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#### Research Paper



## Postharvest application of Methyl Jasmonate to extend shelf-life on yellow kiwifruit (Actinidia chinensis cv. Jinyan)

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#### ABSTRACT

Methyl Jasmonate (MeJA) is a naturally occurring plant hormone derived from jasmonic acid which may play a pivotal role in regulating various physiological processes, including plant defence mechanisms, fruit ripening and senescence, as well as responses to biotic and abiotic stress factors. This study investigates the effects of MeJA treatments at two different concentrations (0.01 mM and 0.1 mM) on the post-harvest of yellow kiwifruit storage for 28 days at  $20^{\circ}$ C. Treatment with 0.1 mM methyl jasmonate (MeJA) significantly delayed fruit ripening during storage. This effect was associated with the suppression of ethylene production and a concomitant increase in  $CO_2$  release. As a result, fruits treated with the highest MeJA concentration exhibited significant differences in pulp color parameters (L\*, a\*, b\*, and  $\Delta E$ ) and firmness compared to the control, maintaining their quality traits for up to 21 days of storage. These findings were further supported by hedonic analysis, which confirmed acceptable marketability of the fruits for up to 28 days of shelf life. In contrast, the 0.01 mM MeJA treatment resulted in ripening behavior similar to that of the control group, indicating limited efficacy at this concentration in delaying senescence. Nevertheless, the lower concentration led to a significant increase in polyphenol production, which contributed to the inhibition of postharvest pathogens such as *Botrytis cinerea and Aspergillus niger*.

In conclusion, this study demonstrates that different concentrations of MeJA can distinctly influence both fruit ripening and the control of major postharvest diseases, highlighting its potential application in postharvest management strategies.

#### 1. Introduction

Commercial kiwifruit primarily comes from cultivars of *Actinidia chinensis:* A. chinensis var. chinensis (yellow-flesh kiwifruit) and A. chinensis var. deliciosa (green flesh kiwifruit) (previously known as A. deliciosa C.F. Liang et A.R. Ferguson). Green kiwifruit genotypes, namely Hayward, have traditionally led the global market, but yellow ones are gaining popularity due to its colour, milder and less acidic banana-like flavour and rich nutritional profile (Asadi et al., 2024). However, one of the main challenges in yellow kiwifruit production is their postharvest shelf life. Kiwifruit are classified as climacteric fruit due to the significant role of ethylene in the ripening and softening processes (Crisosto and Crisosto, 2001). Commercial maturity of yellow kiwifruit occurs when TSS are > 8 °Brix and fruit dry matter is  $\geq$  16,5 %, at this stage the ethylene production is still relatively low (Mitalo et al.,

2019). Thus, the subsequent postharvest changes of fruit are dependent on both the physiological state of the fruit at harvest and the postharvest management applied. Storage of green kiwifruit in a normal atmosphere does not exceed 90–120 days, while controlled atmosphere storage, at 0.5°C can extend their shelf life up to six months (Huang, 2016). The fruit is also highly sensitive to cold temperatures, and the exposure to temperatures below 0°C can cause chilling injury, negatively affecting flavour and flesh texture. To slow down ripening and extend storage life, 1-Methylcyclopropene (1-MCP), has been used, as an ethylene inhibitor (Cantin et al., 2011; Huang et al., 2019). Ethylene is a key hormone in fruit ripening, and its suppression helps maintain fruit firmness and delay senescence. In addition to 1-MCP, elicitors such as Methyl Jasmonate (MeJA), a naturally occurring plant hormone derived from jasmonic acid (JA), has been gainig a growing attention, over the past few decades, in the food industry due to their potential in improving fruit

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quality, extending shelf life, and enhancing postharvest performance, particularly in fresh-cut fruit markets (Muengkaew et al., 2016). Methyl jasmonate (MeJA) is recognized for inducing oxidative stress and promoting the accumulation of secondary metabolites within plant cells (Ho, et al., 2020). MeJA application in in plant has been shown to trigger the release of volatile compounds and alter lipid and carbohydrate levels in plants. The effect of exogenous MeJA can strengthen plants' innate resistance against pathogen attacks and abiotic stresses such as cold temperatures (Wang et al., 2021). MeJA plays a pivotal role in regulating physiological processes such as fruit ripening, senescence, and responses to biotic and abiotic stress factors (Xie et al., 2024; Wang et al., 2021). It has been, also, identified as a key signaling molecule in regulating fruit defence responses to various environmental stressors (Scognamiglio et al., 2012). Han et al. (2019) reported that MeJA application accelerated the ripening process in strawberries, while Wei et al. (2017) demonstrated that exogenous MeJA treatment promoted anthocyanin accumulation in peaches and Saracoglu et al. (2017) reported a delayed ripening and improved quality in sweet cherry. In addition to its role in ripening, MeJA enhances the sensory attributes of fruits and extends postharvest longevity by triggering the synthesis of secondary metabolites such as phenolic compounds and antioxidants (Yang et al., 2025), which contribute to fruit flavor, aroma, and nutritional quality (Wang et al., 2021). Eventually, MeJA has been shown to delay fruit senescence, which is essential for reducing postharvest losses and maintaining fruit quality during transportation and storage. Li et al. (2023) demonstrated that the combination of MeJA application and low storage temperatures significantly reduced chilling injury in papaya, enhancing its antioxidant activity and overall quality. Similar result has been observed in Japanese plums (Khan and Singh, 2007), where MeJA played a crucial role in ethylene synthesis and fruit preservation. In kiwifruit, Pan et al. (2020) found that postharvest MeJA treatment enhanced resistance to soft rot, while Niu et al. (2023) reported that combining MeJA with salicylic acid improved the storage quality of green-fleshed 'Hayward' kiwifruit. However, the effect of MeJA on ethylene production is concentration-dependent. Wu et al. (2020) observed that applying 100 µM/L of MeJA for 12 hours on 'Hayward' kiwifruit accelerated ethylene production when exposed to exogenous ethylene, due to preferential activation of AdACS1 and AdACS2. Beyond its role in ripening regulation, MeJA has also shown antifungal properties. Cao et al. (2008) reported that MeJA reduced anthracnose rot caused by Colletotrichum acutatum in loquat fruit, while Wang et al. (2015) demonstrated its effectiveness against Penicillium citrinum in Chinese bayberry. Pan et al. (2020) further reported that MeJA reduced postharvest fruit rot in green kiwifruit caused by Botryosphaeria dothidea. Given its potential in regulating postharvest physiology and disease resistance, this study aims to (i) investigate the postharvest application of two different concentrations of MeJA (0.1 mM and 0.01 mM) to extend shelf life of yellow kiwifruit cv. "Jinyan" and (ii) evaluate the effect of MeJA, on fruit rot development caused by Aspergillus niger and Botrytis cinerea, in artificially inoculated fruit.

#### 2. Materials and methods

The research was conducted during the summer season in 2023, on yellow kiwifruit (*Actinidia chinensis* cv. "Jinyan") harvested manually from 12 trees from a commercial orchard located in Rosarno,(38° 29' N; longitude 15° 58' E; Calabria, Italy), when the fruit had reached harvest maturity (6–7 °Brix). Soil was a sandy clay loam with 7.0 pH, 2.2 % organic matter and 1.7 g kg $^{-1}$  N content. The vines were spaced 5.0 m  $\times$  4.0 m apart (500 vines ha $^{-1}$ ), and interspecific grafting was implemented in 2014 using a scion of *A. chinensis*, cv. "Jinyan", grafted onto *Actinidia deliciosa* (*A. Chev.*), cv. *Hayward*. Vines were trained in a pergola system. The orchard was drip irrigated and managed using the standard integrated pest management tecniques.

A total of 240 kiwifruit, uniform in color and size and free from any visual defects or chemical damage, were carefully graded and selected.

Immediately after harvest, they were transported to the laboratory at the Università degli Studi di Palermo. 30 kiwifruit were selected at random and used to analyse fruit properties at harvest time (day 0). To check the effect of MeJA, 3 treatments (0.1; 0.01 mM MeJA and untreated fruit as control), were applied as soon as the fruit reached the laboratories, 1 day after being harvested. Methyl Jasmonate (Sigma Aldrich, Germany) at 0.01 mM and 0.1 mM concentrations, was diluted in distilled water containing 1 % of Tween 20 and ethanol, respectively. After being immersed for 10 min., treated fruit were left to dry at 20°C for 1 h of relative humidity (RH). Control kiwifruit were immersed in distilled water for 10 min. All fruit were then stored for 28 days at 20  $\pm$  1°C and 85 %.

Fruit quality parameters were measured at 0, 7, 14, 21, 28 d after harvest storage. Respiration rate and ethylene production were observed every two days for 14 days.14 days after the storage, 20 fruits of each treatment were inoculated with *Aspergillus niger* and other 20 with *Botrytis cinerea* using conidial suspension  $(8.0\times10^5 \text{ spores/mL})$ . The induction effect was evaluated for two weeks (on the  $21^{nd}$  and  $28^{th}$  d), after inoculation.

For each treatment and the control 120 kiwifruit were selected (3 treatment x 10 kiwifruit x 4 time of storage + 60 kiwifruit for inoculation essay) and evaluated on the  $21^{nd}$  and  $28^{th}$  d. Moreover, 30 fruit (10 x treatments) for respiration rate and ethylene production were used.

#### 2.1. Respiration rate and ethylene production

Respiration rate and ethylene production were measured at day 0 and every two days during 14 days of storage. CO2 and ethylene production were determined individually on 10 yellow kiwifruit placed in a 0.5 L glass bag for 60 min using the static method. After that, 1 mL of the atmosphere sample was taken in duplicate with a syringe from the headspace as previously described by Ruíz-Aracil et al. (2023). Carbon dioxide was quantified using a gas chromatograph (Shimadzu 14B, Shimadzu Europa GmbH, Duisburg, Germany), and ethylene production was measured using a Shimadzu GC 2010 gas chromatograph. For CO<sub>2</sub> evaluation, the instrument contained a catarometric detector and a 3 m stainless steel column with an inner diameter of 3.3 mM filled with chromosorb 102. The column was maintained at a temperature of 55°C, and the injector and detector were set at  $110^{\circ}$  C. For ethylene production determination, the instrument was equipped with a flame-ionization detector and a 3 m stainless steel column (inner diameter of 3.5 mm) packed with activated alumina of 80/100 mesh. The column was kept at 70°C, with the injector and detector maintained at 110°C.

The respiration rate and ethylene production were expressed as mg of  $CO_2$  kg $^{-1}$  h $^{-1}$  and nL g $^{-1}$  h $^{-1}$ , respectively and were the mean  $\pm$  standard error (SE).

#### 2.2. Fruit quality parameters

## 2.2.1. Weigh loss, firmness, total soluble solids content and titratable acidity

Individual fresh weight was recorded immediately after the treatment (day 0) and at the different sampling times (7, 14, 21 and 28 d during shelf-life). Weight loss was expressed as the percentage reduction with respect to initial time, using the following equation: % Weight loss = [(Initial fruit weight-Final fruit weight) x 100]/Initial fruit weight. At each sampling day (0, 7, 14, 21 and 28 days) flesh firmness was determined using TR 5325 digital penetrometer (Turoni, Forlì, Italy) with a 8 mM diameter stainless steel probe. Results were expressed as kg cm $^{-2}$  and were the mean  $\pm$  SE. Total soluble solids content (TSS, expressed as  $^{\circ}$ Brix) was determined from the juice of each kiwifruit from each tray using a digital refractometer (model PR-101, Atago, Co., Tokyo, Japan) at 20 $^{\circ}$ C. Titratable acidity (expressed as % citric acid) was determined by titration of 10 mL of juice with 0.1 M NaOH to an end point of pH 8.1.

#### 2.2.2. Color and visual appearance score

The flesh color (10 fruits x 3 replicates for each treatment and sampling date) was measured with a portable colorimeter (Minolta CM2500R, Minolta, Osaka, Japan), equipped with an 8 mm measuring head and a C illuminant (6774 K). The instrument was calibrated using the manufacturer's standard white plate. Color changes were quantified in L\*, a\* and b\* color space and  $\Delta E^*$  was calculated as described in previous work (Sortino et al., 2022a). Visual quality (color, structural integrity and appearance) was evaluated on the 30 yellow kiwi fruit after harvest (T0) and 10 replicates for treatment (CTR, 0.1mM and 0.01 mM of MeJA) on the 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>th</sup> and 28<sup>th</sup> days of shelf-life. The sensory analysis was conducted at laboratory of the Università degli Studi di Palermo. Ten panelists (5 males and 5 females), with an average age of 45 years, were recruited from the campus and surrounding community. Panelists were selected based on having previously consumed yellow kiwifruit, having no known allergies to kiwi, and being available to attend all training and tasting sessions (Di Miceli et al., 2010). Visual appearance score resulted from the medium value of color, visible structural integrity and visual appearance. The different descriptors were quantified using a subjective 5-1 rating scale with 5= very good, 4 =good, 3= sufficient (limit of marketability), 2= poor (limit of usability) and 1= very poor (inedible) (Sortino et al., 2022b).

#### 2.3. Polyphenols, carotenoids and DPPH content

Total phenol content analysis was determined according to the Singleton and Rossi (1965) method using the Folin-Ciocalteau reagent (FC) and the gallic acid as a standard. The FC reagent relies on the transfer of electrons in alkaline medium from phenolic compounds to phosphomolybdic/phosphotungstic acid complexes, which are determined spectrophotometrically at 700 nm. For that, 5 grams of yellow kiwifruit fresh tissue for each replicate was homogenized with methanol (1:10, w/v). After filtration through a Whatman grade N. 1 filter paper, methanolic extracts were concentrated under reduced pressure and the residue was suspended in 50 % (v/v) aqueous methanol and used for phenolic content assay. Results were expressed, as mg of gallic acid equivalent (GAE) kg<sup>-1</sup> of fresh weight (FW). The antioxidant activity was determined by the following methods Brand-Williams, Cuvelier, and Berset, 1995. Frozen yellow kiwifruit samples (6.0 g) were homogenized with 15 mL of 50 % methanol and centrifuged at 10,000 x g for 20 min at 4°C. The supernatant was collected for 2,2-Diphenyl-1-picrylhydrazyl (DPPH) analysis. A calibration curve was prepared, using Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) and the results were expressed as mM TEAC kg<sup>-1</sup> FW.

Total carotenoids were measured on 5 g of flesh obtained from each fruit previously ground to a fine powder under liquid nitrogen were mixed for 20 min with 50 mL of extracting solvent (hexane/acetone/ethanol, 50:25:25, v/v). The organic phase containing carotenoids was recovered and then used for analyses after suitable dilution with hexane. Total carotenoid determination was carried out on an aliquot of the hexane extract by measuring absorbance at 450 nm. Results were expressed as mg  $kg^{-1}$  FW.

#### 2.4. Botrytis cinerea and Aspergillius niger inoculation

Botrytis cinerea and Aspergillus niger strains used in this study have been previously isolated in kiwifruit and collected in the Laboratory of the Università degli Studi di Palermo, by the Authors (i.e. Livio Torta). For the inoculation, the surface of all the fruits was sterilized with 75 % (v/v) ethanol and wounded by a sterile needle in two different point. 14 days after the storage, 20 fruits of each treatment were inoculated with *A. niger* and other 20 with *B. cinerea* using conidial suspension at the concentration of  $8.0 \times 10^5$  conidia/mL, counted by a Thomahaemocytometer (Carl Roth, Karlsruhae, Germany) following the manufacture's protocol. After inoculation, fruit were placed for 24 h in plastic bags, such as in a moist chamber (R.H. near 100 %). Both fungal

strains were isolated from naturally infected grapes. For each fruit, the infection development was evaluated after 7 days, measuring the diametral size of the fruit rot in correspondence of the infected wounds by a Vernier gauge using the cross method. To detect eventual differences in the development of the rot symptoms, the following formula (Pan et al., 2020) was used:

$$(\mathit{Mr}) = \left(\frac{\mathit{M}1-\mathit{M}2}{\mathit{M}1}\right)*100$$

where: Mr = reduction of diametral length of rot [ %], M1 = diametral length of rot on untreated fruit [cm], M2 = diametral length of rot on treated fruit [cm].

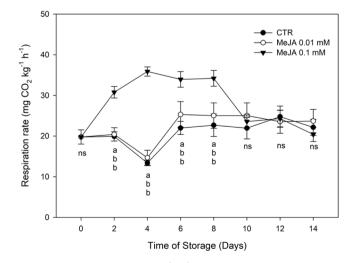
#### 3. Statistical analysis

The experimental design consisted of 3 treatments (0.1, 0.01 mM MeJA and control), with observations made at 7, 14, 21, 28 d after harvest. Respiration rate and ethylene production were observed every two days for 14 days while induction effect was evaluated for two weeks, after inoculation on the 14<sup>th</sup> day of shelf-life. Analysis of variance was applied to data collected. Systat 13.0 for Windows was used as statistical software. Significant differences ( $P \le 0.05$ ) between treatments were evaluated with Tukey's test (n = 10).

#### 4. Results and discussion

#### 4.1. Respiration rate and Ethylene production

Postharvest ripening of kiwifruit involves several key physiological events, which have been delineated in the context of four distinct softening phases (Schröder and Atkinson, 2006). These changes occur in conjunction with ongoing respiration, leading to the degradation of chlorophyll and the catabolism of starch reserves (Wang et al., 2015). Our results showed that fruit respiration rate remained relatively constant in untreated (control) kiwifruit and those treated with the lowest MeJA concentration (0.01 mM) throughout the storage period, with no significant differences observed between both treatments (Fig. 1). In contrast, kiwifruit treated with 0.1 mM MeJA exhibited a sharp increase in respiration rate immediately after treatment application, which persisted until 8 days after storage, showing the highest respiration rate values compared to the other treatments. Subsequently, the respiration rate in 0.1 mM MeJA treated fruit significantly slowed down and no



**Fig. 1.** Respiration rate (mg CO $_2$  kg $^{-1}$  h $^{-1}$ ) of treated (0.1 and 0.01 mM MeJA) and untreated (CTR) yellow kiwifruit (A. chinensis cv 'Jinyan') after 7, 14, 21, 28 days of storage at 20°C. At each sampling date, ns indicate no changes between treatments. p  $\leq$  0.05 at the Tukey's test. Data are means  $\pm$  S.E. (n = 10).

significant differences were observed between the three treatments after 10 days of storage (Fig. 1).

Despite these respiration rate values, ethylene production in kiwifruit treated with 0.1 mM MeJA was very low, being detected just at basal levels during 14 days of storage. In contrast, an increase in ethylene production was observed from 4 days after harvest onwards in kiwifruit treated with 0.01 mM MeJA and from 6 days after harvest in control fruit. No significant differences in ethylene production were observed between the untreated and 0.01 mM MeJA treatments from 6 to 14 days after treatment application (Fig. 2). In a previous study of characterization of kiwifruit cv. "Jinyan" it was observed that ethylene levels remained stable until a substantial increase was observed from 9 to 13 days after harvest (Lin et al., 2024). Similarly, in our study the highest values of ethylene were detected from day 10 until 14, but only for control and kiwifruit treated with the lowest concentration of MeJA (0.01 mM). The differences observed between treatments are in agreement with the results reported in the literature since it has been shown that the effects of exogenous jasmonic acid (JA) or methyl jasmonate (MeJA) application on ripening parameters may change with genotype, concentration applied, and developmental stage of the fruit during treatment (Ziosi et al., 2008). Different studies have showed that jasmonate could accelerate ripening of apple (Li et al., 2017), Japanese plum (Khan and Singh, 2007) and strawberry (Concha et al., 2013), while delayed the ripening process in bayberry (Wang et al, 2010) and peach (Ziosi et al., 2008). Specifically, in a study conducted with 'Hayward' kiwifruit, the addition of MeJA enhanced ethylene production only when combined with exogenous ethylene application, suggesting that the modulatory effect of MeJA on kiwifruit ethylene production is dependent on the presence of an external trigger, such as ethylene itself (Wu et al., 2020). Niu et al. (2024) demonstrated that exogenous MeJA effectively lessened chilling injury in kiwifruit by inhibiting membrane lipid peroxidation and modulating genes related to ethylene biosynthesis, cell wall degradation, hormone signaling, and associated transcription factors.

The observed increase in respiration rate in 0.1 mM MeJA treated kiwifruit and the absence of significant concurrent ethylene production may be attributed to an increase of the metabolic activity after treatment application. Indeed, our data show a dose-dependent effect: 0.1 mM MeJA caused an early and sharp increase in respiration (up to 8 days) but kept ethylene at basal levels throughout the 14 days of monitoring, while 0.01 mM MeJA behaved similarly to control fruit in terms of respiration but allowed the normal climacteric ethylene increase (from day 4 onwards).

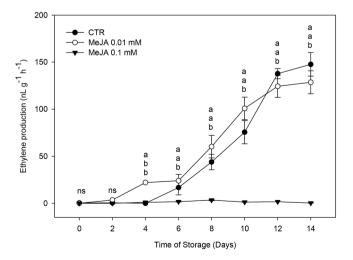


Fig. 2. Ethylene production (nL g $^{-1}$  h $^{-1}$ )of treated (0.1 and 0.01 mM MeJA) and untreated (CTR) yellow kiwifruit (A. chinensis cv 'Jinyan') after 7, 14, 21, 28 days of storage at 20°C. At each sampling date, ns indicate no changes between treatments. p  $\leq$  0.05 at the Tukey's test. Data are means  $\pm$  S.E. (n = 10).

This means MeJA at higher concentration (0.1 mM) can uncouple respiration from ethylene synthesis, stimulating metabolic activity without triggering the ethylene-dependent ripening cascade. Zhu et al. (2024) showed that treatment with 10  $\mu M$  MeJA helps maintain fruit quality and delays ripening in 'Xiahui 8' peaches by suppressing ethylene production. The authors detected that this effect is mediated through jasmonic acid (JA) signaling, which initially increases and then activates a negative feedback loop. As a result, JA signaling is down-regulated over time, leading to reduced expression of PpMYC2, lower ctivities of 1-aminocyclo-propane-1-carboxylate synthase (ACS) and 1-aminocyclopropane-1-carboxylate oxidase (ACO) enzyme activity, and decreased ethylene biosynthesis.

The relationship between respiration and ethylene evolution during ripening has been a subject of investigation, with some researchers suggesting that an increase in respiration and ethylene is anticipated after the climacteric period (Park et al., 2015; Vieira et al., 2010). On the other hand, a rise in ethylene production can occur either before or after the increase in respiration (Lim et al., 2016). In our work kiwifruit treated with MeJA treatment 0.1 mM could have stress-related signal that transiently enhances mitochondrial activity, increasing ATP demand and CO<sub>2</sub> release (Cheng et al., 2021). At the same time, high MeJA suppresses ethylene biosynthesis by downregulating ACS and ACO enzymes via JA signaling and negative feedback loops (Zhu et al., 2024). This could explain the apparent paradox of kiwifruit showing high respiration (energy use) but still with a reduced ethylene accumulation, resulting in a delayed ripening state.

#### 4.2. Fruit quality parameters

Weight loss (%) of untreated fruit was significatively faster and higher than MeJA treated ones during the first two weeks of shelf life (Fig. 3). On the other hand, the weight loss of all samples became faster and almost doubled during the last week of shelf life. At this stage untreated fruit had again significant higher values than MeJA treated ones (Fig. 3), indicating that MeJA application delayed and reduced significatively fruit water loss. This is consistent with Öztürk, and Yüceda, (2021) who showed a decrease in weight loss of *Hayward* kiwifruit soaked with 0.5 mM MeJA. Hence, MeJA application limits water loss in fruit cells during this process, ensuring that they remain fresh, preventing membrane peroxidation by combating free radicals (Baswal

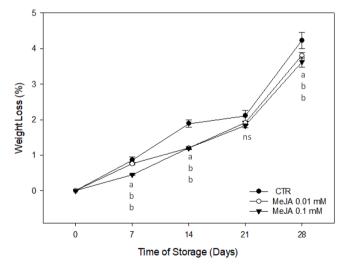


Fig. 3. Weight Loss ( %) of treated (0.1 and 0.01 mM MeJA) and untreated (CTR) yellow kiwifruit (A. chinensis cv 'Jinyan') after 7, 14, 21, 28 days of storage at 20°C. Different letters indicate significant differences between treatments at each sampling date. ns indicate no changes between treatments. p  $\leq 0.05$  was used in the Tukey's test. The data are provided as a mean  $\pm$  S.E. (n = 10).

et al., 2020) and thus reducing the rate of fruit deterioration (Pan et al., 2020). Similar evidence has been shown on mango (Gonzalez-Aguilar et al., 2000), apricot (Ezzat et al., 2017) lemon (Serna et al., 2021). Firmness is a key indicator of the ripening status, since the decline in softening is associated with the decrease in starch and TA and the increase of soluble sugars content and kiwifruit paleability. MeJA has been reported to delay flesh softening depending on concentration (Oztürk, and Yüceda, 2021); we also observed significant differences in firmness between control and MeJA treatments during the four weeks of shelf life, when untreated fruits showed the fastest and greatest decrease of flesh firmness (Fig. 4). On the other hand, the higher the concentration the significatively slower was the decrease of flesh firmness throughout the 4 weeks of shelf life (Fig. 4). During the first week after the treatment, 0.1 mM MeJA treated fruit did not show any significant change in flesh firmness. Yellow kiwifruit treated with 0.1 mM MeJA slowed softening by reducing activity of cell wall-degrading enzymes (polygalacturonase, pectin methylesterase), consistent with JA's role in modulating cell wall metabolism (Niu et al., 2023). However, at the end of the shelf life period, differences between 0.01mM MeJA and control fruits were not significant. On the whole, it appears that MeJA is effective in delaying softening, depending on concentration, but its effects decreased over time. On the other hand, Xie et al., (2024) reported that MeJA combined with 1-MCP promoted the decline in flesh firmness of Hayward kiwifruit.

Starch degradation leading to an increase in TSS, and is a typical characteristic of the kiwifruit climacteric ripening pattern (Xia et al., 2020). MeJA treatment resulted in a significatively slower accumulation of soluble solids during shelf life, only at the highest (0.1 mM) concentration, while no significant differences occurred between 0.01 mM MeJA treated and untreated kiwifruit. Eventually, no differences among treated and untreated fruits occurred at the end of the shelf life period (Fig. 5A). Indeed, during the last week of storage TSS increased sharply in 0.1 mM MeJA, while remained constant in the other treatments. Similar results have been showed by Xia et al. (2020) on yellow "Jinyan" kiwifruit stored at  $4^{\circ}\text{C}$  and  $20^{\circ}\text{C}$  or when treated with 1-MCP and stored at 20°C (Liu et al., 2021). The reduction in acidity is completely mirrored by the increase in soluble solids, as are the differences between the treatments (Fig. 5B). Indeed, as shelf life progressed, a significant decrease in TA was observed, but 0.1 mM MeJA treated fruits delayed the normal reduction in TA that typically is associated with ripening, and showed the highest levels of acidity but at the last sampling date. Other antioxidant compounds, such as carvacrol significantly inhibited

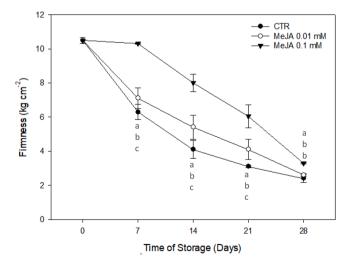


Fig. 4. Firmness (kg cm $^{-2}$ ) of treated (0.1 and 0.01 mM MeJA) and untreated (CTR) yellow kiwifruit (A. chinensis cv 'Jinyan') after 7, 14, 21, 28 days of storage at 20°C. Different letters indicate significant differences between treatments at each sampling date. ns indicate no changes between treatments. p  $\leq$  0.05 was used in the Tukey's test. The data are means  $\pm$  S.E. (n = 10).

the increase in soluble solid content and slowed down the decline of TA in kiwifruit (Luo et al., 2024).

The lightness of yellow kiwifruit was the highest immediately after harvest. However, a gradual decline in L\* values occurred during the shelf life period, reflecting the natural softening of kiwifruit. At the early shelf life stages, up to day 14, this decline in lightness was significatively lower for 0.1 mM MeJA treatment (Fig. 6). This suggests that this MeJA treatment helped slow down the ripening process, potentially preserving L\* values and visual appeal of the fruit for a longer period. However, after 21 days of storage, the decline in lightness became more abrupt and pronounced, regardless the treatment. By the end of shelf-life period, the lightness of both treated and untreated fruits had decreased significantly, indicating that yellow kiwifruit had reached an advanced stage of ripening, characterized by a darker, more subdued appearance. Hu et al. (2018) observed that melatonin at 0.10 mmol/L delayed the decline of yellow kiwifruit color L\* value and effectively reduced the respiration rate and ethylene production rate. Our results demonstrated that 0.1 mM MeJA treatment effectively retards the decline in firmness and L\* value consistently with Niu et al. (2024). Throughout the storage period, a\* values in yellow kiwifruit consistently decreased, signifying the gradual degradation of chlorophyll and the transition toward a pale green hue. This trend was significant for all storage times, reflecting the natural ripening process of the fruit. However, significant differences occurred between the 0.1 mM MeJA treatment and other treatments during the last two weeks of storage (Fig. 7A). Untreated and 0.01 mM MeJA treated kiwifruit showed a more rapid decline of a\* values after harvest, indicating a faster ripening and a more pronounced shift from green to yellow. The b\* value is particularly significant, as it directly reflects the development of the yellow pigmentation during ripening. No differences among treatments occurred during the first two shelf life weeks, while 0.1 mM treated fruit kept significatively lower values than the other treatments during the last two weeks of storage (Fig., 7B). As expected,  $\Delta E^*$  value increased over the course of shelf-life and 0.1 mM MeJA treatment always showed the lowest value, while no difference occurred between the other treatments, but at the last sampling date (Fig. 8). These results obtained through instrumental analysis reflect the data obtained through hedonic analysis. In fact, yellow kiwifruit treated with MeJA 0.1 mM had the best visual appearance scores at all sampling times, while CTR and MeJa 0.1 mM had the fastest decay rates, becoming almost not marketable 14 day for CTR and 21 day for MeJA 0.1 mM during shelf-life condition, respectively (Fig. 9).

#### 4.3. Total polyphenols, antioxidant activity and carotenoids

Total polyphenol content in yellow kiwifruit during shelf-life period under three different treatments showed different trends. Overall, all treatments showed an increase in polyphenol content over the first three weeks after the treatment; during the last week, polyphenol content further increased in 0.01 mM MeJA treatment but decreased in the other ones. Tavarini et al. (2008) measured no changes in polyphenols content in fruit stored for 2 months at 0°C but a sudden rise at about 20°C (Fig. 10). Differences between treatment were not consistent, though the lowest MeJA concentration resulted in highest values three times out of four. The increase in polyphenol content in kiwifruit treated with MeJA, could be attributed to its role as an elicitor of secondary metabolite biosynthesis (Creelman, and Mullet, 1997). Methyl jasmonate is known to activate polyphenol biosynthetic pathways, as it is involved in stress response mechanisms and fruit ripening regulation (Wang et al., 2021; Xu et al., 2019). The 0.01 mM MeJA treatment appears to be more effective in promoting this response, possibly because the higher concentration (0.1 mM) may have triggered negative regulation effects or oxidative stress, ultimately reducing polyphenol synthesis in the long term. These findings suggest that treatment with 0.01 mM MeJA could be an effective strategy to enhance polyphenol content in yellow kiwifruit during shelf life, potentially improving the fruit's nutritional quality and oxidative stress resistance. In fact, different elicitors could

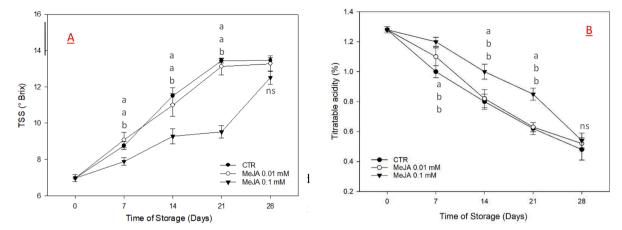
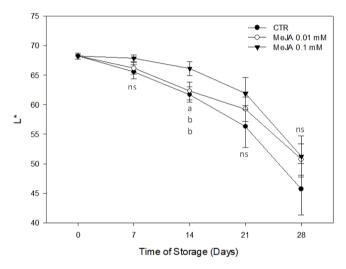


Fig. 5. Total soluble solids (TSS; Brix) (A) and Titratable acidity (%) (B) of treated (0.1 and 0.01 mM MeJA) and untreated (CTR) yellow kiwifruit (A. chinensis cv 'Jinyan') after 7, 14, 21, 28 days of storage at  $20^{\circ}$ C. Different letters indicate significant differences between treatments at each sampling date. ns indicate no changes between treatments.  $p \le 0.05$  was used in the Tukey's test. The data are means  $\pm$  S.E. (n = 10).



**Fig. 6.** L\* of treated (0.1 and 0.01 mM MeJA) and untreated (CTR) yellow kiwifruit (A. chinensis cv 'Jinyan') after 7, 14, 21, 28 days of storage at 20°C. Different letters indicate significant differences between treatments at each sampling date. ns indicate no changes between treatments.  $p \leq 0.05$  was used in the Tukey's test. The data are means  $\pm$  S.E. (n=10).

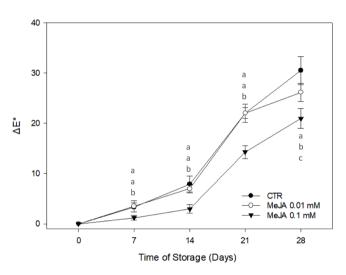


Fig. 8.  $\Delta E^*$  of treated (0.1 and 0.01 mM MeJA) and untreated (CTR) yellow kiwifruit (A. chinensis cv 'Jinyan') after 7, 14, 21, 28 days of storage at 20°C. Different letters indicate significant differences between treatments at each sampling date. ns indicate no changes between treatments.  $p \leq 0.05$  was used in the Tukey's test. The data are means  $\pm$  S.E. (n = 10).

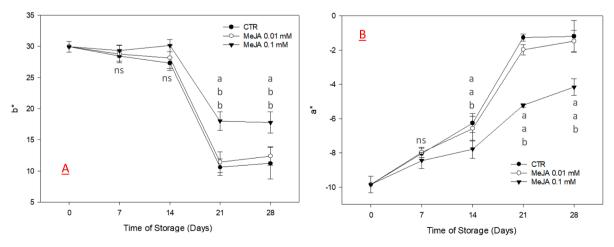


Fig. 7.  $a^*$  (A) and  $b^*$  (B) of treated (0.1 and 0.01 mM MeJA) and untreated (CTR) yellow kiwifruit (A. chinensis cv 'Jinyan') after 7, 14, 21, 28 days of storage at  $20^{\circ}$ C. Different letters indicate significant differences between treatments at each sampling date. ns indicate no changes between treatments.  $p \le 0.05$  was used in the Tukey's test. The data are means  $\pm$  S.E. (n = 10).

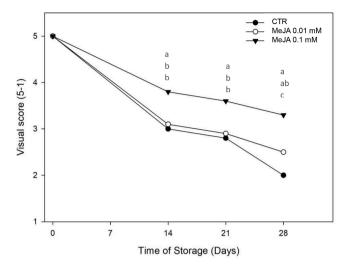
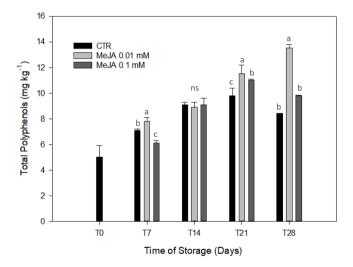


Fig. 9. Visual quality score (5-1) of treated (0.1 and 0.01 mM MeJA) and untreated (CTR) yellow kiwifruit (A. chinensis cv 'Jinyan') after 7, 14, 21, 28 days of storage at  $20^{\circ}$ C. Different letters indicate significant differences between treatments at each sampling date. Rating scale (5-1) corresponding to 5= very good, 4=good, 3= sufficient (limit of marketability), 2= poor (limit of usability) and 1= very poor (inedible). The data are means  $\pm$  S.E. (n=10).



**Fig. 10.** Total polyphenols (mg kg $^{-1}$ ) of treated (0.1 and 0.01 mM MeJA) and untreated (CTR) yellow kiwifruit (A. chinensis cv 'Jinyan') after 7, 14, 21, 28 days of storage at 20°C. Different letters indicate significant differences between treatments at each sampling date. ns indicate no changes between treatments.  $p \leq 0.05$  was used in the Tukey's test. The data are means  $\pm$  S.E. (n = 10).

effectively promote the accumulation of total phenolic content during fruit disease resistance (Li et al., 2016). Indeed, resistance actions induced by MeJA have been associated with the increase of total phenolic content (Wang et al., 2015; Liu et al., 2014).

The decrease, throughout the storage period, in antioxidant activity in the untreated fruits suggests a natural decline as the fruit undergoes senescence. This trend reflects the progressive loss of defence mechanisms as the fruit ages and becomes more susceptible to oxidative stress.

In contrast, fruits treated with 0.01 mM MeJA exhibited significantly higher antioxidant activity during the early shelf life stages, particularly up to day 14. This early response may be attributed to MeJA's known role as a signaling molecule that activates defense pathways, leading to the synthesis of antioxidant compounds such as polyphenols. However, beyond day 14, antioxidant activity in the 0.01 mM treatment began to

decline, and during the last two weeks no significant differences were observed between the two MeJA-treated groups. This slight reduction in antioxidant activity observed might be explained by the accumulation of polyphenols acting as a protective mechanism to stress. The antioxidant activity remained relatively stable throughout the shelf life period in 0.1 mM MeJA treated fruits, with only a slight increase observed by day 28. This more sustained response may be linked to the activation of long-term stress-related pathways, which help maintain redox balance under prolonged storage conditions (Cao et al., 2009). The initial lower antioxidant levels in this treatment may be due to an early oxidative stress response induced by the higher concentration of MeJA, which temporarily suppresses antioxidant activity. Over time, however, the fruit appears to adapt to this stress, leading to stabilization in terms of antioxidant content. (Fig. 10).

Stress is one of the main regulating factors influencing carotenoid metabolism in fruit during storage at low temperature (Matsumoto et al., 2009). In most cases, low temperature retarded carotenoid accumulation; and high temperature increased carotenoid content, as observed on two cultivars in yellow kiwifruit (Xia et al., 2020). The carotenoid content during shelf-life showed a similar trend across all treatments, with an increase during the first three weeks of storage and a subsequent reduction from then onwards (Fig. 11). It is clear that 0.1 mM MeJA treated fruit had always the lowest values, with no differences between the other treatments at each sampling date. The initial increase in carotenoid content may be linked to fruit ripening and the activation expression of biosynthesis gene PSY CCD1 and NCED1 plays an important role in regulating carotenoid content (Xia et al., 2020). However, the subsequent decline may be attribute to oxidative or degradative processes leading to carotenoid breakdown over time. The similarity between the control and the 0.01 mM MeJA treatment suggests that this concentration have no significant impact on carotenoid biosynthesis or degradation compared to untreated fruit. On the other hand, the 0.1 mM MeJA treatment may inhibit carotenoid synthesis, resulting in the lowest observed values Fig. 12.

### 4.4. Effect of MeJA treatments on the lesion diameter after inoculation by Aspergillius niger and Botrytis cinerea

A different effect of the treatment on fruit rot was observed depending on the concentration used. 7 and 14 days after inoculation with *B. cinerea*, kiwifruit treated with 0.01 mM MeJA seems to be the

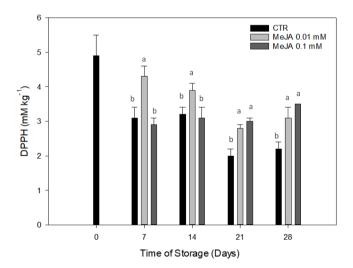


Fig. 11. DPPH (mM kg $^{-1}$ )of treated (0.1 and 0.01 mM MeJA) and untreated (CTR) yellow kiwifruit (A. chinensis cv 'Jinyan') after 7, 14, 21, 28 days of storage at 20°C. Different letters indicate significant differences between treatments at each sampling date. ns indicate no changes between treatments. p < 0.05 was used in the Tukey's test. The data are means  $\pm$  S.E. (n = 10).

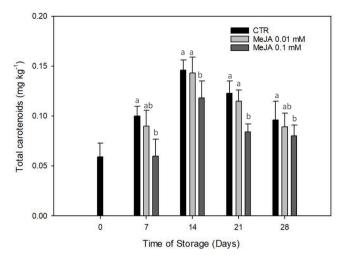


Fig. 12. Total carotenoids (mg kg $^{-1}$ ) of treated (0.1 and 0.01 mM MeJA) and untreated (CTR) yellow kiwifruit (A. chinensis cv 'Jinyan') after 7, 14, 21, 28 days of storage at 20°C. Different letters indicate significant differences between treatments at each sampling date. ns indicate no changes between treatments.  $p \leq 0.05$  was used in the Tukey's test. The data are means  $\pm$  S.E. (n = 10).

most effective inhibiting the proliferation of fungi, both in terms of a reduced lesion diameter and the highest induction effect (Table 1). Pan et al. (2020) proved a similar effect in reducing the diameter of lesions on *Actinidia deliciosa* cv. *Jinkui* treated with 0.1 MeJA, after inoculation with *B. dothidea*. MeJA also reduced disease symptoms in tomato fruit soon after being inoculated with *Botrytis cinerea* (Yu et al., 2009). On the other hand, MeJA induced fruit disease resistance in response of *B. cinerea* infection, by regulating unsaturated fatty acid metabolism and amino acid metabolism pathways (Yan et al., 2022).

The antifungal activity of MeJA, as such or as JA, has been widely demonstrated and applications on post-harvest fruit, generally at concentrations ranging from 100 to 500  $\mu mol/L$  (0,1 – 0,5 mM), but also lower, showed a significant infection control capacity. This activity is due to direct and indirect effects on pathogens, such as inhibition of spore production, germination and germ tube elongation, or induction of JA and ETH signaling defense pathways and production of enzymes such as chitinase,  $\beta$ -1,3-glucanase, polygalacturonase inhibitory proteins and PAL (Wang et al., 2021). Our results indicate that 0.01 mM MeJA played a positive role inducing a defence response on kiwifruit infected with B. cinerea like a reported by Jiang et al. (2015) on grape, while use of a higher concentration delayed the ripening in yellow kiwifruit reducing the mechanism defence.

The defence mechanism may be triggered also by the increase in total phenols, which are closely associated with plant disease resistance (Li et al., 2019). Various elicitors such as MeJA, acibenzolar-S-methyl (ASM), and benzo-(1,2,3)-thiadiazole-7-carbothioic acid s-methyl ester (BTH) can stimulate the accumulation of total phenols in fruits, thereby contributing to the plant's defense response (Prakash, et al., 2007). Phenolic compounds exhibit antimicrobial and antioxidant properties

that help the plant evade pathogenic infections and protect key tissues from the toxic effects of reactive oxygen species. Phenolic compounds also play an important role in pathogen resistance as bioactive or antimicrobial compounds. In response to microbial attack, the defence mechanism induces the synthesis of broad-spectrum active compounds that trigger a site-specific hypersensitivity response to protect against the spread of infection and future attacks (Mandal et al. 2010, Kumar et al., 2020, Tuladhar et al., 2021). In our work, kiwifruit treated with 0.01 mM MeJA, the phenolic content was significantly higher compared to the control (Fig. 10). These findings indicate that elicitors not only increase total phenol levels but also activate the plant's defence mechanisms such as on Chinese bayberry induced by MeJA (Wang et al., 2015), muskmelon induced by (ASM) (Liu et al., 2014) and mango fruits by BTH (Zhu et al., 2010) were accompanied by the increase of total phenolic content. In grape berries, low concentration of MeJA (10 µmol/L) triggered a priming defence mechanism, while higher concentrations of MeJA (50 or 100 µmol/L) directly activated defence responses, thus enhancing disease resistance (Wang et al., 2015). Eventually, when inoculated with A. niger kiwifruit treated with 0.1 mM MeJA did not prove to be as effective as for B. cinerea.

#### 5. Conclusion

This study highlights the effects of two different concentrations of MeJA on the post-harvest quality of yellow kiwifruit during shelf-life. MeJA 0.1 mM treatment was more effective in delaying ripening, reducing weight loss, maintaining higher firmness, and slowing the accumulation of TSS. Additionally, it better preserved TA and kiwifruit color, contributing to greater quality stability during storage. The application of 0.01 mM MeJA had no effects in terms of fruit ripening and shelf-life at  $20^{\circ}\mathrm{C}$ , but promoted higher polyphenol accumulation and direct or indirect effects on the growth of the fungal pathogens, reducing development of rot symptom. Future research could explore combined strategies to further optimize yellow kiwifruit shelf life.

#### CRediT authorship contribution statement

Alessio Allegra: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Paolo Inglese: Writing – review & editing, Supervision, Formal analysis. Livio Torta: Writing – original draft, Validation, Investigation, Data curation. Pedro Javier Zapata: Writing – original draft, Supervision, Investigation, Formal analysis, Data curation, Conceptualization. Maria José Gimenez: Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation. Giuseppe Sortino: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation.

#### Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Table 1 Effect of treatments (0.01mM and 0.1mM MeJA) on the lesion diameter and induction effect (%) in yellow kiwifruit (A. chinensis cv 'Jinyan') after 21 and 28 days of storage at  $20^{\circ}$ C after inoculation, on the  $14^{th}$  day, of B. cinera and A. niger. Different letters indicate significant differences between treatments at each sampling date. ns indicate no changes between treatments. The data are provided as a mean  $\pm$  S.E. (n = 10).

Inoculation on the 14 <sup>th</sup> day	(day)	CTR	MeJA	MeJA		Induction effect	
		0.01mM	0.1mM	0.01mM	0.1 mM	0.01mM	
(Lesion diameter cm)							
Botrytis cinerea	T21	$1.55{\pm}0.1a$	$0.91 \pm 0.2b$	$1.58 \pm 0.1a$	43.9 %a	-2.1 %b	
	T28	$2.92{\pm}0.2a$	$1.1 \pm 0.3c$	$2.0 \pm 0.03b$	61.5 %a	32.1 %b	
Aspergillius niger	T21	$0.87 \pm 0.2b$	$0.70 \pm 0.3b$	$1.33{\pm}0.2a$	19.9 %a	-52.8 %b	
	T28	$1.33{\pm}0.1b$	$1\pm0.2b$	$2.05{\pm}0.1a$	26.4 %a	-55.4 %b	

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#### Data availability

Data will be made available on request.

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