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Preharvest Elicitors as a Tool to Enhance Bioactive Compounds, Quality in Both Peel and Pulp of Yellow Pitahaya (*Selenicereus megalanthus* Haw) a Harvest and during Postharvest Storage

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Abstract: Yellow pitahaya is a tropical fruit that has gained popularity in recent years. Natural elicitors are compounds that can stimulate the resistance and quality of fruits. The objective of this study was to evaluate the effects of natural elicitors: methyl salicylate (MeSa), methyl jasmonate (JaMe), salicylic acid (SA) and oxalic acid (OA) at concentrations of 0.1 mM (SaMe and JaMe) and 5 mM (SA and OA), applied to the yellow pitahaya fruits, under greenhouse conditions. After full blossom, 4 applications were made with a frequency of 15 days. At time of harvest and after storage, the following variables were evaluated: firmness (whole fruit), total soluble solids (TSS), total acidity (TA), phenolics and carotenoids (in the pulp), while phenolics, carotenoids, macronutrients and micronutrients were determined in the peel. The results showed that JaMe presented a delay of the ripening process, according to TSS, TA, firmness, and total phenolic compounds results, while MeSa advanced the fruit maturation. Regarding the alternative use of the peel as by-product, the application of natural elicitors significantly increased the content of polyphenols, carotenoids, macronutrients and micronutrients in the peel, which can be used as a bioactive compound in the food industry. In conclusion, the results indicate that natural elicitors can be an alternative to improve the quality and shelf life of yellow pitahaya fruits.

Keywords: methyl salicylate; salicylic acid; methyl jasmonate; oxalic acid; bioactive compounds; phenolics; carotenoids

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1. Introduction

The pitahaya (*Selenicereus megalanthus*) is a native plant of the *Cactaceae* family, known worldwide as the "dragon fruit" [1]. In the province of Morona Santiago, especially the Palora canton, is among the main pitahaya producing areas in Ecuador, this activity being an important source of employment and economic income for the country [2]. Yellow dragon fruit has gained popularity in the food industry and export due to its water content, nutrients and bioactive compounds, such as glucose, vitamins, organic acids, soluble dietary fibre and constituent minerals [3]. Pitahaya is considered an exotic fruit that originates from Central America and part of South America. It was initially found in the wild by the Spanish conquistadors, who baptized it with the term "pitahaya", which refers to its scaly appearance. Currently, the existence of more than one species of pitahaya is recognized, given that human intervention and influence have given rise to a diversity that encompasses both morphological and organoleptic aspects: red pitahaya (*Hylocereus undatus*) and the yellow. Compared with the red pitahaya, the Palora ecotype has higher values of firmness, total acidity (TA), total soluble solids (TSS), vitamin C, antioxidant capacity and total polyphenols [4]. Yellow pitahaya is characterized by its attractive colour and the composition of bioactive moieties such as flavonoids and other polyphenolic compounds [5] When consumed, it can provide functional actions, supporting immunity and performing antimicrobial, antioxidant, hepatoprotective, hypoglycaemic, healing and antiproliferative activities [6].

Although the cultivation and commercialization of the yellow pitahaya has gained relevance in the agri-food industry, due to the growing demand in both the national and international markets, there are significant challenges in relation to the quality and management of this exotic fruit, especially with regard to optimizing its production and sustainable use, the growth and ripening process, and postharvest handling [7]. In this sense, the use of natural elicitors compounds could be a good alternative. Elicitors present a viable option to promote sustainable agriculture to replace the use of agrochemicals in the production of food and other resources useful to people. To date, there has been no report indicating that the use of elicitors, regardless of whether they are of biotic or abiotic origin, generates adverse effects on plants, human health or the surrounding environment [8].

Agricultural crops are very sensitive to different abiotic stresses due to abnormal climatic changes, the increasing of atmospheric temperature being one of the most crucial factors, affecting crop yield performance, food quality, security, availability, and nutrient deficiencies, among others [9,10]. Therefore, it is mandatory to mitigate the climate change and adopting efficient measurements. Although there are many research limitations, the use of natural elicitors has been proved to be effective counteracting the negative effects of the climate change. Among these elicitors include methyl salicylate (MeSa), methyl jasmonate (JaMe), oxalic acid (OA) and salicylic acid (SA). The preharvest application of these compounds were effective on increasing crop yield and quality at time of harvest and after storage in several fruit commodities, such as table grape, pomegranate and sweet cherry [11–15]. These elicitors can stimulate the defence responses of plants, by the activation of phenolic compounds and antioxidant enzymes, which have the ability to counteract the negative effects of reactive oxygen species (ROS) [16].

As yellow pitahaya production advances, its relevance for industrial uses increases. This process involves the extraction of the pulp and generating a vast amount of disposal by-product, including epicarp and part of mesopcarp [17,18]. The pitahaya peel, which accounts a 35-45% of the total fruit, contains fibre, polyphenols, macro- and micronutrients, which represent a potential source for various industries, such as pharmaceuticals, cosmetics and nutraceuticals, among others [19]. The pitahaya peels contain 75.2% fibre, high amounts of vitamin C and magnesium, which makes them suitable for the production of products rich in fibre, such as cookies and fritters [20].

Given the above, as there is little scientific evidence on the effect of the application of natural elicitors on the quality of yellow pitahaya fruit and its use of the peel as a source of bioactive compound for composting. This research evaluated the effect of four natural elicitors methyl salicylate (MeSa), methyl jasmonate (JaMe), oxalic acid (OA) and salicylic acid (SA) in the quality of pitahaya at time of harvest and after storage, as well as the peel bioactive compounds. By addressing these aspects, the aim is to contribute to the development of more sustainable agricultural practices and the improvement of the dragon fruit value chain, benefiting both producers and consumers and the environment.

2. Results

The objective of this study was to evaluate the effects of the natural elicitors methyl salicylate (MeSa), methyl jasmonate (JaMe), salicylic acid (AS) and oxalic acid (AO) at concentrations of 0.1 mM and 5 mM, respectively, applied 4 times during the growth of yellow pitahaya fruits under greenhouse conditions located in the Palora, Ecuador. The efficacy of these treatments on pitahaya quality at harvest was evaluated, as well as the peel to be used as by-product. Finally, pitahaya fruits were stored to evaluate the fruit quality and the expected shelf-life.

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2.1. Fruit Quality at Harvest and during Postharvest Storage

The pitahaya quality at harvest and over storage at 10 °C was evaluated by 3 parameters: TSS and TA (**Figure 1**) and fruit firmness (**Figure 2**).

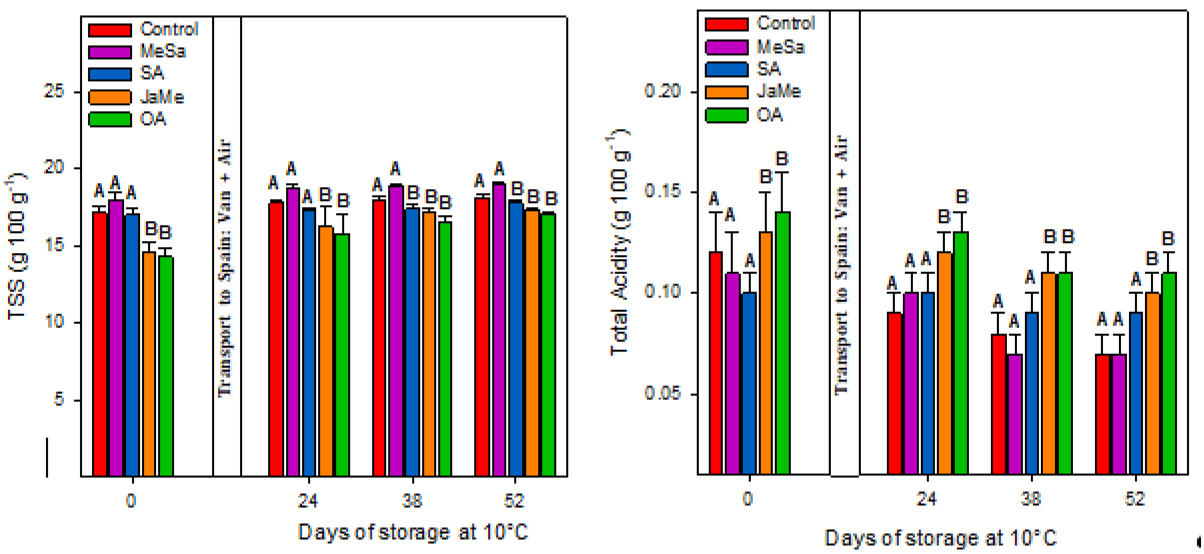

Figure 1. Total soluble solids (TSS) and total acidity (TA) concentration in pitahaya fruits as affected by preharvest treatments with methyl salicylate (MeSa), salicylic acid (SA), methyl jasmonate (JaMe), oxalic acid (OA) and control (untreated). For each sampling date, bars (mean ± SE) with different letters show significant differences at $P \leq 0.05$.

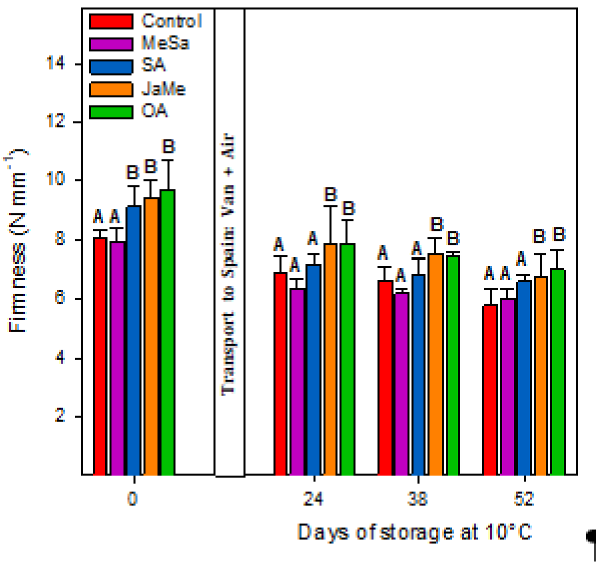

Figure 2. Firmness (N mm^{-1}) values in pitahaya fruits as affected by preharvest treatments with methyl salicylate (MeSa), salicylic acid (SA), methyl jasmonate (JaMe), oxalic acid (OA) and control (untreated). For each sampling date, bars (mean ± SE) with different letters show significant differences at $P \leq 0.05$.

At harvest time, the fruits treated with MeSa, SA and control showed significantly higher TSS (17-18 °Brix) and lower TA (≈ 0.10 g 100 g^{-1}) compared with those treated with JaMe and OA, in which lower TSS (14-15 °Brix) and higher TA (≈ 0.14 g 100 g^{-1}) were obtained. Similarly, firmness (**Figure 2**) was higher in those pitahayas treated with SA, JaMe and OA (≈ 9-19 N mm^{-1}) while the contrary occurred with those fruit treated with MeSa and control (8 N mm-1). During postharvest storage the same behaviour was observed, with MeSa and control being the treatments that exhibited higher TSS and lower TAA and firmness compared with those pitahayas treated with SA, JaMe and OA. It was noticeable that for all treatments TSS slightly increased during storage, while for TA and firmness, a significant diminution was obtained.

2.2. Mineral Composition and Bioactivos Content of the Peel

The peel of the recently harvested pitahayas was used for the determination of the mineral composition (**Figure 3**), and total phenolics and carotenoids (**Figure 4**).

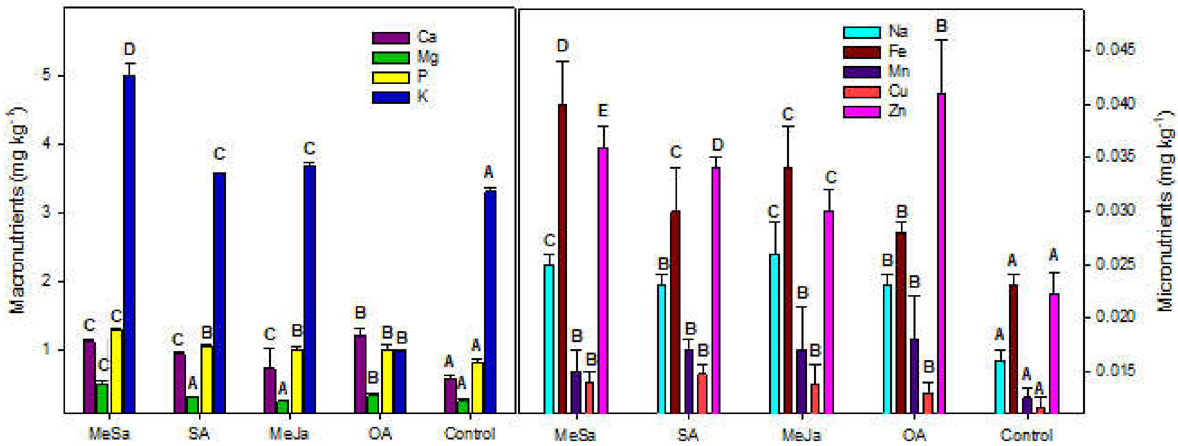

Figure 3. Concentration of Ca, Mg, P and K (macronutrients) and Na, Fe, Mn, Cu and Zn (micronutrients) in the peel of pitahaya fruits as affected by preharvest treatments with methyl salicylate (MeSa), salicylic acid (SA), methyl jasmonate (JaMe), oxalic acid (OA) and control (untreated). For each mineral, bars (mean ± SE) with different letters show significant differences among treatments at *P ≤ 0.05*.

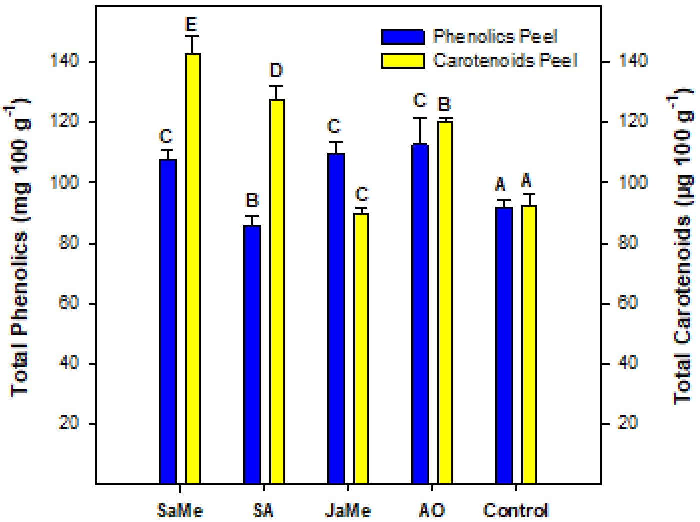

Figure 4. Concentration of total phenolics and carotenoids in the peel of pitahaya fruits as affected by preharvest treatments with methyl salicylate (MeSa), salicylic acid (SA), methyl jasmonate (JaMe), oxalic acid (OA) and control (untreated). For each bioactive compound bars (mean ± SE) with different letters show significant differences among treatments at *P ≤ 0.05*.

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The content of all macronutrients Ca, P and K was significantly higher in the peel of all pitahaya-treated fruits than in control samples, while Mg was significantly higher in MeSa- and OA- treated peels (**Figure 3**). The cation K was found as the major mineral followed by P, the content of Mg being the minor macronutrient. Among treatments, MeSa was the most effective elicitor on increasing the content of all micronutrients.

With respect to the bioactive compounds, all treatments were effective on increasing the concentration of total carotenoids in the peel of the pitahaya fruits (**Figure 4**). On the other hand, the same behaviour was observed for the concentration of total phenolics, with exception of SA-treated peels which showed the lowest content.

2.3. Bioactive Compounds and Antioxidant Activity at Harvest and during Postharvest Storage

At harvest, the content of total phenolics and total antioxidant activity (hydrophilic) was higher in SA, JaMe and OA treated pitahayas than those obtained in MeSa and control fruits (**Figure 5**).

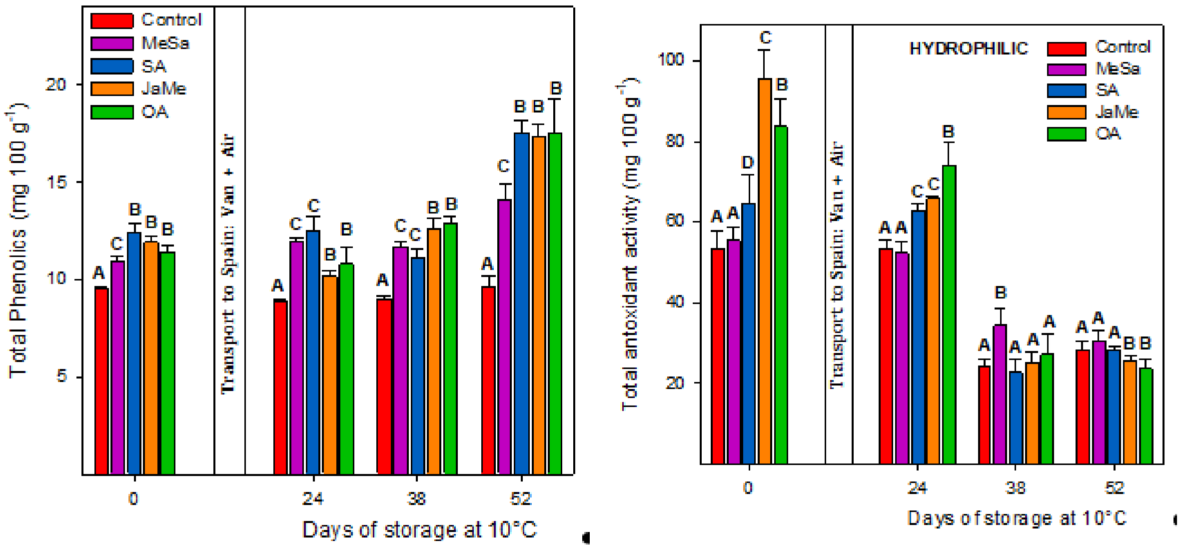

Figure 5. Total phenolics and hydrophilic antioxidant activity concentration in the pulp of pitahaya fruits as affected by preharvest treatments with methyl salicylate (MeSa), salicylic acid (SA), methyl jasmonate (JaMe), oxalic acid (OA) and control (untreated). For each sampling date, bars (mean ± SE) with different letters show significant differences at $P \leq 0.05$.

During storage, the phenolic concentration for control fruits remained unchanged while total antioxidant activity showed a significant diminution, reaching the lowest activity (≈ 30 mg of Trolox eq. 100 g^{-1}). On the contrary, the total polyphenol content (**Figure 5**) increased along storage for all treated pitahaya fruits (≈ 18 mg of gallic acid eq. 100 g^{-1}). The determination of total antioxidant activity revealed that all treated-pitahayas significantly decreased their values, as did the control fruits and also reached the minimum activity at the end of storage (≈ 30 mg of Trolox eq. 100 g^{-1}).

At harvest, the content of total carotenoids in the pulp (**Figure 6**) was significantly higher in SA-, JaMe- and OA- treated pitahayas (≈ 40-60 µg β-carotene eq. 100 g^{-1}) than those obtained in MeSa and control fruits (≈ 20 µg β-carotene eq. 100 g^{-1}). The highest carotenoid content was shown for pitahayas-treated with SA. With respect to total antioxidant activity (lipophilic) at harvest, all pitahayas treated showed significantly higher activity (≈ 5-7 mg of Trolox eq. 100 g^{-1}) than control fruits (≈ 3 mg of Trolox eq. 100 g^{-1}).

During storage, the carotenoid concentration for control fruits remained unchanged, while treated pitahayas showed a significant increase, especially with the application of OA (≈ 90-100 µg β-carotene eq. 100 g^{-1}) and JaMe (≈ 90 µg β-carotene eq. 100 g^{-1}).

The total antioxidant activity (lipophilic) along storage revealed that for all control and pitahaya-treated fruits a significant reduction was shown, although values were always significantly higher in treated than in control fruits. Interestingly, the elicitor that induced the highest lipophilic activity was MeSa.

Finally, the hydrophilic antioxidant activity was higher (≈ 50-fold) lipophilic one in the pulp of yellow pitahayas. The comparison between peel and pulp demonstrated that the concentration of total phenolics and carotenoids was 10-fold and 2.5-fold higher, respectively. in the peel than in the pulp.

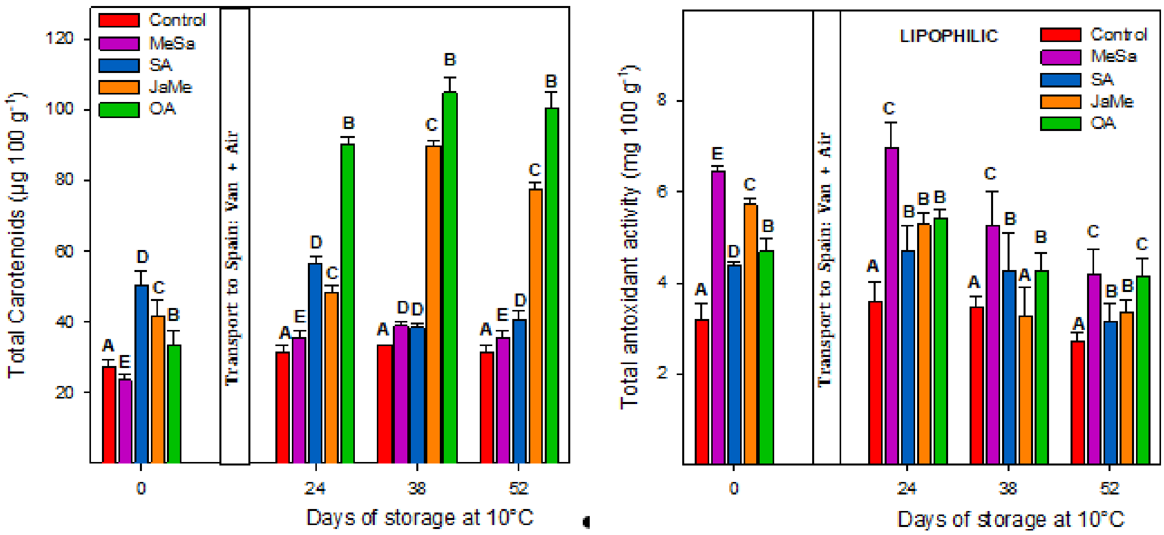

Figure 6. Total carotenoids and lipophilic antioxidant activity concentration in the pulp of pitahaya fruits as affected by preharvest treatments with methyl salicylate (MeSa), salicylic acid (SA), methyl jasmonate (JaMe), oxalic acid (OA) and control (untreated). For each sampling date, bars (mean ± SE) with different letters show significant differences at $P \leq 0.05$.

3. Discussion

The pitahaya is divided in two genera: *Hylocereus* (red skin and white or pink flesh) and *Selenicereus* (yellow skin and white flesh). Most of the research was carried out on red varieties [3,17], compared to the limited knowledge about the growth process, maturation and postharvest potential and shelf life [4].

With the aim of providing new knowledge to the quality of yellow pitahaya at the time of harvest and its behaviour during postharvest storage, in this study we consider the use of different elicitors, applied at preharvest, and their influence on several organoleptic, nutritional, functional quality parameters. In addition, some properties of the peel of yellow pitahaya were evaluated with the objective to be used as by-product.

At harvest time, the preharvest application of the elicitors affected differentially the quality traits in yellow pitahaya, since JaMe and OA showed higher values of firmness and TA, and lower TSS, while MeSA and SA did the contrary and similar behaviour to control fruits. These results demonstrate that on-tree ripening process was delayed in the fruits treated with JaMe and OA, while MeSa and SA advanced the ripening process of pitahaya. The ripening process is accompanied by fruit softening and related to degradation of the cell wall components, such as pectin and cellulose, by the action of the cell wall enzymes polygalacturonase (PG), pectin methylesterase (PME) and cellulase (CEL) causing decomposition of cellulose and hemicelluloses [21]. This softening process is accompanied by the increase in TSS parallel to the TA diminution. The differences found at harvest date were also maintained during postharvest storage, in which after 52 days pitahayas treated with JaMe and OA maintained higher firmness and TA and lower TSS than MeSa and SA.

SA and its derivative MeSa are natural-occurring considered as safe [22], and when applied as preharvest treatment could have roles as plant growth regulators and as inducers of SAR, which in turn alleviating the devastating effects of the abiotic stresses [16,23]. During pitahaya growth, ripening and postharvest storage, both SA and MeSA enhanced TSS although induced lower TA y firmness, these effects being related to an advancement of the ripening process. TSS and TA are good indicators of sweetness and sourness, respectively, being the most important parameter associate taste and determining the consumer acceptability and purchase decision [21]. During storage, the decrease in TSS and TA is mainly caused by the increase in respiration rate by utilising the reserved substances [24]. SA and MeSa have reported to modulate these quality traits although the effects depended of fruit species, type of elicitor, concentration and number of applications. For instance, preharvest SA (1, 2 and 3 mM) applied to lime resulted in hastening maturity for all doses [25], similarly to grapes at 1, 1.5 and mM [26], and in contrast SA at 1 mM accelerated ripening but at 2 mM a delay was observed in peach [27]. Table grapes treated with SA (0.01 mM) and MeSa (0.1 mM) increased TSS while ASA (acetylsalicylic acid 1 mM) which is SA derivative decreased the TSS at harvest [28].

In our previous report on pitahaya [29], preharvest SA and MeSa applied at 1, 5 and 10 mM increased TSS with the doses of 1 and 10 mM of MeSA and SA at 1 and 5 mM. In addition, the elicitors increased yield productivity and fruit weight. These results justify the concentration used on this study for storage. It seems that SA could increase the translocation of sugars from the leaves to the pitahaya and thus enhancement of TSS. During fruit growth and development, sucrose is accumulated due to the increase of the enzymes sucrose phosphate synthase and sucrose phosphatase [30], but during ripening sucrose levels decreased and parallelly to the increased levels of nonreducing sugar content, mainly glucose and fructose.

Methyl jasmonate (MeJA) is a volatile hormone derived of jasmonic acid involved in wide range of plant function, acting as a signal in response to abiotic stresses and modulating the biosynthesis of other plant growth regulators [31,32]. Plants synthesise this hormone in response to defence against biotic and abiotic stress but also modulate the fruit growth and ripening [33]. The higher TA and firmness and the lower TSS in MeJA-treated pitahaya may be attributed to ripening-retarding effects of MeJA and a delay in the senescence process. It is noteworthy that MeJa positively affects fruit growth and crop quality, this issue being extensively reported in several fruit commodities. Also, the MeJa effects on these quality traits in pitahaya applied at preharvest remained also during postharvest storage. Accordingly, postharvest MeJA treatments have been demonstrated to modify the properties of fruits during postharvest storage [34].

In this sense, MeJA increased the sugar content in peach leading to an enhancement of nutritional quality [35]. MeJa applied to Kinnow mandarin as preharvest treatment at 0.1, 0.3, 0.5 and 0.7 mM showed higher fruit firmness and TA and lower TSS/TA ratio (indicative of ripening index), the 0.5 mM. being the most effective in delaying the ripening [36]. Also, preharvest MeJA treatments resulted in higher fruit firmness values at harvest for all the evaluated cultivars, including 'Early Lory', 'Prime Giant', and 'Sweetheart', during four (2019-2022) different growing seasons [37]. It seems that MeJa leads to firmer fruit by a direct action, since this volatile compound can enhance the integrity of the cell wall [38]. Moreover, and indirect action has been also proposed by which MeJa delayed fruit softening due to an elevated and stable level of Ca^{2+} content in the cell walls. Other authors suggest that MeJa activates the pectin methylesterase (PME) enzymes, with the liberation of methyl esters from the pectins and generation of free pectins that could cross-linked with Ca^{2+}, and thus increasing cell wall firmness [39].

On the other hand, oxalic acid (OA) is a naturally-occurring organic acid belonging to the Krebs cycle with multiple functions by altering the plant metabolism. Research carried out during the last two decades, OA has proved the antioxidant activity focused essentially on the enhancement of crop yield and quality, but also showed a delay in postharvest ripening and senescence [40,41]. In yellow pitahaya, preharvest application of OA showed similar results to those obtained for MeJa-treated fruits, that is a delay of the ripening based on the lower TSS and higher TA and firmness. The role of organic acids in general, and particularly OA, play an essential role modulating the fruit ripening

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and delaying senescence during postharvest storage, as well as the upregulation the resistance against both abiotic and biotic stress. Pre- and postharvest OA application has been widely used with the objective to improve fruit quality at harvest. Preharvest OA in sweet cherry [14] and pomegranate [13] showed a clear delay in ripening and senescence with net benefits in terms of quality. The higher firmness in OA-treated pitahayas probably related to the biosynthesis of oxalate-soluble pectin and the inhibition of pectin solubilization and this maintaining higher fruit firmness [21]. This effect was shown in other fruits such as peach and lemon [42,43]. In relation to higher levels of TA could be related to lower respiration rate, since the organic acids are the primary substrates to be used in the respiration physiological process.

The edible part of the pitahaya is the pulp, which is mainly consumed as fresh fruit, juices, jams, ice cream and dessert [44]. However, the fruit has an important part of peel (which account between 40-50% of the total mass), and currently is considered waste, but could have some potential to be used as a by-product. In the case of yellow pitahaya there is almost no literature about the composition of the peel, although there is knowledge in the case of red varieties [45].

According to the latest figures, about 1.3 billion tonnes of food and food by-products are wasted each year, the agroindustry sector being very significative, since fruit peels are a good source of high value-added functional compounds to be used in food, pharmaceutical or cosmetic industries [46]. The fruit of yellow pitahaya is formed by the peel or skin, which account 35-45% of the total mass, the pulp that has a mass of 50-55 % and black seeds. Dragon fruit peels are by-products of juice production that are usually wasted, but they are rich in polyphenols, vitamins, and dietary fibers [47]. Several studies have shown that pitahaya can alleviate some diseases including cardiovascular diseases and metabolic syndrome due to occurrence of bioactive compounds such as polyphenols, betacyanins or vitamins that can be found in both in the peel or pulp of red pitahayas species [48]. However, there is no available literature about the use of peel of yellow pitahaya, which could have the potential to be used in the food industry as functional ingredients, nutraceutical compounds or edible films. Thus, the peels may be used as fat substitutes, enhancing the nutritional value and functional properties of food.

As a first approximation we analyzed the mineral composition, total phenolics and total carotenoids in the peel of yellow pitahaya cv. Palora. With respect to mineral composition in the peel of pitahaya, the preharvest elicitors contained higher concentration of the macronutrients Ca, P and K, the Mg being significantly higher in the peel of MeSa- and OA-treated pitahayas. All micronutrients (Fe, Mn, Cu, Zn and Na) were higher in treated-pitahaya, the MeSa being the most effective elicitor on increasing the content of all minerals. Numerous studies found that pitahaya (red species) has more minerals content (K, P, Na, Mg, Fe and Ca) than other tropical fruits, such as pineapple or mango [49]. Shah et al., 2020 [50] reported that the total ash and mineral content of the peel were 2-fold higher than those obtained in the pulp studying several pitahayas species either red or yellow fruits.

All treatments induced increased concentration of total carotenoids in the peel of the pitahaya fruits, the MeSa being the most effective elicitor. There is no literature reporting the carotenoids in the peel of yellow pitahaya for comparative purposes, although there are some evidences in other red pitahayas. Total carotenoids content in the peel of *H. costaricensis* (red pitahaya) at commercial ripening were ≈2 mg 100 g^{-1} [51]. In a comparative study with 3 pitahaya peels (*Hylocereus undatus*, *Hylocereus costariscensis* and *Hylocereus megalanthus*), the total carotenoids were found in the range of 18-24 μg 100 g^{-1}, the main compounds xantophyll and β-Carotene [52]. The market of natural pigments market is growing in the last decade as consumer demand alternatives to synthetic colorants, which are considered as harmful. Then, exploration natural and eco-friendly pigments is therefore necessary [53]. Contrarily to red pitahaya, which is rich in red pigments (betalains and anthocyanins), the peel of yellow pitahaya (rich in carotenoids) has not studied in depth and merits to be considered as potential source of carotenoids.

The preharvest application of MeSa, JaMe and OA showed higher content of total phenolics in the peel, while SA-treated peels had lower concentration. On average, total polyphenol concentrations ranged between 90-100 mg gallic acid eq. 100 g^{-1} in the peel of yellow pitahaya. It has

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been reported that total phenol and flavonoid (6 and 20 mg g^{-1}, respectively) contents reached their maximum at stage 1 (immature fruits) and decreased progressively as fruit development advanced recaching the lowest levels at harvest time [54].

The main phenolic compounds found in the peel of red pitahaya belongs to the flavonoid group. Recent studies identified 16 phenolic acids including derivatives of benzoic and ellagic acid [5,55]. The peel of 3 cultivars of *Hylocereus undatu* increased gradually the content of total phenolics during fruit ripening until reach the maximum at time of harvest, the concentration being higher than those obtained in the pulp, suggesting that pitaya peels could be considered as good source of natural phenols [56].The phenolic content in the peel of red (*Hylocereus monacanthus*) and yellow (*Hylocereus megalanthus*) pitahaya ecotypes confirmed that the peel had higher total polyphenols (2-fold) than the pulp of the red species, but the contrary occurred in the yellow pitahaya since the pulp had a 12% more than the peel [57]. The higher content of total phenolics in the peel of yellow pitahaya treated with the elicitors support the idea that the peel could have a potential in the food industry used in nutritional supplements. Recently, the mucilage of the peel of yellow pitahaya has been postulated as an innovative hydrocolloid to be used in the food industry because is good source of dietary fiber with a potent antioxidant activity, together with a good solubility, a high water-retention efficiency and excellent capacity to form emulsions [58]. Also, biscuits made with 50% refined wheat flour by 50% peel powder from pitahaya increased 5-fold the fiber content as well as the gallic acid, the biscuit been considered as palatable and with good quality [59].

In recently harvested fruits, the concentration of total phenolics and total antioxidant activity (hydrophilic) was higher in the pulp treated with SA, JaMe and OA, and lower in those treated with MeSa and control fruits. These differences were maintained for the entire period of postharvest storage (52 days at 10°C), although the content of total phenolics increased along storage while the contrary occurred for the total antioxidant activity. The highest concentration of total phenolics. Interestingly, the levels of total phenolics in control pitahayas remained unchanged along storage, while MeSa-treated fruits enhanced the total phenolics. Accordingly, application of MeSa during fruit growth has shown to increase the content of polyphenols of several fruits at harvest and during cold storage, such as grape and sweet cherry, among others [11,16,28]. Similarly, JaMe and OA enhanced the total phenolics table grapes, pomegranate and sweet cherry at time of harvest and also during postharvest storage [12,13]. MeJa applied as postharvest treatment in red pitahaya induced higher total flavonoids, phenolics, anthocyanins and antioxidant activity measured by FRAP and DDPH assays [60]. In a comparative of 3 species (*H. costaricensis*, *H. undatus* and *H. megalanthus*) the content of total phenolics in the pulp was 33, 23 and 22 mg gallic acid eq. 100 g^{-1}, the *H. costaricensis* being the fruit with the highest antioxidant activity, 15-fold higher than *H. undatus* and *H. megalanthus* [61]. The 3 species differ in the colour of the peel and flesh, and it can be concluded that total phenol content and antioxidant capacity are notably higher in red-fleshed fruits than white-fleshed. This was confirmed by [62] with the results in *Hylocereus polyrhizus Hylocereus undatus*, in which the main phenolic compound in both pitahayas was quercetin.

At time of harvest, the total carotenoid content was affected by preharvest treatment, with SA, JaMe and OA being the elicitors showing higher concentration than those with MeSa and control fruits. The concentration of carotenoids in pitahaya species, either red or yellow, has not been investigated in depth, although some evidences exits. Four xantophylls (lutein, neoxanthin, violaxanthin and dihydroxy dihydrozeaxanthin) and two carotenes (lycopene, β-carotene) have been identified in both peel and pulp of red pitahayas [63]. The pulp of 3 species (*H. undatus*, *H. costariscensis* and *H. megalanthus*) revealed a range of 32-60 μg 100 g^{-1} of β-carotene and 18.24 μg 100 g^{-1} of xantophyll [52]. In Indian species of dragon fruits (*H. costariscensis* and *H. megalanthus*) β-carotene have been found as major carotenoid. In white flesh dragon fruits from Vietnam variety (white flesh), β-carotene, lycopene and vitamin E at concentration of 1.4, 3.4 and 0.26 μg 100 g^{-1}, respectively were reported [64].

During storage, the carotenoids increased in OA- and JaMe-treated pitahayas, while the lipophilic antioxidant activity decreased along storage, although all treated fruits had higher concentration than control ones, the MeSa treatment being the elicitor induced the highest

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carotenoids. Then, the continuous increase of carotenoids through postharvest storage of yellow pitahaya maybe is due to the normal ripening process, in which the acceleration of chlorophyll degradation leads to increased in carotenoids. Generally, there is a close relationship between carotenoids and lipophilic antioxidant activity, given the lipophilic nature of the carotenoids [21]. The increase in β-carotene and lycopene has been associated to enhancement of capacity to scavenge the ROS species that can be generated during the postharvest storage of fruits [65]. There is no literature about the role of the elicitors on carotenoid content in red or yellow pitahaya, although our results confirm that JaMe applied at preharvest or postharvest increased the concentration of total carotenoids of mandarin [36]. On the other hand, preharvest SA and its derivatives MeSa and acetylsalicylic acid (AAS) treatments induced a significant increase in total carotenoids in 2 plum cultivars at harvest and during storage [66].

4. Materials and Methods

4.1. Plant Material, Treatments and Experimental Design

The experiments were carried out in a commercial farm (Algro Farm, located Palora, Province of Morona Santiago, Ecuador) of yellow pitahaya (*Selenicereus megalanthus* Haw.) plants grown under greenhouse conditions. The pitahaya plot consists in 4-years old 1200 plants in an area of 2.5 ha (located at 1° 41' 00'' South Latitude, 77° 58' 56.8'' West Longitude, and altitude of 839 m.

For the experiments, a bifactorial randomized complete block design (4 treatments and 1 concentration) was used, executing 3 repetitions (n = 3) (**Figure S1**). A total number of 135 plants were chosen, from which 3 yellow pitahaya plants were selected per block and 3 fruits per plant were marked (9 fruits for each block) to measure fruit growth (total number of fruits was 27).

Treatments were applied with a frequency of 15 days, starting at 57 days after full blossom (DAFB), and after 71, 86, 102 DAFB, while harvest was carried out at 126 DAFB. The elicitors were methyl salicylate (MeSa) and methyl jasmonate (MeJa) (both at 0.1 mM), while salicylic acid (SA) and oxalic acid (OA) were applied at 5 mM, based on previous experiments (Erazo-Lara et al, 2024) [29]. Treatments were performed by spraying 1.5 L per plant of freshly prepared solutions of MeSa, SA, MeJa and OA (purchased from Sigma-Aldrich, Madrid, Spain) containing 0.5% Tween-20 as surfactant. Treatments were applied early in the morning and under favourable weather conditions (no rain or wind were forecasted).

Pitahaya fruits were manually harvested at commercial ripening stage based on fruit size, fruit weight (≈ 360 g), colour (light-green or yellow with green bracts) and the content of total soluble solids (TSS) over 15 °Brix [21]. In addition, the thorns were manually removed with a brush. For each treatment, 27 fruits (9 fruits per replicate) were picked and transferred by refrigerated van to the airport and then shipped to Spain for storage experiment. Another group of pitahayas (2 fruits per replicate and treatment) was used to determine TSS, total acidity (TA) and fruit firmness at harvest (Day 0). The fruits were peeled to separate the pulp from the peel, in which the mineral content (macro- and micronutrients) and total phenolics were determined.

4.2. Postharvest Storage

Once the fruits arrived to Spain, they were stored 10°C and relative humidity of 85%. After 24, 38 and 52 days, one lot from each treatment (9 pitahayas) was taken from the cold room to analyse TSS, TA, firmness, total phenolic compounds, total carotenoids, and antioxidant activity from hydrophilic and lipophilic extracts.

4.3. Mineral Composition of the Peel

The peels corresponded to the fruits at harvest were submitted to dehydration in a heater at 65°C until constant weight. 0.25 g of peel samples from each treatment (in triplicate) were microwave-digested (CEM Mars One) after the addition of 10 ml of 1% nitric acid for 3 hours, and afterwards up to 50 mL with distilled water. Then, aliquots of each samples were used to quantify the mineral concentration using the method of Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

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(Shimadzu icpms-2030). The mineral quantification was carried out by the use of standard curves of Ca, Mg, Na, P and K (macronutrients) and Fe, Mn, Cu and Zn (micronutrients) and results were expressed as mg kg^{-1} dried weight.

4.4. Measurement of Quality Traits

Quality was evaluated in the pitahaya pulp obtained from the 9 fruits of each replicate by obtaining subsamples of 3 fruits. TSS was determined as °Brix and expressed as g 100 g^{-1} with a Kem brand refractometer (RA-620 model). The percentage of TA (g 100 g^{-1}) was determined by by automatic titration (785 DMP Titrino, Metrohm, Herisau, Switzerland) by the use of 0.1 N NaOH up to pH 8.1. Fruit firmness (N mm^{-1}) was evaluated with a Texture Analyzer (Model TA.XTplus, Stable Micro Systems Ltd, UK) by compression (3% of the pitahaya diameter) with the aid of a 75 mm flat plate probe and recording the force (N) and the distance (mm).

4.5. Bioactive Compounds and Antioxidant Activity

The methods described by Habibi et al. (2021) [XX] and adapted to the pitahaya fruit. Total phenolics were calculated by the Folin-Ciocalteau protocol by homogenising 2 g of peel and pulp with 10 mL of extractant (20:80. water: methanol), then submitted to centrifugation at 10.000 *x g* at 4 °C during 20 min. The supernatant was added the Folin-Ciocalteau reagent, and results expressed as mg of gallic acid equivalent 100 g^{-1}.

Total antioxidant activity was determined in both aqueous solvent (hydrophilic) and organic solvent (lipophilic) by using the method of ABTS. Briefly, 5 g of pulp was added of 5 mL phosphate buffer (pH=6.8) and 10 mL of ethyl acetate, homogenising for 2 minutes and then centrifuging at 10.000 *x g* at 4 °C during 20 min. After separation of the phases, each extract was measured in duplicate with ABTS-peroxidase system, and results were expressed as mg 100 g^{-1} of Trolox equivalents. For total carotenoids, the organic phase was used submitted to saponification with 10% KOH in MeOH solvent followed by extraction with diethyl ether and finally drying and dissolving in acetone. Total carotenoids were quantified by reading the absorbance at 450 nm in a spectrophotometer (UNICAM Helios-α spectrophotometer, UK) and expressed as μg β-carotene equivalent 100 g^{-1}.

4.6. Statistical Analysis

A completely balanced subplot design was used in this study. All data in the paper were expressed as the mean ± standard error (SE) of three replicates. The data were subjected to an analysis of variance (ANOVA). Comparisons of means were performed using a multiple range test (Tukey test) to find significant differences ($P \leq 0.05$). All analyses were performed with the SPSS version 22 software package and the SigmaPlot 11.0 software program was used for graphs

5. Conclusions

It is clear that the postharvest storage of fruits is affected by preharvest factors, such as ripening stage at harvest and quality traits (TSS, TA and firmness). Then, the elicitors used in this study (MeSa, SA, JaMe and OA) have induced better quality of yellow pitahaya. The on-tree ripening process was affected by type of elicitor, since SA, JaMe and OA delayed while MeSa advanced the ripening. The peel of the yellow pitahaya is rich in minerals and bioactive compounds (polyphenols and carotenoids), all of them showed a net enhancement with the use the elicitors. So far, peel was considered as waste, but given the great amount of both nutritional and phytochemical compounds, the peel of yellow pitahaya would be a perfect candidate to be recycled and applied in the food industry.

Since carotenoids and phenolics have been proved to have beneficial effects against degenerative diseases, preharvest treatments with these elicitors would provide healthier yellow pitahayas for human consumption. The preharvest sprays is recommended as the optimal method due to the fast absorption and high effectiveness as alternative of postharvest treatments. The preharvest elicitors have been demonstrated an improvement of yellow pitahaya quality during postharvest storage quality through maintaining fruit firmness, TSS and TA. Tropical fruits are very sensitive to develop chilling injury (CI) when stored at sub-optimal temperatures. For that reason, we stored at 10 °C (non CI-temperature), but in future the use of these elicitors and the possibility to use lower temperatures for storing yellow pitahaya deserves further investigation.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Figure S1: Distribution of the pitahaya plants in 3 blocks at random for the elicitor applications.

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