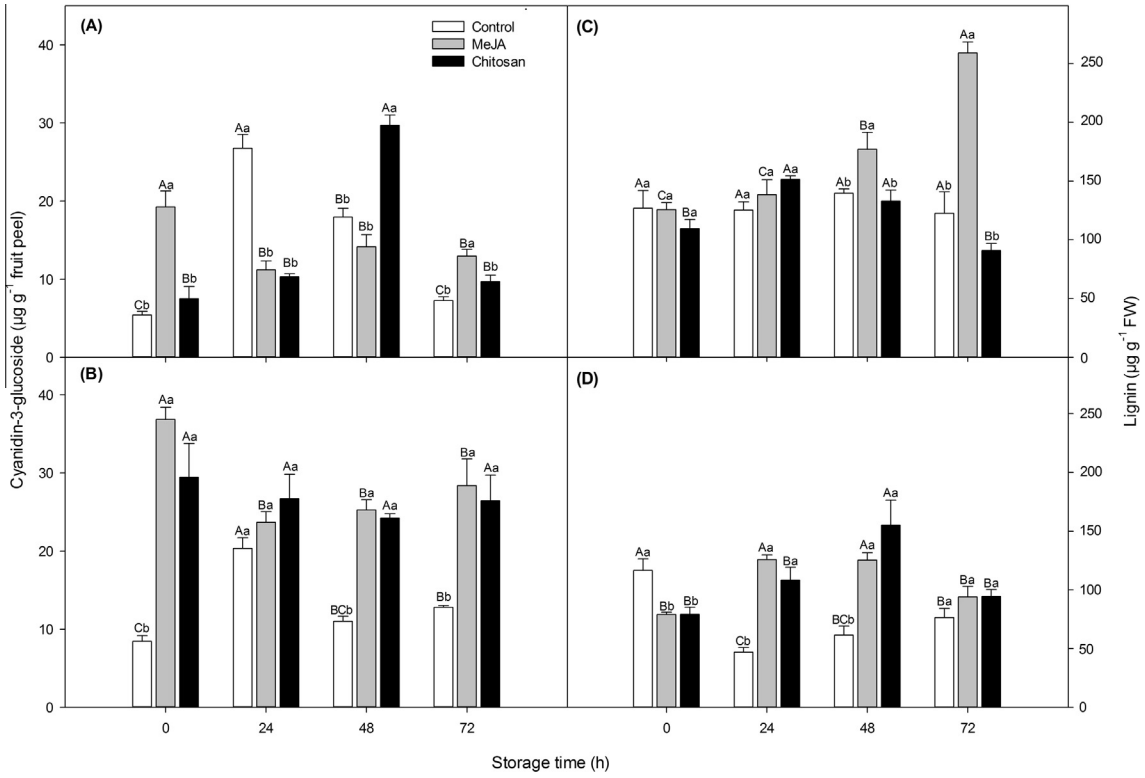

Fig. 3. Changes in anthocyanin (A, B) and lignin contents (C, D) of *F. chiloensis* fruit treated with MeJA, chitosan, and water (control) during preharvest. Legend as in Fig. 1.

effect of both elicitors. As far as we know, no effects of chitosan in lignin formation have been reported.

3.3. Effect of MeJA and chitosan on fruit decay

The effect of MeJA and chitosan on fruit decay incidences during postharvest storage was presented in Table 1. An important finding of our study is that only the control fruits showed signs of decay at 24, 48, and 72 h in the first harvest, suggesting that MeJA and chitosan are effective treatments against naturally occurring pathogens of Chilean strawberry fruit. The fruits of the second harvest show decay incidence under chitosan and MeJA treatments at 48 and 72 h, respectively, indicating a possible loss of the long-term effect of these compounds against postharvest pathogens. Different studies have demonstrated that chitosan had the potential to inhibit decay and hence prolong the storage life of several species, including strawberries (Romanazzi, Feliziani, Santini, & Landi, 2013). In concomitant with our results, it has been reported that MeJA applications inhibit gray mold infection in strawberries (Moline, Buta, Saftner, & Maas, 1997) and reduced postharvest decay in guava fruit (González-Aguilar et al., 2004).

Table 1
Effect of preharvest applications of MeJA and chitosan on decay incidence of *F. chiloensis* fruit during postharvest storage.

Harvest	Treatment	Storage time (h)			
		0	24	48	72
1st	Control	–	+	+	+
	MeJA	–	–	–	–
	Chitosan	–	–	–	–
2nd	Control	–	–	++	+++
	MeJA	–	–	–	++
	Chitosan	–	–	++	++++

Visual evaluation (–: absence; +, ++, +++, ++++: 0–25%, 25–50%, 50–75%, or 75–100% of fruit infected, respectively). Treatments and analysis details are described in Section 2.

4. Conclusions

The findings of the present study have shown that, in general, MeJA and chitosan applications have a positive effect on postharvest quality and reduced decay incidences in Chilean strawberry fruit. Treated fruits maintained higher levels of firmness, anthocyanin and lignin contents during postharvest storage suggesting a residual effect and activation of the phenylpropanoid pathway by both MeJA and chitosan. Moreover, both elicitors showed preserving functions against decay in fruits of the first harvest, showing no incidence of decay till 72 h at 20 °C. These results suggest that MeJA and chitosan applications could be used as favorable treatments to extend shelf life of *F. chiloensis* fruits and represent a promising alternative as an environment-friendly compounds to be used in the complementation or partial substitution of the chemical fungicides presently practiced in this crop and others.

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