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Effect of Fertigation on the Physicochemical Quality and Antioxidant System of 'Fino' Lemons during Postharvest Storage

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Abstract: Fertigation is a technique of injecting fertilizers along the irrigation lines, allowing a precise control of the moisture and the application. Nowadays, the main fertilizers used are nitrogen and potassium. Usually, an excess of fertigation is applied to achieve an early harvest of the lemon fruit. However, there is no literature available regarding the effects of excess fertigation on lemon fruit quality and antioxidant systems at harvest and during cold storage. Therefore, the present study aims to investigate these effects. This experiment was developed by comparing two plots: the first one with standard fertigation (SF) and the second one with intensive fertigation (IF). The yield per tree in the early harvest was higher in the IF than the SF plot; however, total yield was similar under both fertigation strategies. Lemons from the SF plot maintained higher total phenolic content and total antioxidant activity compared with lemons from the IF plot. In addition, firmness, total soluble solids and titratable acidity were also higher in SF lemons. Furthermore, weight loss, ethylene production, colour (parameter a^*) and decay incidence were reduced in lemon fruits from the SF plot. Early lemons harvested from the standard fertigation plot showed high physicochemical quality and antioxidant properties, reducing lemon fruit decay during cold storage and providing high-quality fruits to consumers.

Keywords: quality lemon; fertigation; total antioxidant activity; total phenolic content; decay



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1. Introduction

Lemon fruit (*Citrus limon* L. Burm.f.) is one of the most important crops worldwide. It is the third main Citrus species produced after oranges and mandarins. The main requirement demanded by consumers is a high-quality lemon fruit during the entire year, which is an important consideration for the producers [1]. The external quality of lemon fruit is attributed to visual appearance, freshness and firmness, while internal quality properties are related to fruit flavour, taste and antioxidant capacity. Lemon fruit is well known as an important source of bioactive compounds, such as vitamin C, phenolics and flavonoids. These compounds are involved in fruit senescence and decay resistance, and they provide health benefits to humans [2,3]. Thus, the development of strategies focused on increasing these bioactive compounds could be interesting for producers and consumers.

The lemon crop is classified into early and late season cultivars, the most important being 'Eureka', 'Meyer', 'Fino', 'Femminello' and 'Kütdlken'. 'Fino' grafted on *Citrus macrophylla* is an early season cultivar grown in Spain. The use of this cultivar allows a harvest season from September to December. These lemon fruits are harvested when their size reaches a diameter greater than 55 mm. Therefore, producers use intensive fertigation strategies to promote a fast fruit growth, which could affect the fruit quality [4]. Drip fertigation is commonly used for the application of fertilizers and water in all fruit and vegetable crops. An optimal fertigation management allows a consistent application of nutrients, improving its efficiency. Such planning of strategies to create synergies between

different system components is an emerging feature of agroecological systems. In this sense, water supply is an essential resource for crop management, which is applied in high doses during the different growth and development stages of the fruit to achieve, in this way, an early start of the harvest. However, excessive use of water needs to be controlled to maximize the efficiency of this valuable resource [5]. Different levels of irrigation can affect fruit quality, especially the organoleptic parameters, such as changes in acidity or sugar content [6]. Previous results have shown that modifying the water supply in grapefruits affected their phenolic content and acidity [7]. However, the phenological stage at which the irrigation changes are applied, and its intensity and duration are critical for the effectiveness of the irrigation strategy [4]. The primary inorganic nutrients, nitrogen (N) and potassium (K), are involved in the control of plant vigour, yield and fruit quality [8,9]. Nitrogen availability can determine crop growth and yield since it promotes the synthesis of amino acids and proteins in the plant. However, an excess of N fertilization can negatively affect the fruit quality after harvest [10]. In this sense, the N excess could be a factor that disrupts the balance between reactive oxygen species (ROS) and the antioxidant protection system in plants. Meanwhile, K is an abundant component of most soil types, although its availability for the plant is very low. The uptake of K depends on different factors, such as an adequate soil moisture supply, solid mass flow and plant factors, including genetics and developmental stage [11]. The use of an optimal K concentration has some benefits such as the increase in yield, fruit size and bioactive compounds. However, the most important effect of K fertilization is the colour improvement of the fruit [12]. In tomatoes [13], sweet peppers [14] and grapes [15] treated with K, the colour changes have been directly related to an increased biosynthesis of carotenoids.

Finally, growers are applying intensive strategies to promote the early harvest of the lemon fruit; however, the effect on the fruit quality during cold storage is still unknown. Therefore, the aim of this work is to elucidate the quality parameter changes produced at harvest and during cold storage of lemon fruits treated with an excess of fertilization.

2. Materials and Methods

2.1. Experimental Field Design and Postharvest Storage Conditions

The experimental field is located in Cartagena (Murcia, Spain). The weather data were collected during the whole experiment, resulting in an average temperature of 18 °C and 250 mm of rainfall during the 2021–2022 season. The soils are aridisol type composed of 45% clay, 25% loam and 33% sand, with a pH of 8.03 and an electric conductivity of 0.32 dS m⁻¹. This field has been cultivated using standard agronomical practices. Two plots of 8-year-old ‘Fino’ lemon trees grafted on *Citrus macrophylla* rootstock with an independent drip fertigation system were selected for this experiment. One plot that included 721 trees was cultivated with an intensive fertigation (IF) strategy during flowering, fruit growth and fruit ripening stages. In the second plot of 816 trees, a standard fertigation (SF) was applied (Table 1) coinciding with the lemon growth stage from mid-June to early September. The fertilizers used for this experiment were organic liquid nitrogen and potassium oxide (K₂O).

Lemon fruits with a green colour and a diameter of about 55 mm were harvested on the 10th of October, 2021. The number of fruits per tree with the optimal diameter (55 mm) and the total yield (kg tree⁻¹) was measured and the fruit mass was estimated. Fruit samples were taken from three blocks of 10 trees randomly distributed along the control and treatment plots. These fruits were transferred to the laboratory within a 2 h period and a degreening treatment was conducted in a chamber at 25 °C and a high relative humidity (RH) of 90–95% for eight days. Then, six lots of 10 fruits uniform in size and colour and not presenting any visual physical damage were selected from each of the fields and stored for 35 days at 10 °C and 85% RH. One set of lemons from each field was randomly selected at different sampling days (0, 7, 14, 21, 28 and 35) during the cold storage period for the analytical determinations.

Table 1. Total irrigation ($\text{m}^3 \text{ Ha}^{-1}$) 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–September) and fruit ripening (October–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

2.2. Physiological and Quality Parameters

Ethylene production was determined using a gas chromatographer (TMGC-2010, Shimadzu, Kyoto, Japan) fitted with a flame ionization detector. Each fruit was placed in a 0.5 L airtight glass jar for 60 min. Then, a volume of 1 mL of the headspace was injected into the GC and the data are reported as $\text{ng Kg}^{-1} \text{ h}^{-1}$ [16]. A Radwag WLC 2/A2 precision scale with two digits of accuracy was used for measuring weight loss (WL). These data are presented in percentage (%) of weight loss, as a result of the difference of each fruit weight at the start (day 0) and at each sampling day of the experiment. Firmness was measured using a TX-XT2i texture analyzer (Stable Microsystems, Godalming, UK) coupled to a steel rod, where a 5% of deformation was applied and results were reported in N mm^{-1} . A Minolta colourimeter (CRC200; Minolta, Osaka, Japan) was used to measure the colour parameter a^* at three different points on the equatorial perimeter of the fruit. This parameter allows the accurate measurement of the colour changes from green to yellow that occur in lemon fruit, an essential parameter during the degreening treatment. Total soluble solids (TSS) were determined in lemon juice using a digital refractometer (Hanna Instruments, Rhode Island, USA). The titratable acidity (TA) was also measured in the lemon juice using an automatic titrator (785 DMP Titrino, Metrohm, Herisau, Switzerland) where 0.5 mL of juice was neutralized with NaOH 0.1 mM until a pH of 8.1 was achieved. The TSS and TA results are reported as g sucrose equivalent Kg^{-1} and g citric acid equivalent Kg^{-1} , respectively. These parameters were analyzed individually in ten fruits from each lot, and the results are presented as mean $\pm$ SE. The incidence of decay was evaluated at each sampling day. The lemon fruits with disease symptoms were discarded and the percentage of decay was calculated using the formula presented below:

$$\text{Decay (\%)} = (\text{decayed fruits} / \text{total evaluated fruits}) \times 100 \quad (1)$$

2.3. Total Phenolics and Total Antioxidant Activity

Total phenolics were extracted in the flavedo by homogenizing 2 g of tissue with 15 mL of a solution of water: methanol (2:8) containing 2 mM NaF to inactivate the polyphenol oxidase activity. The extracts were centrifuged at 10,000 g for 15 min at 4 °C. Then, the Folin–Coicalteau reagent was used for the analysis of the total phenolic content (TPC) in duplicate in the supernatant, as previously reported [2]. The results are expressed in g gallic acid equivalent (GAE) Kg^{-1} of fresh weight (FW). In addition, the total antioxidant activity (TAA) was measured in duplicate in each flavedo extract using the ABTS-peroxidase system as previously described [17]. The results are expressed as g Trolox equivalents Kg^{-1} FW. These results are expressed as mean $\pm$ SE.

2.4. Statistical Analysis

The results obtained are expressed as the mean $\pm$ SE of three randomized replicates for all the physiological and bioactive parameters. An analysis of variance (ANOVA) was conducted on the data and the significance of these differences among treatments was

evaluated using Tukey's range test ($p < 0.05$) with the SPSS software package, version 22 (IBM Corp., Armonk, NY, USA).

3. Results and Discussion

3.1. Total Phenolic Content and Antioxidant Activity

Phenolic compounds as well as ascorbic acid are responsible for most of the antioxidant properties attributed to lemon fruits. These antioxidant compounds play an important role as they decrease the effects of free radicals produced during the senescence process [18]. Changes in total phenolic content occur during the development cycle of lemon fruit, with higher levels in early lemons compared with those harvested at the end of the season [19]. These differences could be associated with an increase in the polyphenol oxidase enzyme activity in the ripened lemon fruits [20].

The results showed that TPC in the flavedo of lemons cultivated with IF at day 0 was $2.22 \pm 0.04 \text{ g Kg}^{-1}$, being significantly lower ($p < 0.05$) than those lemons raised in the SF plot with a decrease of 18% (Figure 1A). The effect of different fertigation strategies on phenolics during growth and ripening on the tree is still unknown. Previous results have shown the importance of the water volumes and the nutrient solutions applied through drip fertigation on the fruit quality [17]. For example, increasing the nutritive value (N, K, P and organic matter) in the soil substrate of peppers reduced TPC [21]. In mandarins, the application of K solutions increased TPC in juice [22]. Meanwhile, in peaches fertigated with different doses of N it was found that at high doses of N, lower concentrations of phenolics were observed [23]. These differences could be related to the different ripening stages of fruits at harvest, which is in accordance with the results shown in Figure 1A. Furthermore, a decrease in TPC and flavonoid content with an increase in N application has been previously reported in tomatoes and broccoli [24,25]. These studies showed the importance of an optimal N concentration in the solution used during the fertigation schedule to maintain a high TPC. In addition, previous results on nectarines showed the effect of precise management to reduce the polyphenol oxidase (PPO) activity and maintain a high TPC [26]. During cold storage, TPC increased in both treatments, reaching final levels of 2.62 ± 0.06 and $3.18 \pm 0.07 \text{ g Kg}^{-1}$ in lemons from the IF and SF plots, respectively (Figure 1A). This effect could be attributed to the stimulation of the phenylalanine ammonia-lyase (PAL) activity, a key enzyme in the phenolic biosynthesis pathway [27]. Previous authors have reported that a rise in the PAL activity increases fruit tolerance to chilling injury [28]. Furthermore, TAA in lemon fruit is strongly related to the accumulation of phenolic compounds [29]. Therefore, the TAA results were similar to TPC (Figure 1B), showing an increasing tendency throughout the storage period. Lemons from the SF plot had significantly higher ($p < 0.05$) levels of TAA than those from the IF plot, 30% on average. Neither fertigation strategies affected the evolution of TAA during cold storage. Therefore, the differences observed at harvest were maintained (Figure 1B). These results were similar to those published for oranges, where fruits that presented a higher level of ripening had the lowest antioxidant activity and TFC [30]. Therefore, lemon fruits produced using the IF strategy could accelerate the ripening process compared with those grown using the SF strategy.

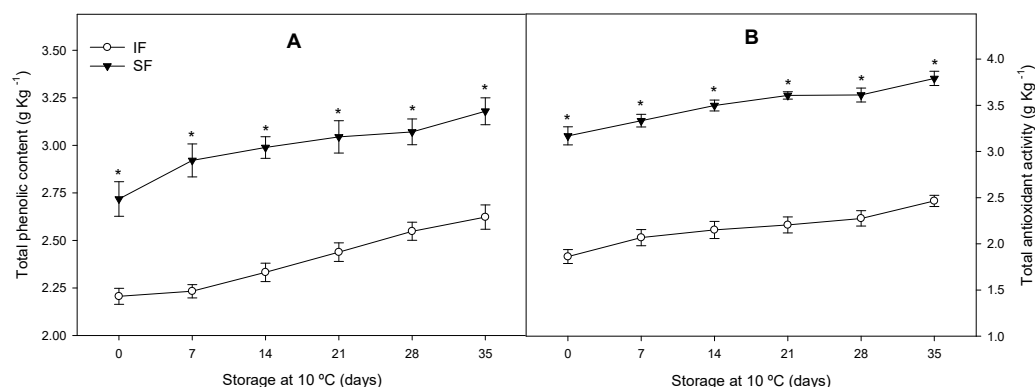


Figure 1. Effect of standard fertilization (SF) and intensive fertilization (IF) on the total phenolic content (A) and total antioxidant activity (B) in lemon fruits stored for 35 days at 10 °C. Asterisks (*) represent significant differences between lemon fruits cultivated with IF and those with SF at $p < 0.05$.

3.2. Crop yield and Quality Parameters

The number of early lemons harvested with the targeted fruit size from the SF plot was significantly ($p < 0.05$) lower than in the IF plot (Table 2). The number of fruits up to 55 mm harvested per tree was reduced by 10% in the SF plots compared with the plots cultivated with an IF strategy (653 ± 18 fruit tree⁻¹). Likewise, yield and fruit mass were higher in the IF than in the SF plot (Table 2). However, total yield at the end of the season was the same for both fertilization strategies (data not presented). These results are in accordance with previous results on tomatoes [31]. Similarly, the size of mangoes was affected when different fertilization strategies were applied during the fruit growth stage [32]. Nowadays, consumers demand fruits and vegetables with high-quality attributes throughout the whole year. These demands determine the agronomic strategies that are applied in the field. Hence, strategies such as the use of early cultivars and high fertilization levels may affect the quality of the fruit at harvest and during storage [33]. Furthermore, the application of intensive cultivation strategies could increase the price of fruits and their environmental sustainability.

Table 2. Effect of standard fertilization (SF) and intensive fertilization (IF) on yield (kg tree⁻¹), number of fruits per tree (fruit tree⁻¹) and fruit mass (g) in the early harvest. Different lowercase letters show significant differences among treatments.

Strategy	Yield (kg tree ⁻¹)	Fruit Tree ⁻¹	Fruit Mass (g)
IF	85.27 ± 3.34 a	653 ± 18 a	130.12 ± 2.87 a
SF	68.17 ± 2.55 b	589 ± 12 b	117.29 ± 3.10 b

Weight loss is an important quality parameter of lemon fruit determining consumer acceptability. It is mainly due to transpiration through the fruit peel and the senescence process [29]. The results show that the WL of the lemon fruit increased during cold storage, although significant differences ($p < 0.05$) were only detected after 28 days. At the end of storage, the WL in lemons harvested from the SF plots was significantly less (28%) than for lemons from the IF plots (Figure 2A). These results were similar to a previous study in mandarins where the concentration of nutrients applied through fertilization during the fruit growth was reduced [34]. In addition, a reduction of WL in SF lemons could be related to the conservation of cell structural integrity, considering that it was directly associated with lemon fruit development and ripening stages [35]. Fruit firmness presented a decreasing tendency during cold storage in lemons harvested from both plots (Figure 2B). However, firmness was 20% significantly higher in IF lemons than in the SF ones at day 0. These differences at harvest could be due to cell turgor, considering that fruits can act as a water deposit [20]. After 21 days of cold storage, firmness was significantly higher in

lemons grown under SF management than the IF strategy. This effect could be related to the influence of the pectin, hemicellulose and cellulose content, which are the main components of the cell wall of the flavedo and determine the fruit firmness. The activity of the main enzymes (polygalacturonase, pectin methyl esterase and cellulase) that are involved in fruit softening is increased during the senescence process [29]. It has been previously reported that high N and K fertigation applied in tomatoes increased the size of fruits. In addition, it was observed that a decrease in the calcium concentration, when high doses of N were applied to tomatoes, resulted in a loss of cell wall integrity and the tissue becoming less firm during storage [36]. In this sense, ‘Murcott’ mandarins cultivated with 75% of the recommended N dose achieved the optimal fruit firmness and peel thickness [37]. These differences in WL and firmness between both irrigation strategies did not concur with the respiration rates of the fruits, which did not show significant differences ($p < 0.05$) during cold storage (data not presented). Ethylene production showed different tendencies between treatments during cold storage (Figure 2C). Lemons from the SF plot presented very low ethylene production. Conversely, lemons grown under IF management showed a high peak at the beginning of the experiment. The ethylene production was maintained at high levels in IF lemons compared with SF ones during cold storage. Lemon fruit is a non-climacteric fruit that does not present respiration or ethylene production peaks associated with ripening. However, when lemon fruits are exposed to stress, basal production can increase [29]. Previous results in pears showed that a high concentration of N in the fertigation solution increased ethylene production, whereas different doses of K did not affect this parameter [11]. In addition, during *Penicillium digitatum* infection in orange fruits, ethylene production was induced as a consequence of the interaction between the pathogen and the fruit [38]. Lemon fruits from both plots were harvested with a green skin colour and exposed to a degreening treatment in controlled chambers. This postharvest storage promotes chlorophyll degradation and increases carotenoid metabolism, changing the green skin to a more favourable yellow colour [19]. The colour changes are represented with the parameter a^* in Figure 2D. The results show that lemons from the IF plot presented 6-fold higher values compared with those from the SF plot. These results are similar to those of a previous study in ‘Newhall navel’ oranges, where K and N fertigation improved the fruit surface colour [39].

Total soluble solids (TSS) in fruits are directly associated with the photosynthetic rate of the tree, which could involve a sugar accumulation in the fruit [28]. Figure 3A presents the TSS results, which show a slight decrease during cold storage in lemons harvested from both plots. However, the TSS content was 28% higher in SF lemons compared with IF ones at day 0. These significant differences ($p < 0.05$) were maintained during the cold storage (Figure 3A). Similar results were reported when an excess of fertigation with N or K solutions was applied, where a reduction in the TSS content was observed in tomatoes [31]. However, other studies reported that high concentrations of K during fertigation could produce an increase in the TSS content, promoting the palatability of tomatoes [40]. Titratable acidity (TA) is associated with citric acid, malic acid and fumaric acid, the main organic acids used in the pyruvate decarboxylation reaction as a carbon source [35]. The results of TA are presented in Figure 3B, which show significantly higher ($p < 0.05$) TA in lemons from the SF plot compared with the IF plot at day 0 and during the whole period of storage. Titratable acidity was 15% higher in SF lemons after 35 days of cold storage (Figure 3B). Therefore, the catabolism of citric acid could be higher in SF lemons than in IF ones, which could be an indicator of different ripening stages in the fertigation strategies. In addition, an excess of fertigation with N and K solutions has been shown to induce fruit growth, hence the decrease in acidity could be due to an increase in the juice percentage of the fruit [22]. Furthermore, N is closely related to the accumulation of sugars and organic acids in fruits. Previous results in *Citrus reticulata* showed that applying medium rates of N achieved fruits with high TSS/TA ratios [41].

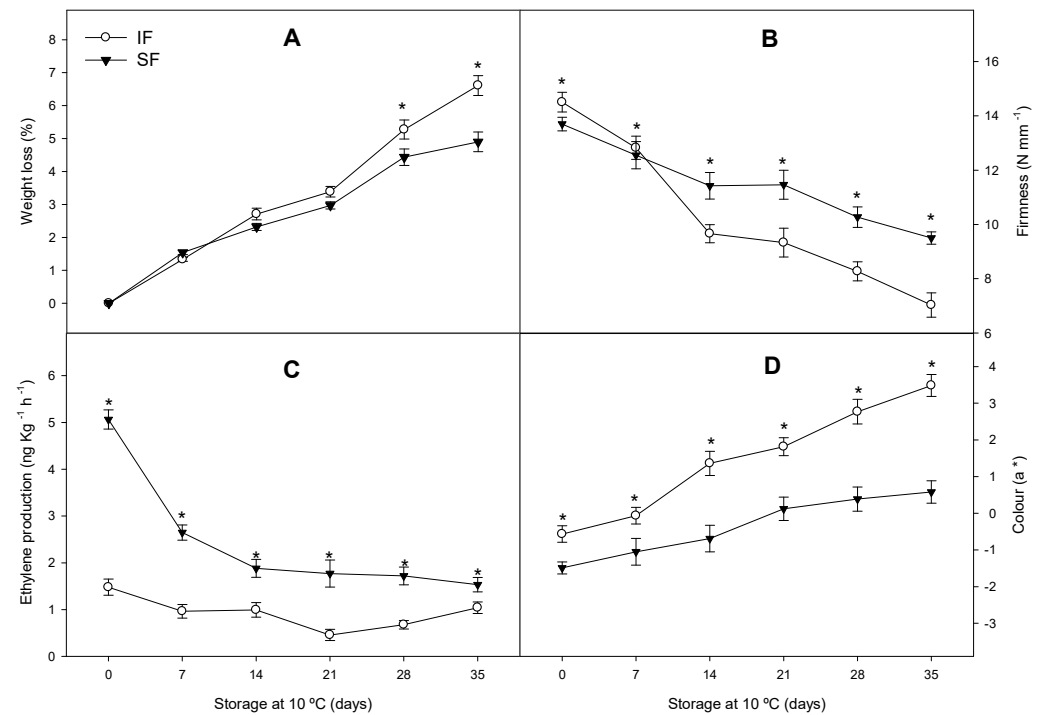


Figure 2. Effect of standard fertilization (SF) and intensive fertilization (IF) on weight loss (A), firmness (B), ethylene production (C) and colour (D) in lemon fruits stored for 35 days at 10 °C. Asterisks (*) show significant differences between lemon fruits cultivated with IF and those with SF at $p < 0.05$.

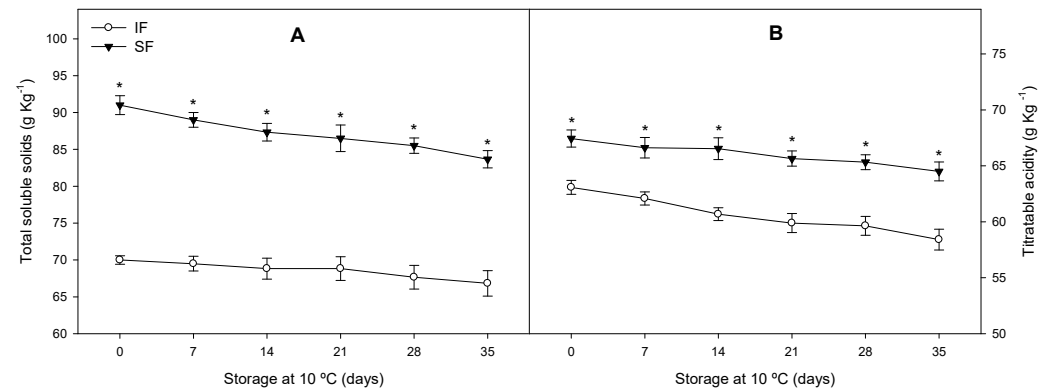


Figure 3. Effect of standard fertilization (SF) and intensive fertilization (IF) on total soluble solids (A) and titratable acidity (B) in lemon fruits stored for 35 days at 10 °C. Asterisks (*) show significant differences between lemon fruits cultivated with IF and those with SF at $p < 0.05$.

Decay incidence was evaluated as the percentage of lemon fruits that showed symptoms of fungal decay. The results show that after 35 days of storage at 10 °C, the SF lemons had a significantly lower ($p < 0.05$) decay incidence than the IF lemons, a decrease of approximately 75% (Figure 4). Lemon fruit decay is mainly due to the presence of two phytopathogenic fungi, *Penicillium digitatum* and *Penicillium italicum*. The oxidative balance of the cells can be altered by both fungi destabilizing the cell wall [42]. In this sense, there is evidence that indicates the important role that the primary metabolism and the antioxidant systems play in the pathogen resistance of fruits [29]. Previous results confirmed that an optimal nutrition supply was very important in the shelf life of apples, concluding that the development stage and an excess of N fertilization could increase decay incidence during cold storage [22]. In addition, it has been observed that high concentrations of N induced calcium deficiency in tomato skins, increasing blossom-end rot incidence [36]. In contrast, K applied through fertigation reduced decay incidence in pepper stored for 28 days at 10 °C.

In oranges, this effect could be related to the role of K in increasing antioxidant systems, such as vitamin C accumulation, antioxidant enzymes and phenolics [39]. Furthermore, optimizing the fertigation strategies could prevent the decay associated with humidity excess and skin defects [43] as the fruit skin can develop microscopic breaks in the cuticle when high fertigation strategies are applied, allowing the fungi to infect the fruit.

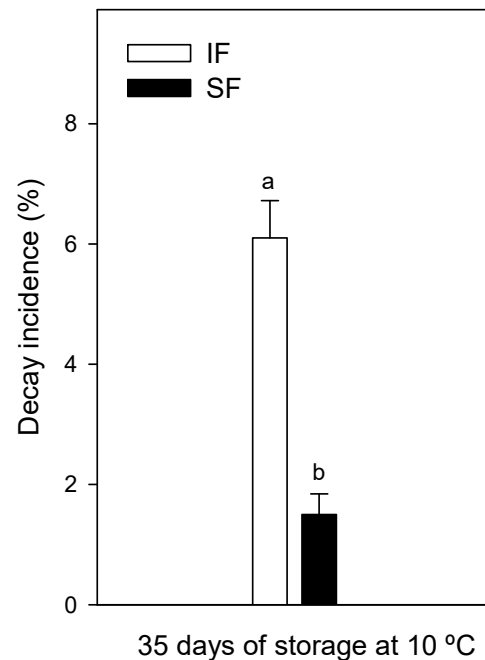


Figure 4. Effect of standard fertigation (SF) and intensive fertigation (IF) on the decay incidence of lemon fruits. Lowercase letters indicate significant differences between lemon fruits cultivated with IF and those with SF at $p < 0.05$.

4. Conclusions

This work showed that the agronomic practices that growers apply to promote optimal fruit size are drastically reducing its quality. Overall, the application of the standard fertigation strategy during the lemon fruit growth stage significantly improved the physicochemical quality of lemon fruits. In addition, that strategy maintained a high antioxidant capacity in the flavedo of lemons, delaying the senescence process and reducing their susceptibility to decay during postharvest cold storage. Therefore, growers must regulate the fertigation strategy to improve the sustainability of their crops and provide high-quality fruits to consumers.

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