1 **1-Methylcyclopropene inhibits ethylene perception and biosynthesis: a theoretical and**
2 **experimental study on cape gooseberry (*Physalis peruviana* L.) fruits**

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25

26 **Abstract**

27 Theoretical and experimental studies were conducted to determine the role of 1-MCP in the
28 biosynthesis of ethylene in cape gooseberry fruits (*Physalis peruviana* L.), ecotype
29 Colombia. A completely randomized experimental design was used with 3 treatments:
30 ethylene (1000 μL ethephon L^{-1}), 1-MCP (1 μL L^{-1}), and a control with no applications. At
31 1, 6 and 11 days after treatment, volatile compounds, firmness and color were measured.
32 The enzymatic activities of ACC oxidase (E.C. 1.14.17.4) and histidine kinase (HK, E.C.
33 2.7.13.3) were also determined *in vitro*. Subsequently, molecular docking studies with the
34 enzymes, their respective substrates and 1-MCP were carried out. It was found that 1-MCP
35 decreased the emission of volatile esters, color index, loss of firmness, and ACC oxidase
36 and histidine kinase activities. Results indicated 1-MCP acted as a competitive inhibitor of
37 ACC oxidase and as a noncompetitive inhibitor of HK. The coupling free energy was
38 higher for 1-MCP in both the ACC oxidase (8.31) and the HK (4.22), compared to their
39 respective substrates. The results also suggested that 1-MCP was able to decrease both the
40 biosynthesis and activity of ethylene in cape gooseberry fruits.

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42 **Keywords:** *Physalis peruviana* L., ethylene signaling, 1-MCP, histidine kinase, ACC
43 oxidase.

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45 **1. Introduction**

46 Ethylene is a plant hormone that regulates ripening and senescence at the molecular,
47 biochemical and physiological levels (Kesari et al., 2007) because it stimulates the
48 expression of genes that code for enzymes related to changes during ripening and/or

senescence, such as chlorophyllase and alcohol acyl transferase. Ethylene has a double role in the postharvest period: it causes fruits to acquire optimal organoleptic characteristics for consumption but is also responsible for the senescence of tissues, generating adverse effects on the fruit quality (Bapat et al., 2010).

Ethylene signaling begins with its binding to a group of protein receptors that act as a negative regulator of the response to ethylene (Binder, 2008). When ethylene is perceived by the receptors, the ethylene signal is transduced by a sequence of biochemical events that regulate the expression of genes related to the activity of enzymes involved in the different processes of ripening (Lacey and Binder, 2014; Binder, 2008; Kesari et al., 2007). Ethylene receptors are membrane proteins of the endoplasmic reticulum (An et al., 2020), encoded by a multigene family that is functionally redundant (Chen et al., 2005; Bouzayen et al., 2010). In tomato, seven ethylene receptors (ETR1 to ETR7) have been identified (Liu et al., 2015).

1-methylcyclopropene (1-MCP) is a chemical ripening retardant that irreversibly binds to ethylene receptors on cell membranes (Serek et al., 2006; Watkins, 2006) and is therefore able to reduce the effects of ethylene and/or interfere with its function in plants, blocking the transduction signal cascade that leads to the expression of genes related to the response to ethylene (In et al., 2013). 1-MCP has an affinity for ethylene receptors that has been reported to be 10-times greater than that of ethylene and acts as inhibitor at concentrations as lower as 5 nLL^{-1} to $100 \text{ }\mu\text{LL}^{-1}$ (Blankenship and Dole, 2003), with no toxic effects on humans or the environment (Huber, 2008). 1-MCP also regulates ethylene biosynthesis through inhibition of the autocatalytic process of the hormone (Alos et al., 2019,

Blankenship and Dole, 2003). Recently, Cheng et al. (2019) found that 1-MCP reduced ethylene production in *Pyrus bretschneideri* Rehd. Cv fruits, whereas Dal Cin et al. (2006) and Yang et al. (2013) reported that 1-MCP significantly reduced ethylene synthesis and expression of the 1-aminocyclopropane-1-carboxylate synthase (ACS) gene in apples, which was attributed to a reduced expression of the *Md-ETR1* and *Md-ERS1* genes. In addition, Amornputti et al. (2016) found that 1-MCP inhibited ethylene synthesis in *Durio zibethinus* Murr fruits, a decrease that was correlated with a lower activity of the enzyme aminocyclopropane-1-carboxylate (ACO) in the epidermis. It seems then that by blocking ethylene receptors, 1-MCP is able to de-regulate the expression of ethylene biosynthetic genes controlled by the hormone (Alos et al., 2019).

Interestingly, because of the structural similarity between 1-MCP and 1-aminocyclopropane-1-carboxylic acid (1-ACC; the immediate precursor in ethylene biosynthesis), it is possible that 1-MCP also decreases ethylene biosynthesis by the competitive inhibition of the ACO enzyme, which catalyzes the conversion of 1-ACC into ethylene (Cara and Giovannoni, 2008). In this regard, Chiriboga et al. (2012) stated that the inhibition of ethylene production by 1-MCP has been well described, but the exact site in the ethylene biosynthesis pathway in which 1-MCP is involved is not yet clearly defined, although apparently it is through the repression of the ACO expression and activity (Chiriboga et al., 2012), as reported by Yang et al. (2013) for apples.

This study used cape gooseberry fruits (*Physalis peruviana* L., Solanaceae family) because they have a climacteric respiratory behavior, with an increase in ethylene synthesis (Balaguera-López et al., 2014; Valdenegro et al., 2012; Gutiérrez et al., 2008). There is

evidence that ethylene is associated with different processes during the ripening of these fruits, such as softening, antioxidant activity, color change, and volatile compounds, among others (Balaguera-López et al., 2017; Valdenegro et al., 2012; Gutiérrez et al., 2008). Another important aspect is that, according to previous studies, the cape gooseberry is affected by the application of 1-MCP during ripening (Balaguera-López et al., 2015 and 2017). In addition, these fruits are very desirable because of their flavor, nutritional, functional and medicinal properties (Fischer et al., 2011). These fruits are also a valuable and convenient experimental system to evaluate the involvement of ethylene in many ripening-related processes. Therefore, theoretical and experimental studies have been conducted with the objective of determining the role of 1-MCP in the activity and biosynthesis of ethylene in cape gooseberry fruits.

2. Materials and methods

2.1. Plant material, treatments and storage conditions

The cape gooseberry fruits, ecotype Colombia, were harvested from a commercial crop in the municipality of Ventaquemada (Boyacá, Colombia) located at 2630 meters above sea level, 5°21'59" N and 73°31'19" W, with an average temperature of 14°C.

A completely randomized design was used with 3 treatments: ethylene (ethephon, 1000 $\mu\text{L L}^{-1}$), 1-methylcyclopropene (1-MCP; 1 $\mu\text{L L}^{-1}$), and a control with no applications. Each treatment had 4 repetitions, for a total of 12 experimental units (EU). Each EU was composed of 125 g of fruits without calyx, obtained at harvest maturity with the following

color values: $L^* = 65.85 \pm 4.5$, $a^* = 10.66 \pm 1.2$, $b^* = 52.6 \pm 3.8$ and color index (CI) = 3.10 ± 0.5 . CI was calculated as

$$CI = \frac{1000xa^*}{L^*xb^*}, \text{ equation 1.}$$

For the ethylene application, the fruits were immersed in 2-chloroethylphosphonic acid (ethylene-releasing agent, EthrelTM 480 g L⁻¹, Bayer Crop Science) for 10 minutes. For 1-MCP (Rohm and Haas), the fruits were placed in a 2 L hermetic chamber with 1-MCP for 12 h. The application conditions were carried out in accordance with Balaguera-López et al. (2015). The fruits were exposed to the treatments for 24 h at room temperature. After the treatments, all the fruits, including those in the control, were packed in a thermoformed box of polyethylene terephthalate and left at room temperature (20°C, 75% Relative Humidity).

2.2. Quantification of volatile compounds, instrumental color and firmness

Measurements were taken at 1, 6 and 11 days after harvest. The analyzed major volatile compounds were ethyl octanoate, ethyl butanoate, ethyl decanoate and butyl butanoate, for which headspace solid-phase microextraction (HS-SPME) was used for 80 g of fruit. The equilibrium time was 1 h, and the polydimethylsiloxane (100 µm, SUPELCO) microextraction fiber was exposed for 30 minutes. For the identification of the volatile compounds, a 7890A gas chromatograph (Agilent Technologies, USA) coupled to a 5975C mass spectrometer (Agilent Technologies, USA) was used; the spectra were compared with those in the NIST 8 library (NIST, 2013). In addition, the Kovats index (KI) was used, which was calculated using the homologous series of n-alkanes under the same operating conditions.

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144 For color, three measurements were made in the equatorial zone of each fruit, using the
145 CIELab system parameters L^* , a^* and b^* , with a Konica-Minolta CR-410 digital
146 colorimeter (Konica Minolta, Osaka, Japan). With these parameters, the CI was calculated
147 (equation 1).

148 The fruit firmness (expressed as N) was determined using a Lloyd LS1 digital texture
149 analyzer (Lloyd LS1, Bognor Regis, UK) with a 100 N load cell, a 3 mm cylindrical die
150 and Nexygen Plus software.

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152 *2.3. Extraction and partial purification of the enzymes histidine kinase (HK) and ACC*
153 *oxidase (ACO)*

154 The extraction and partial purification of the HK and ACO enzymes was carried out using
155 the methodology established by Dupille and Zacarías (1996). To obtain the crude enzyme
156 extract, approximately 1.0 g of cape gooseberry tissue was weighed and crushed with liquid
157 nitrogen until a homogeneous powder was obtained. Then, 10 mL of 0.50 M Tris-HCl
158 buffer (pH 7.50) with 15% w/v glycerol, 30 mM sodium ascorbate, 5 mM dithiothreitol
159 (DTT) and 5% w/v polyvinylpyrrolidone (PVPP) were added. The mixture was stirred
160 for 60 min at 4°C and then centrifuged at 4,000 x g and 4°C for 30 min. To fractionate the
161 crude enzyme extract with size exclusion chromatography, the Sephadex G-25M filtration
162 gel (particle size distribution of 20-80 µm) was activated with 15 mL of the extraction
163 buffer at 4°C. A 3.0 mL aliquot of the crude enzyme extract was eluted through the
164 filtration gel with the extraction buffer at a flow rate of 1.0 mL min⁻¹. Sixty fractions of 1
165 mL were collected, and the protein concentration was determined for each fraction with the

Bradford method linearized by Zor and Selinger (1996) using bovine serum albumin (BSA) as a standard, along with the activity of the ACC oxidase and HK enzymes.

2.4. Assay for in vitro enzyme activities

The ACO enzyme activity was determined using the methodology established by Dupille and Zacarías (1996). In a 5 mL glass vial, 300 μ L of a solution containing 1.5 mM 1-ACC, 255 μ M FeSO₄, 30 mM sodium ascorbate and 100 mM NaHCO₃ was added, followed by the addition of 400 μ L of deionized water and 300 μ L of partially purified enzyme extract obtained in section 2.3. The vial was hermetically closed with a silicone seal, and the reaction medium was incubated at 30°C for 30 min. Afterwards, the ethylene accumulated in 500 μ L of the headspace was analyzed by gas chromatography using the methodology established by Fiserová et al. (2008). An Agilent 6820 gas chromatograph with a flame ionization detector (FID) was used. An Agilent J&W Alumina 30 m x 0.53 mm x 0.25 mm capillary column was used. The chromatographic conditions were: injector temperature 70°C, oven temperature 50°C (isothermal) and FID detector temperature 250°C. Helium was used as a carrier gas at a flow of 7.0 mL min⁻¹, and the combustion gases in the FID detector were dry air and hydrogen with flows of 200 and 20 mL min⁻¹, respectively. For the quantification, an ethylene standard (100 ppm in nitrogen, AGA, Colombia) was used. The activity of the ACC oxidase enzyme was expressed as the μ mol of ethylene generated per minute per mg of protein (μ mol C₂H₄ min⁻¹ mg⁻¹ protein). The protein concentration of the partially purified enzyme extract was determined with the Bradford method linearized by Zor and Selinger (1996).

The HK enzyme activity was determined with reversed-phase high efficiency liquid chromatography with a refractive index detector (RID) thermostated at 40°C. Five hundred μL of a solution containing 1.0 mM histidine, 50 μM KCl, 10 mM MnCl_2 and 1 mM ATP was mixed with 200 μL of extraction buffer and 300 μL of partially purified enzyme extract. The reaction medium was incubated at 30°C for 45 min, the reaction was stopped by adding 500 μL of 1% trichloroacetic acid (TAC) with 0.5 mM EDTA, and the mixture was centrifuged at 4000 x g and 4°C for 60 min (Gamble et al., 1998). For the detection of histidine consumed during the reaction, a high efficiency liquid chromatograph, a Shimadzu LC-20AT (Shimadzu, Tokyo, Japan), was used, with an RID detector and a 20 μL injection loop. The isocratic elution was performed with the mobile phase for amino acid analysis, ACCQ Tag Eluent A Concentrate (Waters Inc.), composed of acetonitrile with 0.2% formic acid, a pH of 4.95, and a flow rate of 1.0 mL min^{-1} . A RP-18 Phenomenex 250 mm x 4.6 mm x 5 μm reversed-phase column was used at room temperature (20°C). The activity of the HK enzyme was expressed as the μmol of histidine consumed per minute per mg of protein ($\mu\text{mol HisP min}^{-1} \text{mg}^{-1} \text{protein}$). The protein concentration of the partially purified enzyme extract was determined with the Bradford method linearized by Zor and Selinger (1996).

2.5. Determination of the inhibition kinetics of 1-MCP against HK and ACO enzyme activities

This determination was performed *in vitro* with the enzymatic extract of the cape gooseberry fruits, as explained in the previous section, and used two substrates: 1-ACC (carboxy-aminocyclopropane acid) for the ACO enzyme and histidine for the HK enzyme.

For ACO, the kinetic profiles were constructed with six concentrations of the 1-ACC substrate (0, 20, 40, 60, 80, and 100 μ M); for HK, the kinetic profiles were constructed with six concentrations of histidine (0, 20, 40, 60, 80, and 100 μ M). In both experiments, different concentrations of 1-MCP were used: 0, 20, 40, 60, 80, and 100 μ M. The applications were performed in triplicate. Subsequently, the activities of the ACO and HK enzymes were measured as reported in the previous section. With these data, the Lineweaver-Burk double reciprocals plot was calculated for the kinetic constants (K_m , V_{max} , K_i) and the mechanism of inhibition (competitive, noncompetitive or self-competitive) was identified.

2.6. Theoretical studies

The structure of each enzyme was obtained from the Protein Data Bank (<http://www.rcsb.org/pdb>). The enzymes included the ETR1 ethylene receptor from *Arabidopsis Thaliana* (pdb code 1DCF) and ACC oxidase from *Petunia hybrida* (EC 1.14.17.4, pdb code 1W9Y).

The molecular structures of ethylene, 1-ACC, and 1-MCP have been fully optimized using density functional theory (DFT) with the B3LYP functional, as implemented in GAMESS (General Atomic and Molecular Electronic Structure System) version May 01, 2012 (Schmidt et al., 1993) and the 6-311G(d,p) basis set. The solvent effects were simulated by placing the solute in a dielectric medium simulating water using the IEF-PCM model (Cossi et al., 1998). Both geometry optimization and energy calculations were performed in this medium.

The basic docking protocol was used with the default settings provided by AutoDock Tools (ADT) according to Neuhaus (2010). The enzymes and substrates were converted to pdbqt format in ADT. The Lamarckian Genetic Algorithm with a population size of 150 dockings and five millions energy evaluations was used. All other parameters, e.g., crossover rate and mutation rate, were run with the default settings. The grid size for specifying the search space was set at 21 x 21 x 21 Å³ in a centered position with a default grid point spacing of 0.375 Å. Autodock 4.0 was launched from ADT in the Devian-Linux operating system, and the docking logs were analyzed in the ADT graphical user interface. The docked energy was defined as the sum of the intermolecular and internal energies. For a representative docking instance, the orientation or pose with the lowest estimated free energy (ΔG) of binding corresponding to the docking energy and the unbound system's free energy was chosen in each calculation. The binding free energy was expressed as kcal mol⁻¹.

2.7. Data analysis

The univariate ANOVA was conducted using treatment as a factor (control, ethrel, 1-MCP). Then the multiple comparison test of Fisher's LSD averages ($P < 0.05$) were performed using Statgraphics statistical software.

3. Results and discussion

3.1. Physiological responses of cape gooseberry fruits

The effect of 1-MCP on volatiles was transitory (Fig. 1). Ethyl octanoate (KI=1097, volatile compound of greatest abundance in cape gooseberry fruits), as well as ethyl butanoate (KI=801), butyl butanoate (KI=981) and ethyl decanoate (KI=1296), showed the

same behavior, where the application of 1-MCP generated a decrease from day 1 to 6; on day 6, there was no emission of these compounds (except for ethyl octanoate, which presented a very low value). Then, an increase was observed until the end of the postharvest period. Conversely, in the control fruits and with ethylene, the concentration of these volatile compounds was high, significantly higher with ethylene; however, after day 6, the volatile compounds decreased in these two treatments. Ethyl butanoate (KI=1097) showed a similar behavior but did not decrease after day 6 in any treatment (Fig. 1). In general, it was found that the esters evaluated in the cape gooseberry fruits increased their concentration with the application of ethylene, while a decrease was observed with 1-MCP. These results agree with reports for apples and cape gooseberry fruits in which volatile production was inhibited by the application of 1-MCP or accelerated by ethylene treatments (Defilippi et al., 2005; Balaguera-López et al., 2017).

Previous studies have reported the role of ethylene in the induction of volatile esters in climacteric fruits (Beekwilder et al., 2004; Defilippi et al., 2004), particularly the control of the enzyme alcohol acyl transferase (Yahyaoui et al., 2002; Defilippi et al., 2005; Li et al., 2016), an enzyme that catalyzes the last step in the biosynthesis of esters (Pérez and Sanz, 2008). It has also been reported that ethylene regulates the reduction of fatty acids and aldehydes and, partially, the step of esterification in the life of aliphatic esters, which may indicate that the aldehyde dehydrogenase enzymes involved in this pathway are ethylene-dependent (Flores et al., 2002; Li et al., 2016) and possibly explain the increase in esters through ethylene activity and the decrease in the presence of 1-MCP in the cape gooseberry fruits. However, other ethylene-independent pathways of volatile emission have been also observed (Alos et al., 2019)

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284 The color index of the cape gooseberry fruits increased continuously during storage, but
285 this change was significantly lower in fruits with 1-MCP, compared to ethylene and the
286 control (Fig. 2). Studies have shown that 1-MCP decreases the activity of the enzyme
287 chlorophyllase, and therefore the loss of chlorophyll is lower (Watkins, 2006; Sun et al.,
288 2012). Gutierrez et al. (2008) reported that 1-MCP delays color change in cape gooseberry
289 fruits, which coincides with the results in this study. For the effect of ethylene, it has been
290 found to accelerate the degradation of chlorophyll and is involved in the expression of
291 genes that code for carotenoids and other compounds (Rodrigo and Zacarías, 2007).

292

293 The firmness of the cape gooseberry fruits decreased during the postharvest period.
294 However, this decrease was smaller ($p < 0.05$) with the application of 1-MCP and greater
295 with the ethylene treatment (Fig. 2). The main cause of the loss of firmness in fruits is the
296 activity of enzymes that hydrolyze structural elements and preserve the polysaccharides. In
297 cape gooseberry fruits, the activities of polygalacturonase (PG), pectinmethylesterase and
298 some glycosidases apparently are related to the loss of fruit firmness, where PG correlates
299 with the presence of ethylene in fruits (Majumder and Mazumdar, 2002); this may indicate
300 a possible relationship between the presence of ethylene and the loss of firmness in cape
301 gooseberry fruits, which in turn, would explain the lower loss of firmness with the 1-MCP
302 treatment. Similar results were also found in cape gooseberry fruits by Gutiérrez et al.
303 (2008).

304

305 The previous results indicate that ethylene is associated with several physiological
306 processes during the ripening of cape gooseberry fruits, but there is also strong evidence

that 1-MCP generates an antagonistic effect on ethylene during fruit ripening, as stated by Balaguera-López et al. (2017), who used 1-MCP as an inhibitor of ethylene activity in cape gooseberry fruits.

3.2 Protein profile of the enzymes HK and ACO

The fractions 17-21 exhibited the activities of the HK and ACC oxidase enzymes (Fig. 3). Therefore, these fractions were used to determine the activities in the enzyme kinetic experiments.

3.3 HK enzyme activity

The HK enzyme activity was significantly lower in the cape gooseberry fruits treated with 1-MCP, followed by the control, in this treatment, the HK enzyme activity is due to endogenous ethylene, which can be in concentrations between 77.11 and 266.76 $\mu\text{L L}^{-1}$ (Balaguera-López et al., 2016). With the highest activity observed in the fruits with the ethylene applications (Fig. 4). When ethylene is sensed by the receptors, the ethylene signal is sent through a sequence of biochemical events that regulate the expression of genes related to the activity of enzymes involved in fruit ripening (Lacey and Binder, 2014; Binder, 2008; Kesari et al., 2007). Genetic experiments suggest that the binding of ethylene to the ETR1 receptor activates HK (Hall et al., 2012); for this reason, the increase in HK activity is thought to be indicative of the binding between ethylene and the receptor, which would explain the increase in HK in the cape gooseberry fruits in the presence of ethylene.

Conversely, when 1-MCP was added, the HK activity in the cape gooseberry fruits decreased, and no response to ethylene was generated, as expected since 1-MCP was made

in order to irreversibly bind to ethylene membrane receptors and to therefore inhibit its subsequent function (Serek et al. 2006). In this regard, both the abundance and activity of the receptors are affected by the stability of the protein (Kevany et al., 2007) and by changes in the phosphorylation level in the receptor (Kamiyoshihara et al., 2012). Other studies have reported that the treatment of 1-MCP decreased the levels of ethylene receptor mRNA in plums (El-Sharkawy et al., 2007), apples (Yang et al., 2013) and tomatoes (Mata et al. 2018).

3.4. ACC oxidase enzyme activity

A lower activity of the ACO enzyme was observed with the application of 1-MCP, while the fruits treated with ethylene had the highest activity of this enzyme (Fig. 4). In apple and peach fruits, climacteric ripening ethylene is accompanied by an increase in the accumulation of ACS and ACO transcripts, and the genes involved are induced by ethylene and participate in the autocatalytic synthesis of this hormone (Dal Cin et al., 2006). The increase in ACO activity observed in the cape gooseberry fruits with an exogenous application of ethylene may indicate the presence of autocatalytic biosynthesis of ethylene in this species since it is a climacteric fruit (Valdenegro et al., 2012), as an increase in ACO gene transcription may generate an increase in ethylene synthesis (Cara and Giovannoni, 2008).

1-MCP, by decreasing the activity of ethylene, also decreases the expression and activity of ACO (Chiriboga et al., 2012), as found in apples (Yang et al., 2013), and therefore ethylene biosynthesis is decreased. Cape gooseberry fruits treated with 1-MCP might produce less

ethylene and potentially have a longer postharvest life, which coincides with that reported by Valdenegro et al. (2012).

3.5. Determination of the inhibition kinetics of 1-MCP against ACC oxidase and HK enzyme activities

The ACO activity increased as a function of the concentration of the 1-ACC substrate, with the ACO activity being higher with the higher concentration of 1-ACC (100 μ M); nevertheless, the ACO activity decreased as the concentration of 1-MCP increased from 0 to 100 μ M (Fig. 5a and b); this result was confirmed, as shown in Fig. 4e, where the $V_{\max\text{app}}$ value remained constant with the addition of 1-MCP but the K_m increased linearly as a function of the increase of 1-MCP. This behavior is characteristic of a competitive inhibition mechanism.

The kinetics of the HK enzyme indicated that its activity increased with the increase in the histidine concentration; nevertheless, the increase was greater when histidine increased from 0 to 20 μ M. For the effect of 1-MCP, it was observed that HK activity decreased markedly with the increase of 1-MCP from 0 to 20 μ M, but from 60 μ M to 100 μ M, the HK activity was constant (Fig. 5c). However, Fig. 4d shows that the HK inhibition was proportional to the concentration of 1-MCP. Likewise, it was found that the K_m remained constant as a function of the 1-MCP concentration, while the $V_{\max\text{App}}$ decreased (Fig. 5f), which is characteristic of a noncompetitive inhibition mechanism.

Inhibition of the enzyme ACC oxidase by 1-MCP followed a competitive mechanism because 1-MCP (inhibitor) reversibly binds to the active site of the ACO, forming a

complex enzyme-inhibitor (EI) similar to the enzyme-substrate (ES) complex but one that is catalytically inactive. When comparing the free energy of the binding between 1-ACC and 1-MCP to ACO, it was found that, with 1-MCP, this energy was 8.31 times greater than that of 1-ACC, indicating that ACO had a higher affinity for 1-MCP compared to 1-ACC (Fig. 6).

For HK, the inhibition induced by 1-MCP was found to be noncompetitive because 1-MCP irreversibly binds to the enzyme (EI) and the ES (ESI) complex at a different site than the active site, affecting the three-dimensional structure of the enzyme and avoiding the correct orientation of the substrate (histidine) in the active site. However, it is important to note that HK is part of the methylene membrane receptor (Lacey and Binder, 2014) and that 1-MCP is irreversibly joined to this receptor (Blankenship and Dole, 2003). Consequently, HK becomes inactive, blocking the transduction signal cascade that leads to the expression of genes related to the response to ethylene (In et al., 2013).

The relative affinity between the substrate (ethylene for ETR1 and 1-ACC for ACC oxidase) and the inhibitor (1-MCP) for each enzyme was evaluated. In both cases, the inhibitor (1-MCP) interacted more strongly with the enzymes than their respective substrates, suggesting that 1-MCP had a higher affinity compared to 1-ACC. For ETR1, the inhibitor (1-MCP) had an affinity 4 times greater than the substrate (ethylene), while for ACC oxidase, the inhibitor (1-MCP) showed an affinity 8 times greater than the substrate (1-ACC; Fig. 6 and 7). Therefore, it is possible that 1-MCP is more effective in inhibiting the ACC oxidase than the ETR1. The high affinity (the high inhibition capacity) of 1-MCP for ACC oxidase can be explained by the great structural similarity that exists between the

substrate (1-ACC) and the inhibitor (1-MCP) of this enzyme. These results are consistent with those reported by Blankenship and Dole (2003), who stated that the affinity of 1-MCP for the receptors is 10 times greater than that of ethylene.

The substrates (left) and the inhibitor (right) bound at the same site, suggesting that this site is the active site of the enzyme and is the activity site for the inhibitor (1-MCP). This applies to the two enzymes (Fig. 7, ETR1 (upper panels), ACC oxidase (lower panels)) because their results were similar.

3.6. Theoretical studies

In the case of ACC oxidase, the theoretical results coincide with the kinetic enzyme profiles (experimental). In the experimental results, the observed kinetic profile (Fig. 5 a, b and c) for ACC oxidase suggested that 1-MCP is a competitive inhibitor of this enzyme. This coincides with the theoretical data results because of the structural similarity between the inhibitor (1-MCP) and the substrate (1-ACC); therefore, they coupled at the same active site of the enzyme (Fig. 5c (1-ACC) and 5d (1-MCP)), which is very characteristic of competitive inhibition. Although both 1-ACC (substrate) and 1-MCP (inhibitor) coupled in the same place (competitive inhibition), 1-MCP had a higher affinity (Fig. 7). This means that as soon as the fruit is subjected to 1-MCP treatment, it will be very effective in delaying ripening, because it will be able to compete with ethylene and 1-ACC at the active sites of the ETR1 receptor and the ACC oxidase enzyme, respectively.

These results are consistent with Blankenship and Dole (2003) because suggest a higher affinity of ETR1 receptor for 1-MCP compared to that for ethylene and that for binding to

the receptor, 1-MCP inhibits ethylene binding, leading to the inhibition of ethylene-dependent physiological responses. There is strong evidence that 1-MCP can reversibly bind to the active site of ACO because chemically it is very similar to 1-ACC, resulting in competitive inhibition.

1-MCP is a chemical compound that not only interacts with ethylene receptors but also has the ability to inhibit the ethylene biosynthesis pathway. Consequently, it presents two levels of ethylene control in agricultural products, making it a significant product for delaying the processes induced by ethylene in the postharvest period of agricultural products.

4. Conclusion

Treatment with 1-MCP produced opposite effects as ethylene in the cape gooseberry fruits. The application of 1-MCP induced a decrease and/or inhibition of the parameters related to the synthesis and perception of ethylene, which provided additional evidence that the regulation of physiological and biochemical processes, such as color change, loss of firmness, emission of volatile esters, and ACO and HK activity, depend on ethylene production. The enzymatic kinetics and theoretical chemistry indicated that 1-MCP not only acts at the receptor level but can also competitively inhibit ethylene synthesis.

Author contributions

Balaguera-López: conceptualization, investigation, methodology, writing (original draft preparation); Espinal-Ruiz: methodology, software, writing (original draft preparation); Rodríguez-Nieto: methodology, visualization, writing (reviewing and editing); Herrera-

Arévalo: supervision, reviewing and editing; Zacarías: conceptualization, supervision, reviewing and editing.

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.

5. References

- Alos, E., Rodrigo, M.J. Zacarias, L: 2019. Ripening and senescence . In Yahia, E.& Carrillo-Lopez, A. (Eds). “Postharvest Physiology and Biochemistry of Fruits and Vegetables”, Chapter 7, pp 131-155. Elsevier Inc. <https://doi.org/10.1016/B978-0-12-813278-4.00007-5>
- Amornputti, S., Ketsa, S., van Doorn, W.G. 2016. 1-Methylcyclopropene (1-MCP) inhibits ethylene production of durian fruit which is correlated with a decrease in ACC oxidase activity in the peel. *Postharvest Biol. Technol.* 114, 69–75. <https://doi.org/10.1016/j.postharvbio.2015.11.020>
- An, J., Almasaud, R.A., Bouzayen, M., Zouine, M., Chervin, C. 2020. Auxin and ethylene regulation of fruit set. *Plant Sci.* 292, 110381. <https://doi.org/10.1016/j.plantsci.2019.110381>
- Balaguera-López, H., Espinal-Ruiz, M., Zacarías, L., Herrera, A. 2017. Effect of ethylene and 1-methylcyclopropene on the postharvest behavior of cape gooseberry fruits (*Physalis peruviana* L.). *Food Sci. Technol. Int.* 23(1), 86-96. <https://doi.org/10.1177/1082013216658581>

471 Balaguera-López, H.E., Martínez, C.A., Herrera, A. 2016. Comportamiento poscosecha de
 472 frutos de uchuva (*Physalis peruviana* L.): efecto de diferentes dosis y tiempos de
 473 exposición al 1-metilciclopropeno. *Bioagro*. 28(1), 21-28.

474 Balaguera-López, H.E., Martínez, C.A., Herrera, A. 2015. Refrigeration affects the
 475 postharvest behavior of 1-methylcyclopropenetrated cape gooseberry (*Physalis*
 476 *peruviana* L.) fruits with the calyx. *Agron. Colomb.* 33(3), 356-364.
 477 <https://doi.org/10.15446/agron.colomb.v33n3.51896>

478 Balaguera-López, H.E., Martínez, C.A., Herrera, A. 2014. Papel del cáliz en el
 479 comportamiento poscosecha de frutos de uchuva (*Physalis peruviana* L.) ecotipo
 480 Colombia. *Rev. Colomb. Cienc. Hortic.* 8, 181-191.
 481 <https://doi.org/10.17584/rcch.2014v8i2.3212>

482 Bapat, V.A., Trivedi, P.K., Ghosh, A., Sane, V.A., Ganapathi, T.R., Nath, P. 2010.
 483 Ripening of fleshy fruit: Molecular insight and the role of ethylene. *Biotechnol. Adv.* 28,
 484 94-107. <https://doi.org/10.1016/j.biotechadv.2009.10.002>

485 Beekwilder, J., Alvarez-Huerta, M., Neef, E., Verstappen, F.W., Bouwmeester, H.J.,
 486 Aharoni, A. 2004. Functional characterization of enzymes forming volatile esters from
 487 strawberry and banana. *Plant Physiol.* 135, 1865-1878.
 488 <https://doi.org/10.1104/pp.104.042580>

489 Binder, B. 2008. The ethylene receptors: complex perception for a simple gas. *Plant Sci.*
 490 175, 8-17. <https://doi.org/10.1016/j.plantsci.2007.12.001>

491 Blankenship, S.M., Dole, J.M. 2003. 1-Methylcyclopropene: a review. *Postharvest Biol.*
 492 *Technol.* 28, 1-25. [https://doi.org/10.1016/S0925-5214\(02\)00246-6](https://doi.org/10.1016/S0925-5214(02)00246-6)

493 Bouzayen, M., Latché, A., Nath, P., Pech, J. 2010. Mechanism of Fruit Ripening. In: Pua,
 494 E., Davey, M. (Ed). Plant Developmental Biology – Biotechnological Perspectives. Vol.
 495 1. Pp.319-339. Springer-Verlag.

496 Bradford, M.M. 1976. A rapid sensitive method for the quantification of microgram
 497 quantities of protein utilizing the principle of protein-dye binding. Analytical
 498 Biochemistry. 72, 248 – 254.

499 Cara, B., Giovannoni, J. 2008. Molecular biology of ethylene during tomato fruit
 500 development and maturation. Plant Sci. 175, 106–113.
 501 <https://doi.org/10.1016/j.plantsci.2008.03.021>

502 Chen, Y., Etheridge, N., Schaler, E. 2005. Ethylene signal transduction. Annals of Botany
 503 95, 901–915. <https://doi.org/10.1093/aob/mci100>

504 Cheng, Y., Liu, L., Feng, Y., Dong Y., Guang, J. 2019. Effects of 1-MCP on fruit quality
 505 and core browning in ‘Yali’ pear during cold storage. Scientia Horticul. 243, 350–356.
 506 <https://doi.org/10.1177/1082013207075600>

507 Chiriboga, M.A., Recasens, I., Schotsmans, W., Dupille, E., Larrigaudiere, C. 2012. Cold-
 508 induced changes in ACC metabolism determine softening recovery in 1-MCP treated
 509 ‘conference’ pears. Postharvest Biol. Technol. 68, 78–85.
 510 <https://doi.org/10.1016/j.postharvbio.2012.02.006>

511 Cossi, M., Barone, V., Mennucci, B., Tomasi, J. 1998. Ab-initio Study of Ionic Solutions
 512 by a Polarizable Continuum Dielectric Model. Chemical Physics Letters, 286, 253-260.
 513 [https://doi.org/10.1016/S0009-2614\(98\)00106-7](https://doi.org/10.1016/S0009-2614(98)00106-7)

514 Dal Cin, V., Massimo, F., Botton, A., Tonutti, P. 2006. The ethylene biosynthetic and
 515 signal transduction pathways are differently affected by 1-MCP in apple and peach fruit.

516 Postharvest Biol. Technol. 42, 125-133.
 517 <https://doi.org/10.1016/j.postharvbio.2006.06.008>
 518 Defilippi, B.G., Dandekar, A.M., Kader, A.A. 2004. Impact of suppression of ethylene
 519 action or biosynthesis on flavor metabolites in apple (*Malus domestica* Borkh) fruits. J.
 520 Agric. Food Chem. 52, 5694–5701. <https://doi.org/10.1021/jf049504x>
 521 Defilippi, B.G., Kader, A.A., Dandekar, A.M. 2005. Apple aroma: alcohol acyltransferase,
 522 a rate limiting step for ester biosynthesis, is regulated by ethylene. Plant Sci. 168, 1199-
 523 1210. <https://doi.org/10.1016/j.plantsci.2004.12.018>
 524 Dupille, E., Zacarías, L. 1996. Extraction and biochemical characterization of wound-
 525 induced ACC oxidase from citrus peel. Plant Science. 114, 53- 60.
 526 [https://doi.org/10.1016/0168-9452\(95\)04311-X](https://doi.org/10.1016/0168-9452(95)04311-X)
 527 El-Sharkawy, I., Kim, W.S., El-Kereamy, A., Jayasankar, S., Svircev, A.M., Brown,
 528 D.C.W. 2007. Isolation and characterization of four ethylene signal transduction
 529 elements in plums (*Prunus salicina* L.). J. Exp. Bot. 58, 3631–3643.
 530 <https://doi.org/10.1093/jxb/erm213>
 531 Fischer, G., Herrera, A., Almanza, P.J. 2011. Cape gooseberry (*Physalis peruviana* L.) En:
 532 Yahia, E.M. (Ed). Postharvest biology and technology of tropical and subtropical fruits.
 533 Acai to citrus, Vol. 2. Pp. 374-396. Woodhead Publishing.
 534 Fiserová, H., Mikusová, Z., Klems, M. 2008. Estimation of ethylene production and 1-
 535 aminocyclopropene-1-carboxylic acid content in plants by means of gas
 536 chromatography. Plant, Soil and Enviromental. 53 (2), 55-60.
 537 <https://doi.org/10.17221/2782-PSE>
 538 Flores, F., Yahyaoui, F.E., de Billerbeck, G., Romojaro, F., Latché, A., Bouzayen, M.,
 539 Pech, J.C., Ambid, C. 2002. Role of ethylene in the biosynthetic pathway of aliphatic

540 ester aroma volatiles in Charentais Cantaloupe melons. *J. Exp. Bot.* 53, 201-206.
 541 <https://doi.org/10.1093/jexbot/53.367.201>

542 Gamble, R.L., Coonfield, M.L., Schaller, G.E. 1998. Histidine kinase activity of the ETR1
 543 ethylene receptor from Arabidopsis. *Proceedings of the National Academy of Sciences.*
 544 95(6), 7825- 7829. <https://doi.org/10.1073/pnas.95.13.7825>

545 Gutiérrez, M.S., Trincherro, G.D., Cerri, A.M., Vilella, F., Sozzi, G.O. 2008. Different
 546 responses of goldenberry fruit treated at four maturity stages with the ethylene
 547 antagonist 1-methylcyclopropene. *Postharvest Biol. Technol.* 48, 199-205.
 548 <https://doi.org/10.1016/j.postharvbio.2007.10.003>

549 Hall, B.P., Shakeel, S.N., Amir, M., Haq, N.U., Qu, X., Schaller, G.E. 2012. Histidine inase
 550 activity of the ethylene receptor ETR1 facilitates the ethylene response in Arabidopsis.
 551 *Plant Physiol.* 159, 682-695. <https://doi.org/10.1104/pp.112.196790>

552 Herzberg, G. 1966. Electronic spectra and electronic structure of polyatomic molecules III.
 553 Van Nostrand.

554 Hubert, D. 2008. Suppression of ethylene responses through application of 1-
 555 methylcyclopropene: a powerful tool for elucidating ripening and senescence
 556 mechanisms in climacteric and nonclimacteric fruits and vegetables. *HortScience* 43(1),
 557 106-111. <https://doi.org/10.21273/HORTSCI.43.1.106>

558 In, B.C., Strablea, J., Binder, B.M., Falbel, T.G., Patterson, S.E. 2013. Morphological and
 559 molecular characterization of ethylene binding inhibition in carnations. *Postharvest Biol.*
 560 *Technol.* 86, 272–279. <https://doi.org/10.1016/j.postharvbio.2013.07.007>

561 Kamiyoshihara, Y., Tieman, D.M., Huber, D.J., Klee, H.J. 2012. Ligand-induced
 562 alterations in the phosphorylation state of ethylene receptors in tomato fruit. *Plant*
 563 *Physiol.* 160, 488-497. <https://doi.org/10.1104/pp.112.202820>
 564 Kesari, R., Trivedi, P., Nath, P. 2007. Ethylene-induced ripening in banana evokes
 565 expression of defense and stress related genes in fruit tissue. *Postharvest Biol. Technol.*
 566 46, 136-143. <https://doi.org/10.1016/j.postharvbio.2007.04.010>
 567 Kevany, B.M., Tieman, D.M., Taylor, M.G., Cin, V.D., Klee, H.J. 2007. Ethylene receptor
 568 degradation controls the timing of ripening in tomato fruit. *Plant J.* 51, 458–467.
 569 <https://doi.org/10.1111/j.1365-3113X.2007.03170.x>
 570 Lacey, R., Binder, B. 2014. How plants sense ethylene gas- The ethylene receptors. *Journal*
 571 *of Inorganic Biochemistry* 133, 58–62. <https://doi.org/10.1016/j.jinorgbio.2014.01.006>.
 572 Li, Y., Qi, H., Jin, Y., Tian, X., Sui, L., Qiu, Y. 2016. Role of ethylene in the biosynthetic
 573 pathway of related-aroma volatiles derived from fatty acids in oriental sweet melon. *J.*
 574 *Amer. Soc. Hort. Sci.* 141, 327-338. <https://doi.org/10.21273/JASHS.141.4.327>
 575 Liu, M., Pirrello, J., Chervin, C., Roustan, J.-P., Bouzayen, M. 2015. Ethylene control of
 576 fruit ripening: revisiting the complex network of transcriptional regulation. *Plant*
 577 *Physiol.* 169 2380-2390. <https://doi.org/10.1104/pp.15.01361>
 578 Majumder, K., Mazumdar, B. 2002. Changes of pectic substances in developing fruits of
 579 cape-gooseberry (*Physalis peruviana* L.) in relation to the enzyme activity and evolution
 580 of ethylene. *Sc. Hortic.* 96, 91-101. [https://doi.org/10.1016/S0304-4238\(02\)00079-1](https://doi.org/10.1016/S0304-4238(02)00079-1)
 581 Mata, C., Van de Poel, B., Hertog, M., Tran, D., Nicolai, B. 2018. Transcription analysis of
 582 the ethylene receptor and CTR genes in tomato: The effects of on and off-vine ripening
 583 and 1-MCP. *Postharvest Biology and Technology*, 140, 67-75.
 584 <https://doi.org/10.1016/j.postharvbio.2018.02.014>

585 Neuhaus, F.C. 2010. Role of Arg301 in substrate orientation and catalysis in subsite 2 of d-
 586 alanine:d-alanine (d-lactate) ligase from *Leuconostoc mesenteroides*: A molecular
 587 docking study. Journal of Molecular Graphics and Modelling. 28(8), 728-734.
 588 <https://doi.org/10.1016/j.jmgm.2010.01.010>

589 NIST. 2013. NIST Standard Reference Database Number 69URL:
 590 <http://webbook.nist.gov/chemistry/>. Accessed 06/03/2013.

591 Pérez, A.G., Sanz, C. 2008. Formation of Fruit Flavour. In: Fruit and Vegetable Flavour.
 592 Bruckner, B., Grant, W.S. (Ed). Pp.41-70. Cambridge, England.

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

597 Schmidt, M.W., Baldridge, K.K., Boatz, J.A., Elbert, S.T., Gordon, M.S., Jensen, J.H.,
 598 Koseki, S., Matsunaga, N., Nguyen, K.A., Su, S., Windus, T.L., Dupuis, M.,
 599 Montgomery, J.A. 1993. General atomic and molecular electronic structure system.
 600 Journal of Computational Chemistry. 14 (11), 1347 -1363.
 601 <https://doi.org/10.1002/jcc.540141112>

602 Serek, M., Woltering, E.J., Sisler, E.C., Frello, S., Sriskandarajah, S. 2006. Controlling
 603 ethylene responses in flowers at the receptor level. Biotechnology Advances 24, 368-
 604 381. <https://doi.org/10.1016/j.biotechadv.2006.01.007>

605 Sun, B., Yan, H., Liu, N., Wei, J., Wang, Q. 2012. Effect of 1-MCP treatment on
 606 postharvest quality characters, antioxidants and glucosinolates of chinese kale. Food
 607 Chem. 131(2), 519-526. <https://doi.org/10.1016/j.foodchem.2011.09.016>

Valdenegro, M., Fuentes, L., Herrera, R., Moya-León, M.A. 2012. Changes in antioxidant capacity during development and ripening of goldenberry (*Physalis peruviana* L.) fruit and in response to 1-methylcyclopropene treatment. *Postharv. Biol. Technol.* 67,110-117. <https://doi.org/10.1016/j.postharvbio.2011.12.021>

Watkins, C. 2006. The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. *Biotechnol. Adv.* 24, 389-409. <https://doi.org/10.1016/j.biote>

Fig. 1. Emission of volatile ethyl butanoate (a), butyl butanoate (b) ethyl octanoate (c) and ethyl decanoate (d) during the postharvest period of cape gooseberry fruits with the applications of ethylene and 1-MCP. The vertical bars indicate the standard error ($n = 4$).

Yahyaoui, F.E., Wongs-Aree, C., Latché, A., Hackett, R., Grierson, D., Pech, J.C. 2002. Molecular and biochemical characteristics of a gene encoding an alcohol acyltransferase involved in the generation of aroma volatile esters during melon ripening. *Eur. J. Biochem.* 269, 2359-2366. <https://doi.org/10.1046/j.1432-1033.2002.02892.x>

623 Yang, X., Song, J., Campbell-Palmer L., Fillmore, S., Zhang, Z. 2013. Effect of ethylene
624 and 1-MCP on expression of genes involved in ethylene biosynthesis and perception
625 during ripening of apple fruit. *Postharvest Biol. Technol.* 78, 55-66.
626 <https://doi.org/10.1016/j.postharvbio.2012.11.012>

627 Zor, T., Selinger, Z. 1996. Linearization of the Bradford protein assay increases its
628 sensitivity: theoretical and experimental studies. *Analytical Biochemistry.* 236, 302 –
629 308. <https://doi.org/10.1006/abio.1996.0171>

630

Fig. 1. Emission of volatile ethyl butanoate (a), butyl butanoate (b) ethyl octanoate (c) and ethyl decanoate (d) during the postharvest period of cape gooseberry fruits with the applications of ethylene and 1-MCP. The vertical bars indicate the standard error ($n = 4$).

Fig. 2. Behavior of the color index of the epidermis (a) and firmness (b) during the postharvest period of the cape gooseberry fruits with the applications of ethylene and 1-MCP. The vertical bars indicate the standard error (n = 4).

Fig. 3. Profiles of size exclusion chromatography of enzymes HK (a) and ACC oxidase (b) extracted from cape gooseberry fruit using Sephadex G-25M filtration gel as the stationary phase.

Fig. 4. Effect of the application of ethylene and 1-MCP on the activity of HK and ACC oxidase enzymes extracted from cape gooseberry fruits (*Physalis peruviana*). Enzyme activity units are $\mu\text{mol HisP min}^{-1} \text{mg}^{-1} \text{protein}$ for HK and $\mu\text{mol C}_2\text{H}_4 \text{min}^{-1} \text{mg}^{-1} \text{protein}$ for ACC oxidase. Different asterisks indicate significant differences, as established with the Fisher LSD test for significant minimum differences ($p < 0.05$). The vertical bars in each average indicate the standard error ($n = 3$).

Fig. 5. *In vitro* experiments for the determination of the kinetic effect of the concentration of 1-MCP on the activities of the enzymes ACC oxidase (a and b) and HK (c and d). Effects of 1-MCP concentration on the $V_{\text{max app}}$ and the K_m of ACC oxidase (e) and HK (f). The following concentrations of 1-MCP were used: 0 ($\bullet$), 20 ($\circ$), 40 ($\blacktriangle$), 60 ($\triangle$), 80 ($\blacksquare$), and 100 μM ($\square$). The reactions were catalyzed by the enzyme HK (g) or by the enzyme ACO (h).

Fig. 6. Free energy of the binding of ethylene and 1-MCP to the ETR1 receptor and of 1-ACC and 1-MCP to ACC oxidase.

a.

b.

c.

d.

672

673 **Fig. 7.** Diagram of the binding between the ETR1 receptor and ethylene (a), the ETR1
674 receptor and 1-MCP (b), ACO and 1-ACC (c) and ACO and 1-MCP (d). The purple
675 structure is the enzyme, and the red one is the substrate.

676