Plant Physiology and Biochemistry 223 (2025) 109851

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

# Involvement of ethylene production and polyamines in rind pitting of 'Fino' lemon fruit

Eugenia Guccione [b], Vicente Serna-Escolano [a,*], Alessio Allegra [b], Giuseppe Sortino [b], Ana M. Solivella-Poveda [a], María Serrano [a], Daniel Valero [a], Pedro J. Zapata [a], María J. Giménez [a]

[a] *Institute for Agro-food and Agro-environmental Research and Innovation (CIAGRO), EPSO, Miguel Hernández University (UMH), Ctra. Beniel km. 3.2, 03312, Orihuela, Spain*

[b] *Department of Agricultural, Food and Forest Sciences, Università degli Studi di Palermo, Viale delle Scienze ed. 4, ingresso H, Palermo 90128, Italy*

ARTICLE INFO

*Keywords:*
*Citrus limon*
Respiration rate
1-Aminocyclopropane-1-carboxylic acid
Putrescine
Spermidine
Spermine

ABSTRACT

Rind disorders in early cultivars of lemon fruits cause serious economic losses at market since these lemons are very sensitive to develop rind pitting during postharvest. Therefore, the aim of this study was to determine the involvement of ethylene and polyamines (PAs) in rind pitting of 'Fino' lemons grown with an intensive fertigation (IF) and standard fertigation (SF). Results after degreening treatment showed that lemons harvested from the IF system had ca. 48 %, 3.5-fold, 2.5-fold and 28 % more respiration rate, ethylene production, free and total 1-aminocyclopropane-1-carboxylic acid (ACC), respectively, than SF lemons. Furthermore, the concentrations of spermidine and spermine were ca. 30 % lower in IF lemons compared to SF ones, without differences in putrescine levels. After 7 days of storage at 8 °C, the highest values of rind pitting incidence and severity of damage were found in IF lemons. It was observed that lemons with rind pitting harvested from the IF system had the highest concentration of free-ACC and total-ACC, whereas the spermine content was ca. 3-fold higher in fruits without rind pitting independently of the fertigation system. Thus, the results showed that ethylene can be considered as a marker for the lemon fruit susceptibility to suffer rind pitting, while PAs have a protective role.

## 1. Introduction

Lemon (*Citrus limon* L. Burm) is one of the most appreciated fruits worldwide due to its flavour, taste and antioxidant capacity. Lemons must reach a specific size in order to be commercialized in the fresh market. Therefore, growers apply intensive fertigation (IF) strategies to accelerate fruit growth. In addition, lemons harvested from these early cultivars are harvested at green mature stage and undergo a degreening process to achieve the desirable yellow colour. Both strategies can be quite aggressive and may have a negative impact on the external quality of the fruit (Serna-Escolano et al., 2023).

An intact blemish-free rind fruit is an important factor for the fresh markets. However, the external peel quality of lemon fruits is seriously affected by physiological disorders that appear during postharvest storage, such as rind pitting. It is characterized by the appearance of deep depressions and darkening in the epidermis, resulting from the collapse of the albedo and the release of oil from the flavedo that damages adjacent tissues (Zacarias et al., 2020). The causal mechanisms and factors influencing fruit susceptibility to this physiological disorder are unknown. However, previous results in oranges (Alferez et al., 2005) and grapefruits (Alferez et al., 2010) have associated its susceptibility to low relative humidity. Additionally, controlling fruit respiration using cooling systems or wax coatings with an adequate oxygen diffusion has been effective in reducing its incidence (Alquézar et al., 2010). Cronje et al. (2017) proposed that albedo thickness and its ability to control water relations is probably an important factor involved in the development of this disorder. Furthermore, Alferez and Zacarías (2014) showed that maturity stage directly affected the control of water potential, which increased rind disorders in more mature citrus fruits.

Ethylene is a plant hormone synthesized from S-adenosylmethionine (SAM) through 1-aminocyclopropane-1-carboxylic acid (ACC), which plays an important role in regulating stress response (Tosetti et al.,

* Corresponding author. Institute for Agro-food and Agro-environmental Research and Innovation (CIAGRO), EPSO, Miguel Hernández University (UMH), Ctra. Beniel km. 3.2, 03312, Orihuela, Spain.

*E-mail address:* vserna@umh.es (V. Serna-Escolano).

https://doi.org/10.1016/j.plaphy.2025.109851
Received 30 January 2025; Received in revised form 24 March 2025; Accepted 27 March 2025
Available online 27 March 2025

2020). Lemon fruits are non-climacteric and produce very low levels of ethylene during ripening. This hormone does not promote relevant internal changes in citrus fruits, but it is widely used to improve the skin colour changes from green to yellow (degreening) in early lemon season cultivars (Rodrigo and Zacarias, 2007). Therefore, ethylene can have an effect on the expression of multiple genes in tissues peel. In this sense, the application of exogenous ethylene has been used to prevent non-chilling (Establés-Ortiz et al., 2016) and chilling induced peel pitting (Lafuente et al., 2004). This molecule induces numerous and diverse stress-related mechanisms in citrus fruit. A study on the network analysis of postharvest senescence in orange peel revealed that abiotic stress induced endogenous production of ethylene and the expression of transcription factors associated with fruit senescence, which also impacted the overall energy-related metabolism (Ding et al., 2015).

Polyamines (PAs) are linear or aliphatic molecules occasionally branched with polycationic nitrogen compounds. Putrescine (Put), spermidine (Spd) and spermine (Spm) are the most common PAs in plants, while thermospermine and cadaverine are only found in some higher plant species (Jangra et al., 2023). Arginine is the precursor of ornithine and agmatine, which can be transformed into Put. Putrescine is the first point to obtain Spd through the transfer of decarboxylated S-adenosyl methionine (dcSAM) to Put, and Spm is formed by the addition to Spd of another aminopropyl group (Blázquez, 2024). Ethylene and PAs have an interconnected pathway due to their common precursor SAM. Therefore, if this substrate is limited, it could determine the preference for the production of ethylene or Spd and Spm (Puente-Moreno et al., 2025). Although previous results in mutants of tomatoes proved that the presence of SAM could not be a limiting factor, and both pathways could function at the same time (Mehta et al., 2002). In this sense, a parallel biosynthesis of ethylene and PAs has been widely studied by Lokesh et al. (2019) in bananas, concluding that ethylene and PAs do not compete for SAM, since PAs were synthetized through arginine-mediate pathway. It has been proposed that PA accumulation has an adaptive response to protect plants against abiotic stresses (Alcázar et al., 2020). In comparison to the many reports that describe the roles of PAs in climacteric fruits, in non-climacteric fruits the information that highlight how PAs participate in fruit ripening, senescence and quality is limited. In lemons, it has been previously reported the accumulation of Put in flavedo when fruits were stored at chilling injury temperatures (McDonald, 1989; Martínez-Romero et al., 1999). In grapes, endogenous Put and Spd levels usually decline during fruit ripening and senescence, while Spm remains constant (Agudelo-Romero et al., 2013). Results in strawberries showed that Spm levels increased after the fruit turning red, being predominant in ripe fruits (Guo et al., 2018). Furthermore, in strawberries it has been recently described the importance of polyamine oxydase 5 (PAO5) regulating fruit ripening, allowing the accumulation of Spd and Spm (Mo et al., 2020). Exogenous application of Put to 'Verna' lemons at postharvest increased endogenous levels of Put, leading to increased fruit firmness maintenance and reduced weight loss during storage as compared to control fruits (Valero et al., 1998). Also in lemons, the exogenous application of individual Put and Spd and their combination reduced electrolyte leakage, indicating maintenance of the cell membrane structure that protects the fruit from chilling injury (Kawaleguet et al., 2022). Accordingly, in climacteric fruits such as peach fruits, the exogenous application of PA lead to the inhibition of ethylene emission, delaying fruit softening and increasing fungal resistance during postharvest storage (Torrigiani et al., 2012). Therefore, the increase in PA levels could be considered an important adaptive mechanism to delay fruit ripening and senescence (Shi et al., 2010).

The molecular basis that explains the differences in the susceptibility of lemon fruits to rind pitting is still unclear. Therefore, the aim of this research was to study the impact of intensive fertigation on ethylene production and PAs accumulation compared to standard fertigation in order to identify their possible roles in the rind pitting incidence and severity of 'Fino' lemons.

## 2. Material and methods

### 2.1. Experimental design and quality assessment

The experiment was designed to follow the normal agronomical practices used in the field and postharvest handling for lemon fruits under organic production system (Fig. 1). Thus, the experiment was carried out in a commercial field located in Orihuela (Alicante, Spain), under an average temperature of 18 °C and 280 mm/year of rainfall during 2023–2024 season. Two plots with 75 'Fino' lemon trees grafted on *Citrus macrophylla* rootstock of 10 years old with two independent drip fertigation systems (intensive fertigation (IF) and standard fertigation (SF)) were selected for this experiment, according to previous conditions described by Serna-Escolano et al. (2023) summarised in Table 1. The fertilisers used for this experiment were organic liquid nitrogen and potassium oxide ($K_2O$).

Both fertigation systems are used by the lemon fruit growers at the beginning of the season with different purposes; IF is normally applied to promote lemon fruit growth, whereas SF is used to reduce fruit maturation process on tree.

Two thousand lemon fruits with green colour and commercial diameter (higher than 55 mm) were harvested on the 18th of September 2023 from both SF and IF systems. These fruits were transferred to the laboratory in 2 h and were subjected to a degreening treatment (Fig. 1), consisting of 8 days storage in closed chamber at 25 °C and a relative humidity (RH) of 90–95 % in order to acquire the yellow skin colour necessary for its marketing. This degreening treatment is the only allowed for organic lemon fruit production by the European legislation (Regulation (EU) 2018/848). After degreening, the first sample of 30 lemons from the SF and IF systems without visual physical damages and homogeneous in size were selected for the individual measures of respiration rate and ethylene production as shown Fig. 1. In addition, six lots of five fruits were made at random and after peeling, peel samples from each lot were mixed and frozen at −80 °C for ACC and PA determinations.

Furthermore, 1000 lemons from each fertigation treatment were selected after degreening and stored at 8 °C and 85 % RH during 7 days. These storage conditions are used by the lemon fruit industry prior to marketing (Fig. 1). After that storage period, lemons were visually assessed according to the severity of rind pitting symptoms as follows: 0: absence of rind pitting.1: low level (less 25 % of the flavedo affected). 2: medium level (25–50 % of the flavedo affected). 3: high level (more than 50 % of the flavedo affected) (Fig. 2). Then, the second sample (Fig. 1) of 30 lemons from SF and IF systems without rind pitting (WRP) and with rind pitting (RP), 0 and 3 scores, respectively, were selected for the individual measures of respiration rate and ethylene production and the peel of six lots of five fruits were mixed and frozen at −80 °C to measure ACC and PAs as indicated above.

### 2.2. Physiological analysis

The individual respiration rate of 30 lemons was determined at room temperature by placing each fruit individually in a 0.5 L glass jar for 60 min. Then, a volume of 1 mL of the headspace was injected into a gas chromatograph (GC- 14B, Shimadzu, Kyoto, Japan) coupled to a thermal conductivity detector to measure $CO_2$ concentration and the data was expressed as mg of $CO_2$ $Kg^{-1}$ $h^{-1}$ (Martínez-Esplá et al., 2018). For the individual ethylene production another sample of 1 mL from the jar was injected into GC (TMGC-2010, Shimadzu, Kyoto, Japan) fitted with a flame ionization detector and results were expressed in nL $Kg^{-1}$ $h^{-1}$ (Martínez-Esplá et al., 2018).

### 2.3. ACC extraction assay

Total ACC (free and conjugated) was extracted as previously described Zapata et al. (2004) with some modifications by

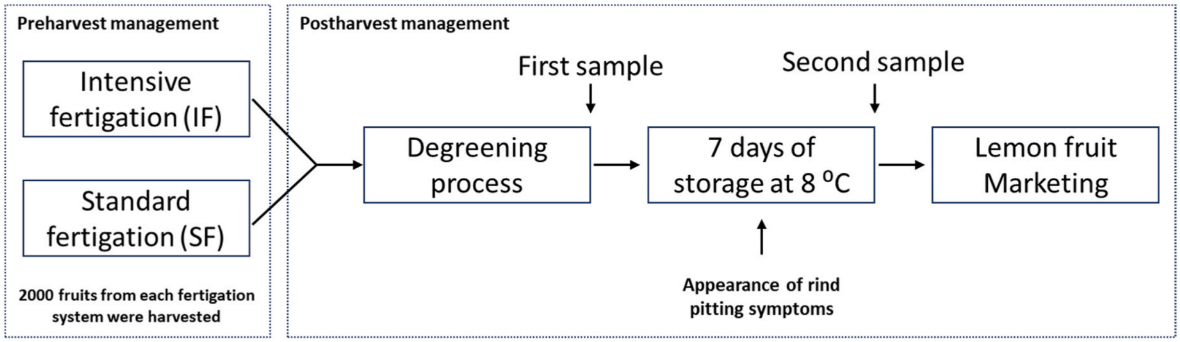


**Fig. 1.** Flowchart of the experimental design to determine the involvement of ethylene production and polyamine accumulation in lemon fruit rind pitting promoted by the most stressful steps of the lemon fruit production in the industry. The first sample was taken after degreening and the second samples after 7 days of cold storage.

**Table 1**
Total irrigation ($m^3$ $ha^{-1}$) and nitrogen/potassium fertilization (Kg $ha^{-1}$) applied in intensive fertigation (IF) and standard fertigation (SF) plots during the flowering (March–June), fruit growth (July–August) and fruit ripening (September–December).

| Stages | IF | | | SF | | |
|---|---|---|---|---|---|---|
| | Irrigation | Nitrogen | Potassium | Irrigation | Nitrogen | Potassium |
| Flowering | 150 | 10 | 20 | 150 | 10 | 20 |
| Fruit growth | 600 | 60 | 120 | 300 | 30 | 40 |
| Fruit ripening | 200 | 0 | 10 | 200 | 0 | 10 |
| Total | 950 | 70 | 150 | 550 | 40 | 70 |

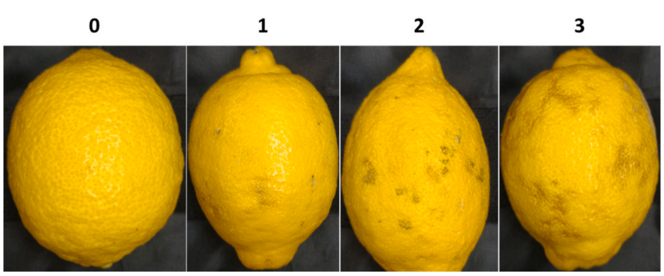


**Fig. 2.** Classification of ‘Fino’ lemons according to the flavedo rind pitting damage from scores 0 to 3.

homogenising 2 g of peel from each sample with 0.2 M trichloroacetic acid (1: 3 w/v) and the extracts were centrifuged at 10000 *g* for 10 min. The supernatants were used to determine its free-ACC through the chemical conversion of ACC to ethylene in a reaction medium that contained 100 μL of 10 mM $ClHg_2$, 100 μL of saturated NaOH, 0.5 mL of 5 % NaOCl and 0.5 mL of extracts. In order to determine total-ACC, 5 mL of the extracts were hydrolysed with 1 mL of 2 N HCl, at 100 °C for 60 min. Then, total-ACC was measured as described above doing the chemical conversión of ACC to ethylene. A calibration curve of ACC from Sigma-Aldrich (Poole, Dorset, England) was used as standard. The results (mean ± SE) of three measurements from each of the six replicates for each peel sample were expressed as nmol per gram of fresh weight (nmol $g^{-1}$ FW).

### 2.4. Polyamine analysis

Free PAs were extracted by homogenising 2 g of peel samples in 10 mL of 5 % perchloric acid and those extracts were centriguged 15 min at 10000 *g*. Benzoylated free PAs were determined as previously reported Zapata et al. (2003) by an HPLC (Hewlett-Packard Company, Wilmington, DE) equipped with a phase reverse column (LiChroCart 250–4.5 μm). The elution system MeOH/$H_2O$ (64: 36) running isocratically with a flow rate of 0.8 mL $min^{-1}$. Calibration was made with hexanediamine (100 nmol $g^{-1}$ FW of tissue) as an internal standard, and standard curves of Put, Spd, and Spm from Sigma-Aldrich (Poole, Dorset, England) were used to determine the Pas content in samples. The results (mean ± SE) of three measurements from each of the six replicates for each peel sample were expressed as nmol per gram of fresh weight (nmol $g^{-1}$ FW).

### 2.5. Total antioxidant activity

The extraction was carried out by homogenising 2 g of flavedo in 10 mL of potassium phosphate buffer 50 mM pH 7 and ethyl acetate (2:1 v/v). The extracts obtained were centrifuged at 10,000×*g* for 12 min at 4 °C. The antioxidant activity was measured in the hydrophobic and hydrophilic phases using the ABTS-peroxidase system as described by Serna-Escolano et al. (2023). The total antioxidant activity (TAA) was the sum of both phases in six replicates for each peel sample, and the results were expressed as mg of Trolox equivalent per 100 g FW.

### 2.6. Statistical analysis

Data for each peel sample were the mean ± SE and were statistically analysed by Student’s unpaired *t*-test and ANOVA with a multiple-range test (Tukey’s test) to determine significant differences between samples ($p < 0.05$). Those statistical analyses were performed using SPSS, version 22 (IBM Corp., Armonk, NY, USA). In addition, the PCA model was constructed with normalized data using Unscrambler 11 software (CAMO AS, Oslo, Norway).

## 3. Results and discussion

Respiration rate is one of the most important parameters that indicates the metabolic processes of the fruit after harvest. Thus, higher values of respiration rate lead to faster comsumption of carbohydrates and organic acids accelerating fruit senescence (Li et al., 2016). Results indicated differences in fruit metabolism reflected in the respiration rate, as it was 37.5 % higher in lemons cultivated with the IF compared to those in the SF system after the degreening process (Fig. 3A). Additionally, results in Fig. 3B showed that ethylene production was 3-fold

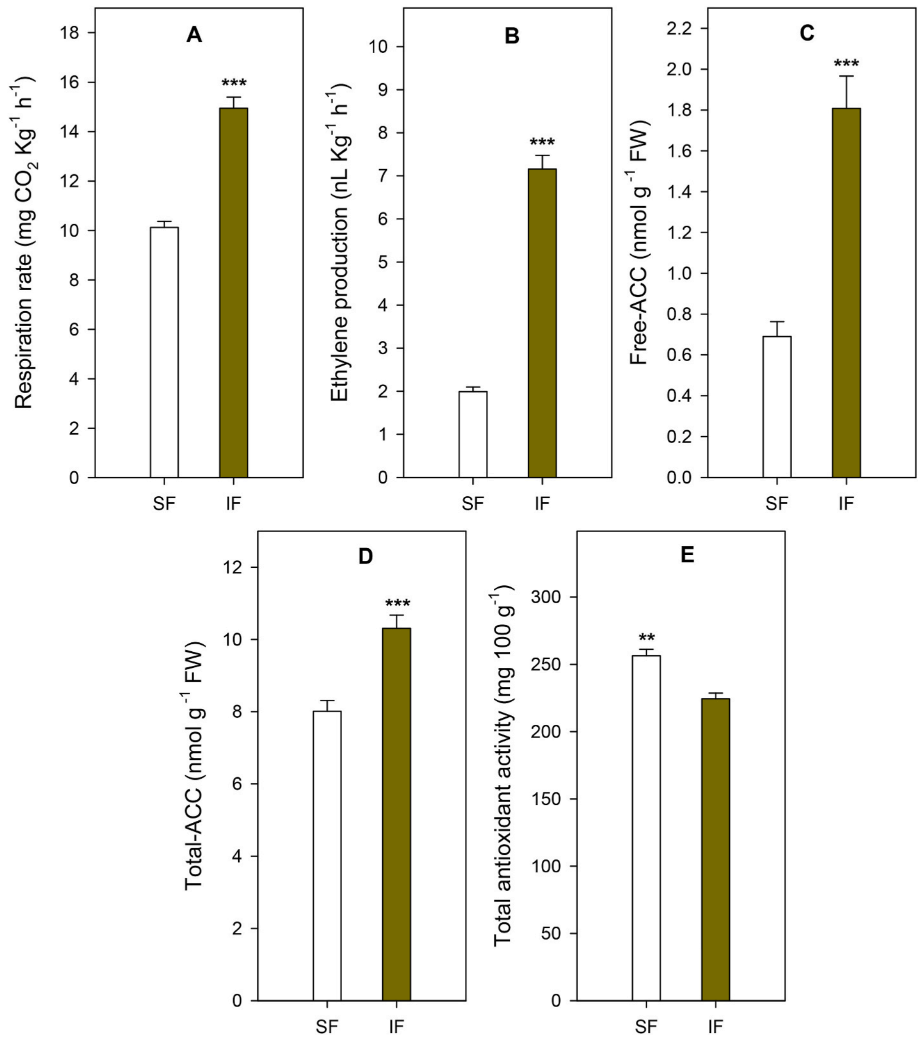


**Fig. 3.** Effect of standard fertigation (SF) and intensive fertigation (IF) on A) respiration rate, B) ethylene production, C) free 1-aminocyclopropane-1-carboxylic acid (Free-ACC), D) total 1-aminocyclopropane-1-carboxylic acid (Total-ACC) and E) total antioxidant activity in lemon peel after degreening. Asterisks show significant differences at * $p < 0.5$, **$p < 0.01$ and ***$p < 0.001$ according to Student's unpaired *t*-test.

higher in the IF lemons compared to the SF ones, 7.15 ± 0.32 and 1.99 ± 0.11 ng kg$^{-1}$ h$^{-1}$, respectively. Furthermore, free-ACC and total-ACC content in the peel of lemons harvested from the IF system showed an increase of ca. 80 % and 37.5 %, respectively, compared to the SF lemons (Fig. 3C and D). Generally, the degreening process in lemons has been associated with fruit quality losses due to exposure to high temperatures and relative humidity over a long period (Serna-Escolano et al., 2022). Temperature plays an important role in modulating ethylene production. Therefore, ethylene could be acting as a stress signal during degreening. Thus, in watermelon the increase of storage temperature from 20 °C to 30 °C enhanced ethylene production through the expression of genes encoding ACC synthase and ACC oxidase (Zhou et al., 2016). Also, in longan fruits stored at 25 °C an increase in ethylene production occurred, which was associated with the transcription of the ethylene-responsive factor-like gene DlERF1, which promotes postharvest decay and fruit senescence (Kuang et al., 2012). Lemon fruit is a non-climacteric fruit that shows no respiration or ethylene production peaks associated with ripening. However, previous results in other non-climacteric fruits, such as strawberry and orange have reported that ethylene was involved in the senescence process of the fruit interacting with abscisic acid through the induction of the NCED1 gene (Tosetti et al., 2020; Feng et al., 2021). Therefore, high ethylene production could act as a signal of stress and trigger lemon fruit senescence. In this sense, the higher ethylene production and free and total-ACC content in peel tissue of lemons from IF system than in those from SF system would indicate a response to stress due to IF. Regarding the antioxidant status of the fruit, lemons harvested from the SF system showed ca. 14 % higher TAA than those harvested from the IF system (Fig. 3E). These results were similar to those published in lemons and oranges where fruits harvested from the IF system showed the lowest levels of TAA, which was associated with an accelerated ripening process during postharvest (Hou et al., 2021; Serna-Escolano et al., 2023).

Regarding the content of PAs in lemon peel after degreening, lemons harvested from the SF system had ca. 20 % more free PAs (Put + Spd + Spm) than IF ones (248.45 ± 5.39 and 195.07 ± 7.44 nmol g$^{-1}$, respectively), with Spm being the major PA (Fig. 4). Besides, the content of Spd and Spm was lower in the IF lemons compared to the SF ones, with a decrease of ca. 30 % in both PAs (Fig. 4B and C), while no significant ($p < 0.05$) differences were found for Put between both fertigation systems (Fig. 4A). Those results could indicate the importance of SAM as a pivotal factor in the biosynthetic pathways of ethylene and PAs in lemons. In fact, lower PA content and higher ACC biosynthesis and ethylene production was found in the IF samples than in SF samples. In *Arabidopsis*, it has been reported that levels of free PAs increase under stress conditions through transcriptional regulation of the genes encoding PA metabolism (Zarza et al., 2017). However, the present results show higher levels of Spd and Spm in SF than in IF samples showing that lemon fruit from SF would be more tolerant to the stressor

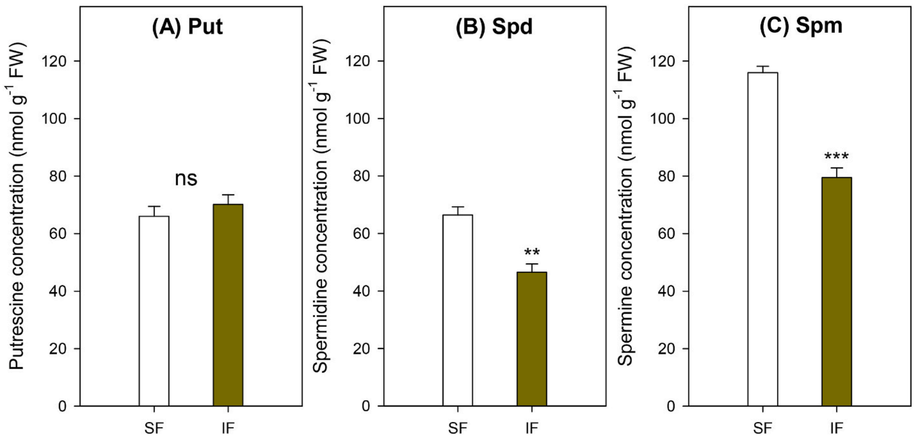


**Fig. 4.** Effect of standard fertigation (SF) and intensive fertigation (IF) on A) putrescine (Put), B) spermidine (Spd) and C) spermine (Spm) concentration of lemon peel after degreening. Asterisks show significant differences at * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and ns: not significant according to Student's unpaired *t*-test.

degreening process, due to their higher PA content. In agreement with the present results, Spm has been evidenced to provide abiotic stress tolerance in plants through the activation of its regulatory enzymes and regulating redox signalling (Kusano et al., 2008; Anwar et al., 2015).

After 7 days of storage at 8 °C, the rind pitting damage was evaluated according to 0–3 scores (Fig. 2). Results showed that the incidence of rind pitting in lemons harvested from the IF system was approximately 2-fold higher than in SF lemons (Fig. 5A). Besides, the severity of damage in lemons with rind pitting harvested from SF system was mainly scored as 1, while in lemons from the IF system, the severity was accounted for score 3 (Fig. 5B). Fertigation practices could impact growth and maturation process of fruit on the tree. Furthermore, previous results in 'Fino' lemons showed that the best physicochemical quality and antioxidant properties were obtained in those samples harvested from the SF system (Serna-Escolano et al., 2023). Therefore, lemons from SF and IF systems could have different responses to postharvest stress. Rind pitting in lemon fruits is associated with the stability loss of the cell wall structure. The effect of high endogenous ethylene could be related to the increased activity of cell wall hydrolytic enzymes, particularly polygalacturonases, pectin methylesterases and cellulases, which hydrolyse the cell wall components of the peel (Wormit and Usadel, 2018). In Navelate oranges, ethylene-mediated mechanisms modified the epicuticular waxes through the modulation of terpenoids and fatty acids, which could negatively affect the peel structure (Romero and Lafuente, 2022).

On the other hand, polyamines are present in living organism as free and conjugated forms. Specifically, non-covalent conjugated PAs are associated with the stability of the membrane structure (Agudelo-Romero et al., 2013). Thus, increased levels of endogenous PAs in Washington Navel and Lane Late sweet orange fruit were

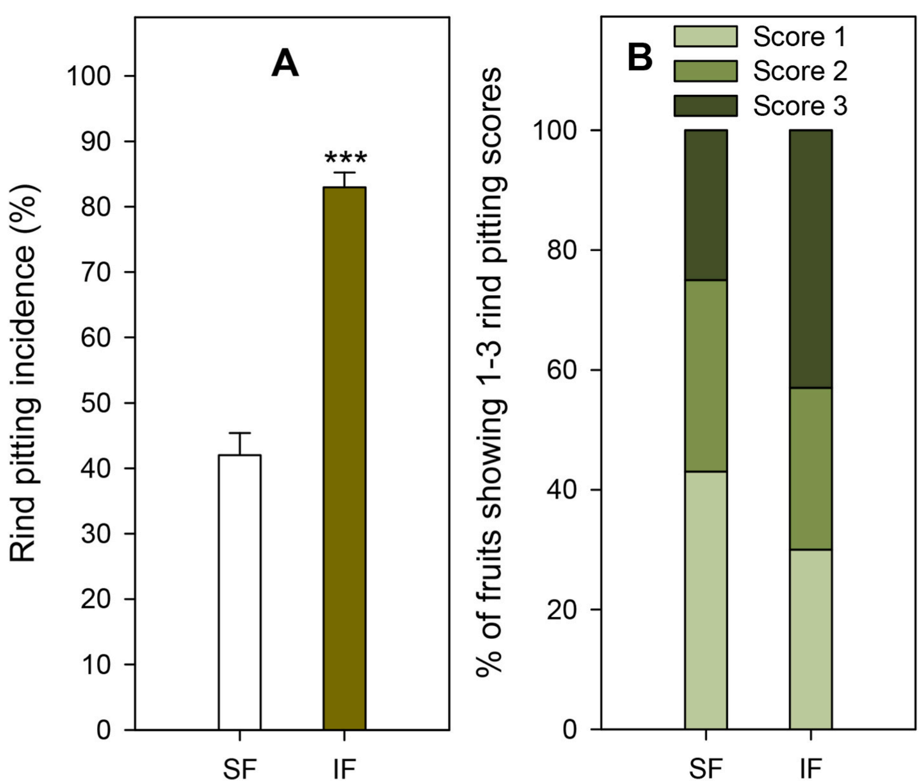


**Fig. 5.** Effect of standard fertigation (SF) and intensive fertigation (IF) on rind pitting incidence (A) and severity (scored from 1 to 3) (B) in lemons stored for 7 days at 8 °C. Asterisks show significant differences at * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ according to Student's unpaired *t*-test.

associated with peel resistance to chilling temperatures (Hussain and Singh, 2015). Thus, the higher ethylene production after degreening in IF lemons with respect to SF ones could be responsible for their increased rind pitting incidence and severity, while the higher Spd and Spm content in SF fruits show a protective role against this physiological disorder.

Lemons from the SF and IF systems without rind pitting (score 0) and with severe rind pitting (score 3) after 7 days of cold storage were used to measure respiration rate, ethylene production and ACC and PAs levels. Results showed that the lowest was found in SF lemons without rind pitting, which was in agreement with results previously observed after the degreening process, and in lemons from IF system showing rind pitting (Fig. 6A). Thus, the lower respiration of lemons from the SF system without rind pitting could be due to a better control of the metabolism associated with maintaining energetic balance during cold storage (Sati et al., 2024), while the results observed in IF lemons with rind pitting could be related to programmed cell death produced during peel collapse (Ding et al., 2015. Regarding ethylene production, this parameter decreased during cold storage in all lemon samples, since values were lower after cold storage (Fig. 6B) than after the degreening process (Fig. 3B). However, the highest values were observed in the SF lemons with rind pitting, without significant ($p < 0.05$) differences between the other samples (Fig. 6B). In addition, lemons from the IF system with rind pitting had approximately 2-fold more free-ACC concentration than other samples, without significant ($p < 0.05$) differences found between them (Fig. 6C). Total-ACC was higher in the IF lemons independently of the presence of rind pitting, followed by lemons from the SF system with rind pitting and the lowest concentrations were found in the SF lemons without rind pitting (Fig. 6D). Therefore, total-ACC has been synthesized to a greater extent in lemons cultivated in the IF system either with or without rind pitting damages. Furthermore, the lack of increased ethylene production in IFRP lemons could be due to a low activity of ACC oxidase enzyme (ACO), which is supported by higher free-ACC found in those samples. In this sense, the

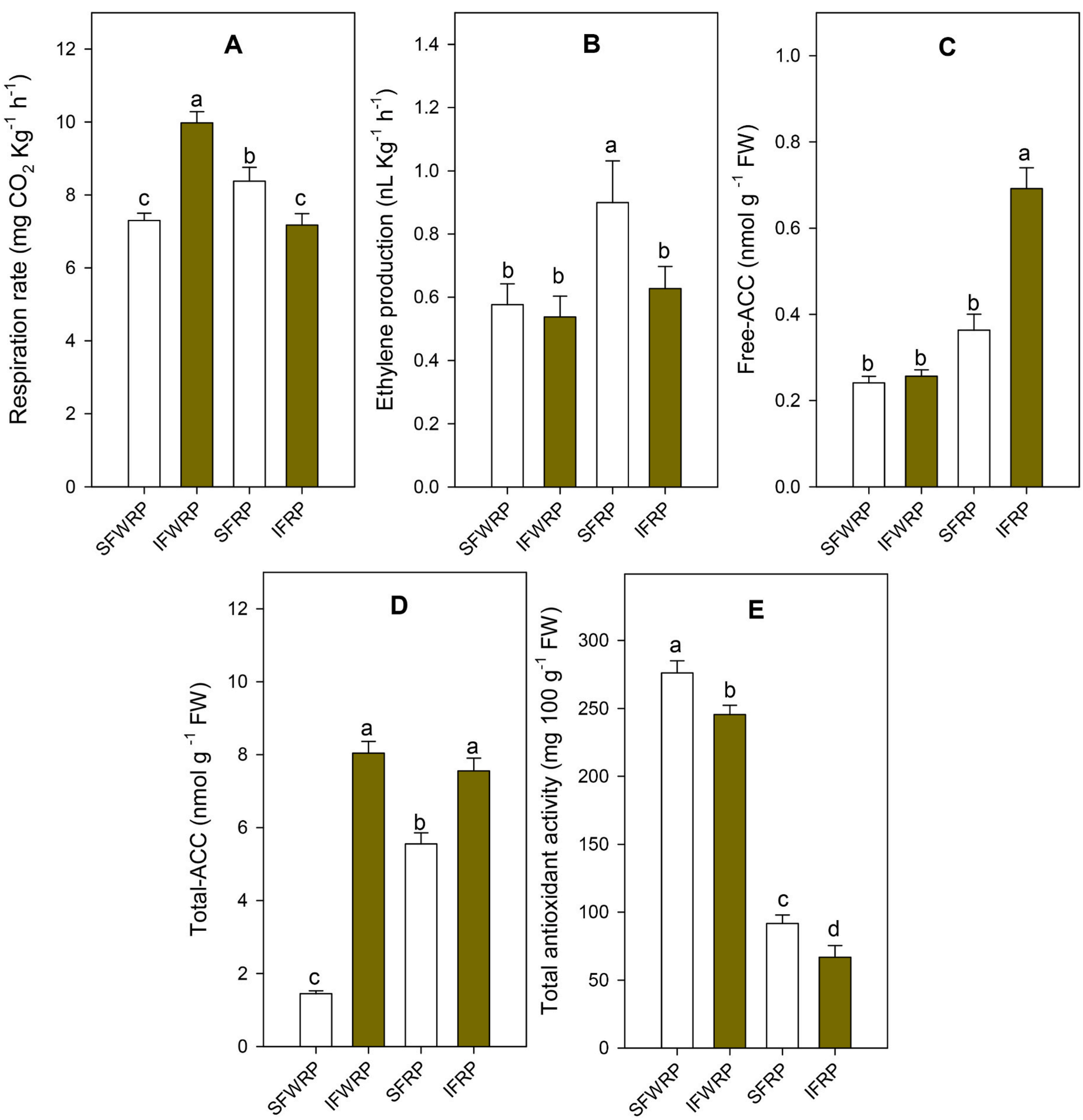


**Fig. 6.** Effect of standard fertigation (SF) and intensive fertigation (IF) on A) respiration rate and B) ethylene production of lemons stored for 7 days at 8 °C without rind pitting (WRP) and with rind pitting (RP). Furthermore, C) free 1-aminocyclopropane-1-carboxylic acid (Free-ACC), D) total 1-aminocyclopropane-1-carboxylic acid (Total-ACC) and E) total antioxidant activity were determined in lemon peel. Different letters show significant differences at $p < 0.05$ according to Tukey's range test.

modulation of the ACO activity during cold storage has been described in transgenic oranges, showing that the increase on ACC content coincided with absence of ACO activity which was directly related to the development of chilling injury symptoms (Wong et al., 2001). These results could be related to others previously published in ‘Fortune’ mandarins, ‘Navelina’ and ‘Valencia’ oranges where endogenous ethylene production was stress-induced (Lafuente et al., 2003). Furthermore, a wound assay induced a rapid increase in ethylene production and ACC content in orange tissues, indicating the importance of ethylene as a damage signalling molecule in citrus (Zacarias and Alferez, 2007). In other non-climacteric fruit, such as zucchini, it has been published that ethylene production occurs not only during fruit rewarming but also under cold storage, which could be partly responsible for peel chilling injury (Megías et al., 2016). In addition, it is worth nothing that the increase of total-ACC observed in lemons from IF system before storage (Fig. 3D) was also evident after a week of cold storage, although it was independent of the rind pitting incidence (Fig. 6D). Thus, the IF system would increase ACC-synthase activity leading to high total-ACC content which was evident after degreening and even after cold storage. The results showed that the TAA was significantly ($p < 0.05$) higher in fruits without rind pitting than in fruits with rind pitting symptoms (Fig. 6E), showing a decrease of ca. 3.5-fold on average. This effect could be related to the scavenging activity of free radicals produced during cell collapse, which consume the antioxidant compounds (Costanzo et al., 2022). Furthermore, an effect of the fertigation system was observed, since the highest TAA levels were found in fruits harvested from the SF system, independently of the rind damage (Fig. 6E), maintaining the differences previously observed in Fig. 3E.

Results in Fig. 7 showed that free PAs decreased during cold storage in all lemon samples as compared with results after degreening (Fig. 4). After cold storage, Put and Spm showed the highest levels in lemons without rind pitting compared to those with rind pitting (Fig. 7A and C). Regarding Spd, lemons from SF system with rind pitting showed the highest levels, while no significant ($p < 0.05$) differences were found between other samples (Fig. 7B). In addition, total PAs content (Put + Spd + Spm) was higher in lemon without rind pitting than in those showing high rind pitting scores, either for SF as for IF systems, with values of 159.63 ± 4.39 and 166.38 ± 5.42 nmol $g^{-1}$ for SFWRP and IFWRP, respectively and 120.11 ± 3.87 and 95.01 ± 4.21 nmol $g^{-1}$ for SFRP and IFRP, respectively. In addition, the results showed that SFWRP and IFWRP lemons had similar Spm values (Fig. 7C), which could be related to the fact that these lemons did not develop rind pitting during the cold storage. Therefore, this experiment shows that in both fertigation systems there are lemons with good quality characteristics to prevent rind disorders during storage. These results highlight the importance of the PA accumulation to protect lemon fruit against rind pitting incidence. Previous studies have providing evidences regarding the role of Spm inducing stress tolerance (Alcázar et al., 2020). This effect could be explained by several mechanisms; the first would be the capacity of PAs to inhibit the oxidation of metals, decreasing the production of oxidative species; the second would be indirectly through the regulation of genes encoding the antioxidant machinery or by increasing the activity of antioxidant enzymes such as superoxide dismutase and catalase; and the third would be through the induction of $H_2O_2$ by PA oxidases acting as a signaling to induce antioxidant enzymes (Shi et al., 2010; Tanou et al., 2014; Moschou et al., 2008). In these results Spm was the PA that showed the highest differences between fruit with and without rind pitting symptoms (Fig. 7C), which has been previously reported by Hasan et al. (2021) in plants under stress conditions. The elevated concentration of Spm could be induced as a part of the antioxidant systems under stress conditions (Capell et al., 2004). Moreover, the involvement of Spd in stress resistance has been also supported by experiments that studied the effects of the mutation of PAO5 gene or by overexpression of genes encoding SAM decarboxylase or Spm synthase which caused higher tolerance to different types of abiotic stresses (Sánchez-Rodríguez et al., 2016). In this sense, the higher level of Spm found in SFWRP and IFWRP lemons (Fig. 7A) could be explained by the Spm synthase activity, since in these samples the lower levels of free-ACC and ethylene production (Fig. 6B and C).

A principal component analysis (PCA) was applied to the whole results showing that PC-1 and PC-2 accounted for 63 % and 21 % of the total variance of the X and Y variables, respectively (Fig. 8). The cumulative variance contribution of PC-1 and PC-2 was 84 %. PC-1 was clearly identified with respiration rate (0.42), ethylene production (0.44), free-ACC (0.39), Put (0.39) and Spd (0.33), while PC-2 was related to TAA (0.70), total-ACC (−0.71), and Spm (0.62). All parameters were found on the positive side of the PC-1. The most important parameters on the positive side of the PC-2 were respiration rate, TAA, Spm, and Put, while ethylene production, Spd, free-ACC and total-ACC were on the negative side. The results showed that PC-1 allowed the differentiation of three groups: two groups consisting of lemons from the SF and IF systems after degreening, and another group consisting of lemons after 7 days of cold storage, regardless of the fertigation system, without rind pitting symptoms, which also included the SF and IF lemons with rind pitting. Regarding PC-2, the results showed four groups: the first group consisted of lemons from the SF system without rind pitting after 7 days of cold storage, the second group consisted of lemons from the IF system without rind pitting after 7 days of cold

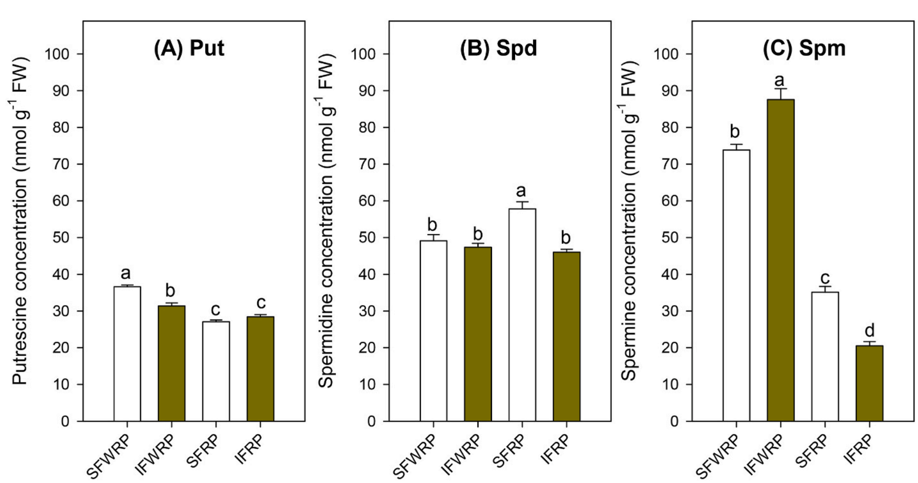


**Fig. 7.** Effect of standard fertigation (SF) and intensive fertigation (IF) on A) putrescine (Put), B) spermidine (Spd) and C) spermine (Spm) concentration of lemon peel taken from fruit without rind pitting (WRP) and with rind pitting (RP) stored for 7 days at 8 °C. Different letters showed significant differences at $p < 0.05$ according to Tukey’s range test.

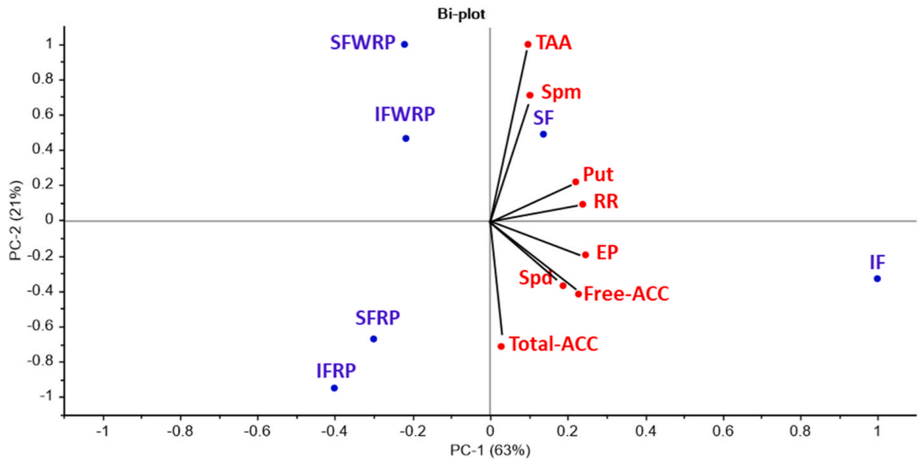


**Fig. 8.** Principal component analysis (PCA) biplot showing the relationships among different samples and the measured parameters. Samples are shown in blue (•) and the parameter vectors are shown in red (•). Lemon samples were abbreviated as follows; standard fertigation (SF), intensive fertigation (IF), standard fertigation without rind pitting (SFWRP), intensive fertigation without rind pitting (IFWRP), standard fertigation with rind pitting (SFRP) and intensive fertigation with rind pitting (IFRP). The parameters included in the analysis were respiration rate (RR), ethylene production (EP), free 1-aminocyclopropane-1-carboxylic acid (Free-ACC), total 1-aminocyclopropane-1-carboxylic acid (Total-ACC), total antioxidant activity (TAA), putrescine (Put), spermidine (Spd) and spermine (Spm). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

storage and those harvested from the SF system after degreening, the third group contained lemons from the IF system after degreening and those from the SF system with rind pitting symptoms, and the fourth group consisted of IF lemons with rind pitting. Therefore, the fertigation system in lemons after degreening, and, the cold storage time were closely related to the respiration rate, ethylene production, Spd, Put and free-ACC while the rind pitting symptoms were more dependent on Spm, TAA and total-ACC.

## 4. Conclusion

The present study revealed that ethylene production through the biosynthesis of its precursor (ACC) was drastically increased in lemons grown under the IF system after degreening, which significantly reduced the accumulation of free PAs in these peel samples compared to lemons from the SF system. In addition, when rind pitting appeared on the lemon fruit surface after 7 days of cold storage, only a basal ethylene production was detected, although total-ACC was high in fruits from the IF system and/or rind pitting. Furthermore, it was observed that free PAs were lower in samples with a high incidence and severity of rind pitting, regardless of the fertigation system. The results also showed the importance of Spm in controlling the severity of the rind pitting. Therefore, the overall data showed that ACC synthesis is induced under stress conditions, while increased PAs levels have a role as an adaptative response to protect against IF, degreening process and cold storage conditions, although further research is needed to understand the molecular mechanism involved in these responses.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

## References

Agudelo-Romero, P., Bortolloti, C., Pais, M.S., Tiburcio, A.F., Fortes, A.M., 2013. Study of polyamines during grape ripening indicate an important role of polyamine catabolism. Plant Physiol. Biochem. 67, 105–119. https://doi.org/10.1016/j.plaphy.2013.02.024.

Alcázar, R., Bueno, M., Tiburcio, A.F., 2020. Polyamines: small amines with large effects on plant abiotic stress tolerance. Cells 9, 2373. https://doi.org/10.3390/cells9112373.

Alferez, F., Zacarias, L., Burns, J.K., 2005. Low relative humidity at harvest and before storage at high humidity influence the severity of postharvest peel pitting in citrus. J. Am. Soc. Hortic. Sci. 130, 225–231. https://doi.org/10.21273/JASHS.130.2.225.

Alferez, F., Alquézar, B., Burns, J.K., Zacarias, L., 2010. Variation in water, osmotic and turgor potential in peel of 'Marsh' grapefruit during development of postharvest peel pitting. Postharvest Biol. Technol. 56, 44–49. https://doi.org/10.1016/j.postharvbio.2009.12.007.

Alferez, F., Zacarías, L., 2014. Influence of fruit maturity in the susceptibility of Navelina oranges to develop postharvest non-chilling peel pitting. Food Sci. Technol. Int. 20, 183–191. https://doi.org/10.1177/1082013213476077.

Alquézar, B., Mesejo, C., Alferez, F., Agustí, M., Zacarias, L., 2010. Morphological and ultrastructural changes in the peel of 'Navelate' oranges in relation to variations in relative humidity during postharvest storage and development of peel pitting. Postharvest Biol. Technol. 56, 163–170. https://doi.org/10.1016/j.postharvbio.2009.12.005.

Anwar, R., Mattoo, A.K., Handa, A.K., 2015. Polyamine interactions with plant hormones: crosstalk at several levels. In: Kusano, T., Suzuki, H. (Eds.), Polyamines. Springer, Tokyo, pp. 267–302. https://doi.org/10.1007/978-4-431-55212-3_22.

Blázquez, M.A., 2024. Polyamines: their role in plant development and stress. Annu. Rev. Plant Biol. 75, 95–117. https://doi.org/10.1146/annurev-arplant-070623-110056.

Capell, T., Bassie, L., Christou, P., 2004. Modulation of the polyamine biosynthetic pathway in transgenic rice confers tolerance to drought stress. Proc. Natl. Acad. Sci. USA 101, 9909–9914. https://doi.org/10.1073/pnas.0306974101.

Costanzo, G., Vitale, E., Iesce, M.R., Naviglio, D., Amoresano, A., Fontanarosa, C., Spinelli, M., Ciaravolo, M., Arena, C., 2022. Antioxidant properties of pulp, peel and seeds of Phlegrean Mandarin (Citrus reticulata Blanco) at different stages of fruit ripening. Antioxidants 11, 187. https://doi.org/10.3390/antiox11020187.

Cronje, P.J.R., Zacarias, L., Alferez, F., 2017. Differential susceptibility to peel pitting in fruit from citrus cultivars with different albedo thickness. Postharvest Biol. Technol. 123, 77–82. https://doi.org/10.1016/j.postharvbio.2016.08.012.

Ding, Y., Chang, Y., Ma, Q., Chen, L., Liu, S., Jin, S., Han, J., Xu, R., Zhu, A., Guo, J., Luo, Y., Xu, J., Xu, Q., Zeng, Y.L., Deng, X., Cheng, Y., 2015. Network analysis of postharvest senescence process in citrus fruits revealed by transcriptomic and metabolomic profiling. Plant Physiol. 168, 357–376. https://doi.org/10.1104/pp.114.255711.

European Union. Regulation (EU) 2018/848 of the European Parliament and of the Council of 30 May 2018 on Organic Production and Labelling of Organic Products and Repealing Council Regulation (EC) No 834/2007. EUR-Lex 02018R0848-20241201.

Establés-Ortiz, B., Romero, P., Ballester, A.R., González-Candelas, L., Lafuente, M.T., 2016. Inhibiting ethylene perception with 1-methylcyclopropene triggers molecular responses aimed to cope with cell toxicity and increased respiration in citrus fruits. Plant Physiol. Biochem. 103, 154–166. https://doi.org/10.1016/j.plaphy.2016.02.036.

Feng, G., Wu, J., Xu, Y., Lu, L., Yi, H., 2021. High-spatiotemporal-resolution transcriptomes provide insights into fruit development and ripening in *Citrus sinensis*. Plant Biotechnol. J. 19, 1337–1353. https://doi.org/10.1111/pbi.13549.

Guo, J., Wang, S., Yu, X., Dong, R., Li, Y., Mei, X., Shen, Y., 2018. Polyamines regulate strawberry fruit ripening by abscisic acid, auxin, and ethylene. Plant Physiol. 177, 339–351. https://doi.org/10.1104/pp.18.00245.

Hasan, M., Skalicky, M., Jahan, M.S., Hossain, M., Anwar, Z., Nie, Z.F., Alabdallah, N.M., Brestic, M., Hejnak, V., Fang, X.-W., 2021. Spermine: its emerging role in regulating drought stress responses in plants. Cells 10, 1–15. https://doi.org/10.3390/cells10020261.

Hou, J., Liang, L., Su, M., Yang, T., Mao, X., Wang, Y., 2021. Variations in phenolic acids and antioxidant activity of navel orange at different growth stages. Food Chem. 360, 129980. https://doi.org/10.1016/j.foodchem.2021.129980.

Hussain, Z., Singh, Z., 2015. Involvement of polyamines in creasing of sweet orange [*Citrus sinensis* (L.) Osbeck] fruit. Sci. Hortic. 190, 203–210. https://doi.org/10.1016/j.scienta.2015.04.013.

Jangra, A., Chaturvedi, S., Kumar, N., Sing, H., Sharma, V., Thakur, M., Tiwari, S., Chhokar, V., 2023. Polyamines: the gleam of next-generation plant growth regulators for growth, development, stress mitigation, and hormonal crosstalk in plants—a systematic review. J. Plant Growth Regul. 42, 5167–5191. https://doi.org/10.1007/s00344-022-10846-4.

Kawaleguet, M.G., Chen, F.Y., Shi, W.L., Wu, Z.B., Wang, L.Y., Ban, Z.J., Liu, L.L., Wang, L.J., Wu, Y.F., 2022. Exogenous polyamines alleviate chilling injury of *Citrus limon* fruit. Int. Food Res. J. 29, 698–706. https://doi.org/10.47836/ifrj.29.3.21.

Kuang, J.F., Chen, J.Y., Luo, M., Wu, K.Q., Sun, W., Jiang, Y.M., Lu, W.J., 2012. Histone deacetylase HD2 interacts with ERF1 and is involved in longan fruit senescence. J. Exp. Bot. 63, 441–454. https://doi.org/10.1093/jxb/err290.

Kusano, T., Berberich, T., Tateda, C., Takahashi, Y., 2008. Polyamines: essential factors for growth and survival. Planta 228, 367–381. https://doi.org/10.1007/s00425-008-0772-7.

Lafuente, M.T., Sala, J.M., Zacarias, L., 2004. Active oxygen detoxifying enzymes and phenylalanine ammonia-lyase in the ethylene-induced chilling tolerance in citrus fruit. J. Agric. Food Chem. 52, 3606–3611. https://doi.org/10.1021/jf035185i.

Lafuente, M.T., Zacarias, L., Martínez-Téllez, M.A., Sánchez-Ballesta, M.T., Granell, A., 2003. Phenylalanine ammonia-lyase and ethylene in relation to chilling injury as affected by fruit age in citrus. Postharvest Biol. Technol. 29, 309–318. https://doi.org/10.1016/S0925-5214(03)00047-4.

Li, L., Lv, F.Y., Guo, Y.Y., Wang, Z.Q., 2016. Respiratory pathway metabolism and energy metabolism associated with senescence in postharvest Broccoli (*Brassica oleracea* L. var. italica) florets in response to O2/CO2 controlled atmospheres. Postharvest Biol. Technol. 111, 330–336. https://doi.org/10.1016/j.postharvbio.2015.09.032.

Lokesh, V., Manjunatha, G., Hegde, N.S., Bulle, M., Puthusseri, B., Gupta, K.J., Neelwarne, B., 2019. Polyamine induction in postharvest banana fruits in response to NO donor SNP occurs via l-arginine mediated pathway and not via competitive diversion of S-adenosyl-l-methionine. Antioxidants 8, 358. https://doi.org/10.3390/antiox8090358.

Martínez-Esplá, A., Zapata, P.J., Valero, D., Martínez-Romero, D., Díaz-Mula, H.M., Serrano, M., 2018. Preharvest treatments with salicylates enhance nutrient and antioxidant compounds in plum at harvest and after storage. J. Sci. Food Agric. 98, 2742–2750. https://doi.org/10.1002/jsfa.8770.

Martínez-Romero, D., Valero, D., Serrano, M., Martínez-Sánchez, F., Riquelme, F., 1999. Effects of post-harvest putrescine and calcium treatments on reducing mechanical damage and polyamines and abscisic acid levels during lemon storage. J. Sci. Food Agric. 79, 1589–1595. https://doi.org/10.1002/(SICI)1097-0010(199909)79:12<1589::AID-JSFA403>3.0.CO;2-J.

McDonald, R.E., 1989. Temperature-conditioning affects polyamines of lemon fruits stored at chilling temperatures. Hortscience 24, 475–477. https://doi.org/10.21273/HORTSCI.24.3.475.

Megías, Z., Martínez, C., Manzano, S., García, A., Rebolloso-Fuentes, M.M., Valenzuela, J.L., Garrido, D., Jamilena, M., 2016. Ethylene biosynthesis and signaling elements involved in chilling injury and other postharvest quality traits in the non-climacteric fruit of zucchini (*Cucurbita pepo*). Postharvest Biol. Technol. 113, 48–57. https://doi.org/10.1016/j.postharvbio.2015.11.001.

Mehta, R.A., Cassol, T., Li, N., Ali, N., Handa, A.K., Mattoo, A.K., 2002. Engineered polyamine accumulation in tomato enhances phytonutrient content, juice quality, and vine life. Nat. Biotechnol. 20, 613–618. https://doi.org/10.1038/nbt0602-613.

Mo, A., Xu, T., Bai, Q., Shen, Y., Gao, F., Guo, J., 2020. FaPAO5 regulates Spm/Spd levels as a signaling during strawberry fruit ripening. Plant Direct 4, e00217. https://doi.org/10.1002/pld3.217.

Moschou, P.N., Paschalidis, K.A., Delis, I.D., Andriopoulou, A.H., Lagiotis, G.D., Yakoumakis, D.I., Roubelakis-Angelakis, K., 2008. Spermidine exodus and oxidation in the apoplast induced by abiotic stress is responsible for $H_2O_2$ signatures that direct tolerance responses in tobacco. Plant Cell 20, 1708–1724. https://doi.org/10.1105/tpc.108.059733.

Puente-Moreno, J., Garrido-Auñón, F., García-Pastor, M.E., Valero, D., Serrano, M., 2025. The renaissance of polyamines: new roles in crop yield and quality properties in freshly fruit. Agronomy 15, 201. https://doi.org/10.3390/agronomy15010201.

Rodrigo, M.J., Zacarias, L., 2007. Effect of postharvest ethylene treatment on carotenoid accumulation and the expression of carotenoid biosynthetic genes in the flavedo of orange (*Citrus sinensis* L. Osbeck) fruit. Postharvest Biol. Technol. 43, 14–22. https://doi.org/10.1016/j.postharvbio.2006.07.008, 2007.

Romero, P., Lafuente, M.T., 2022. Ethylene-driven changes in epicuticular wax metabolism in citrus fruit. Food Chem. 372, 131320. https://doi.org/10.1016/j.foodchem.2021.131320.

Sánchez-Rodríguez, E., Romero, L., Ruiz, J.M., 2016. Accumulation of free polyamines enhances the antioxidant response in fruits of grafted tomato plants under water stress. J. Plant Physiol. 190, 72–78. https://doi.org/10.1016/j.jplph.2015.10.010.

Sati, H., Oberoi, H.S., Pareek, S., 2024. Is ATP a signaling regulator for postharvest chilling tolerance in fruits? Hortic. Res. 11, uhae204. https://doi.org/10.1093/hr/uhae204.

Serna-Escolano, V., Dobón-Suárez, A., Giménez, M.J., Zapata, P.J., Gutiérrez-Pozo, M., 2023. Effect of fertigation on the physicochemical quality and antioxidant system of 'Fino' lemons during postharvest Storage. Agriculture 13, 766. https://doi.org/10.3390/agriculture13040766.

Serna-Escolano, V., Giménez, M.J., García-Pastor, M.E., Dobón-Suárez, A., Pardo-Pina, S., Zapata, P.J., 2022. Effects of degreening treatment on quality and shelf-life of organic lemons. Agronomy 12, 270. https://doi.org/10.3390/agronomy12020270.

Shi, J., Fu, X.Z., Peng, T., Huang, X.S., Fan, Q.J., Liu, J.H., 2010. Spermine pretreatment confers dehydration tolerance of citrus in vitro plants via modulation of antioxidative capacity and stomatal response. Tree Physiol. 30, 914–922. https://doi.org/10.1093/treephys/tpq030.

Tanou, G., Ziogas, V., Belghazi, M., Christou, A., Filippou, P., Job, D., Fotopoulos, V., Malassiotis, A., 2014. Polyamines reprogram oxidative and nitrosative status and the proteome of citrus plants exposed to salinity stress. Plant Cell Environ. 37, 864–885. https://doi.org/10.1111/pce.12204.

Torrigiani, P., Bressanin, D., Ruiz, K.B., Tadiello, A., Trainotti, L., Bonghi, C., Ziosi, V., Costa, G., 2012. Spermidine application to young developing peach fruits lead to a slowing down of ripening by imparing ripening-related ethylene and auxin metabolism and signaling. Physiol. Plantarum 146, 86–98. https://doi.org/10.1111/j.1399-3054.2012.01612.x.

Tosetti, R., Elmi, F., Pradas, I., Cools, K., Terry, L.A., 2020. Continuous exposure to ethylene differentially affects senescence in receptacle and achene tissues in strawberry fruit. Front. Plant Sci. 11, 174. https://doi.org/10.3389/fpls.2020.00174.

Valero, D., Martínez-Romero, D., Serrano, M., Riquelme, F., 1998. Influence of postharvest treatment with putrescine and calcium on endogenous polyamines, firmness, and abscisic acid in lemon (*Citrus lemon* L. Burm Cv. Verna). J. Agric. Food Chem. 46, 2102–2109. https://doi.org/10.1021/jf970866x.

Wong, W.S., Li, G.G., Ning, W., Xu, Z.F., Hsiao, W.L.W., Zhang, L.Y., Li, N., 2001. Repression of chilling-induced ACC accumulation in transgenic citrus by over-production of antisense 1-aminocyclopropane-1-carboxylate synthase RNA. Plant Sci. 161, 969–977. https://doi.org/10.1016/S0168-9452(01)00505-2.

Wormit, A., Usadel, B., 2018. The multifaceted role of pectin methylesterase inhibitors (PMEIs). Int. J. Mol. Sci. 19, 2878. https://doi.org/10.3390/ijms19102878.

Zacarias, L., Alferez, F., 2007. Regulation by carbon dioxide of wound-induced ethylene biosynthesis in the peel of citrus fruit. Food Sci. Technol. Int. 13, 497–504. https://doi.org/10.1177/1082013207087812.

Zacarias, L., Cronjé, P., Palou, L., 2020. Postharvest Technology of Citrus Fruits. Elsevier eBooks, pp. 421–446. https://doi.org/10.1016/B978-0-12-812163-4.00021-8.

Zapata, P.J., Serrano, M., Pretel, M.T., Amoros, A., Botella, M.A., 2003. Changes in ethylene evolution and polyamine profiles of seedlings of nine cultivars of *Lactuca sativa* L. in response to salt stress during germination. Plant Sci. 164, 557–563. https://doi.org/10.1016/S0168-9452(03)00005-0.

Zapata, P.J., Serrano, M., Pretel, M.T., Amorós, A., Botella, M.A., 2004. Polyamines and ethylene changes during germination of different plant species under salinity. Plant Sci. 167, 781–788. https://doi.org/10.1016/j.plantsci.2004.05.014.

Zarza, X., Atanasov, K.E., Marco, F., Arbona, V., Carrasco, P., Kopka, J., Fotopoulos, V., Munnik, T., Gómez-Cadenas, A., Tiburcio, A.F., Alcázar, R., 2017. Polyamine oxidase 5 loss-of-function mutations in *Arabidopsis thaliana* trigger metabolic and transcriptional reprogramming and promote salt stress tolerance. Plant Cell Environ. 40, 527–542. https://doi.org/10.1111/pce.12714.

Zhou, M., Guo, S., Zhang, J., Zhang, H., Li, C., Tang, X., Ren, Y., Gong, G., Xu, Y., 2016. Comparative dynamics of ethylene production and expression of the ACS and ACO genes in normal-ripening and non-ripening watermelon fruits. Acta Physiol. Plant. 38, 228. https://doi.org/10.1007/s11738-016-2248-x.