

## Quality attributes during maturation of ‘Golden Delicious’ and ‘Red Delicious’ apples grown in two geographical regions with different environmental conditions

Francisco J. MOLINA-CORRAL<sup>1</sup>, Miguel ESPINO-DÍAZ<sup>1</sup>,  
Juan L. JACOBO<sup>2</sup>, Scott D. MATTINSON<sup>3</sup>, John K. FELLMAN<sup>3</sup>,  
David R. SEPÚLVEDA<sup>1</sup>, Gustavo A. GONZÁLEZ-AGUILAR<sup>4</sup>,  
Nora A. SALAS-SALAZAR<sup>2</sup>, Guadalupe I. OLIVAS<sup>1\*</sup>

<sup>1</sup>Centro de Investigación en Alimentación y Desarrollo A.C., Av. Río Conchos S/N, Cuauhtémoc, Chih., 31570, México;  
[aster3000@hotmail.com](mailto:aster3000@hotmail.com); [javiermolina@ciad.mx](mailto:javiermolina@ciad.mx); [dsepulveda@ciad.mx](mailto:dsepulveda@ciad.mx); [golivas@ciad.mx](mailto:golivas@ciad.mx) (\*corresponding author)

<sup>2</sup>Universidad Autónoma de Chihuahua, Facultad de Ciencias Agrotecnológicas, Calle Presa de la Amistad 2015 Cuauhtémoc, Chih.,  
31510, México; [jacobocuellarl@gmail.com](mailto:jacobocuellarl@gmail.com); [nora.salas09@yahoo.com.mx](mailto:nora.salas09@yahoo.com.mx)

<sup>3</sup>Washington State University, Department of Horticulture and Landscape Architecture, Pullman, WA 99164-6414, USA;  
[dmattinson@wsu.edu](mailto:dmattinson@wsu.edu); [fellman@wsu.edu](mailto:fellman@wsu.edu)

<sup>4</sup>Centro de Investigación en Alimentación y Desarrollo A.C., Carretera a la Victoria Km 0.6, Hermosillo, Son., México;  
[gustavo@ciad.mx](mailto:gustavo@ciad.mx)

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### Abstract

Apples have distinctive quality attributes that may be defined by environmental conditions of the geographical regions where fruits are cultivated, such as temperature, solar radiation, photoperiod, and photothermic units. A three-year study was conducted to compare ‘Golden Delicious’ and ‘Red Delicious’ apples from two different regions, Washington, USA (WA) and Chihuahua, Mexico (CHIH). Apple samples were harvested weekly from early August to late October (~120-180 days after full bloom - DAFB), and analysed for quality parameters. Geographic environmental data were obtained, and photoperiod, solar radiation, degree-days and photothermal units were calculated. Results show quality differences between CHIH and WA apples. WA shows a ~5-week delay in apple bloom, possibly due to the lower temperatures presented in WA. Apples from both regions required the same photoperiod, ~ 2,222 h, to attain the beginning of the ripening stage, which took more days (three weeks) for CHIH apples, most likely attributed to the higher elevation of CHIH orchards (2,062 vs. 763 masl). The main distinctive quality differences found between WA and CHIH apples were firmness and aroma volatile compounds. CHIH apples presented substantially higher amounts of aroma compounds. WA apples showed greater firmness, probably due to lower photothermal units. Using all firmness data (both varieties, both growing zones) a remarkable correlation was found between firmness and photothermal units ( $R=0.89$ ), which may suggest firmness could be improved by the manipulation of degree days and photoperiod, that is, temperature and light.

**Keywords:** aroma volatiles; elevation; firmness; *Malus domestica*; photoperiod; photothermal units; solar radiation

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## Introduction

Apples are important fruits worldwide, and Washington (WA) and Chihuahua (CHIH) are the top apple-producing states in the US and Mexico. 'Golden Delicious' (GD) and 'Red Delicious' (RD) apples are among the most cultivated varieties in both areas and are highly valued for their quality (USDA, 2020; SIAP, 2020).

Apple quality characteristics may be affected by factors such as environmental conditions (Dal Cin *et al.*, 2007; Timilsina and Tripathi, 2019), which may also modify the blooming time and maturation rate of the fruit (Dhanaraj *et al.*, 1986; Guédon and Legave, 2008). Among the environmental characteristics that can vary between WA and CHIH include temperature, solar radiation, and photoperiod, which are defined in part by the altitude and latitude of the zones (Drogoudi and Pantelidis, 2011; Jin *et al.*, 2017; Alessandrini *et al.*, 2019; Timilsina and Tripathi, 2019).

The onset of flowering is hastened by temperature in pears, apples, and leguminous species such as chickpea, which is explained by the fulfilment of the heat requirements (Keatinge *et al.*, 1998; Guédon and Legave, 2008; Kanchan and Bhatia, 2014). The time span in the apple developmental cycle required to reach a given ripening stage seems to be longer in high altitude zones, probably due to a delay in ethylene biosynthesis (Dhanaraj *et al.*, 1986; Danesin *et al.*, 2004; Dal Cin *et al.*, 2007). The ripening rate may be affected by the photoperiod; according to Keatinge *et al.* (1998), temperate leguminous species matured earlier in zones with more extended photoperiod.

Elevation may affect fruit quality. According to Faniadis *et al.* (2010), cherries of the highest elevations have better quality compared to those cultivated in the lowest elevations (Danesin *et al.*, 2004; Dal Cin *et al.*, 2007). According to Timilsina and Tripathi (2019), lower acidity is observed on mandarins (*Citrus reticulata* Blanco) as the elevation increased. Also, some studies report that elevation has a marked influence on the aroma production of grapes (Jiang *et al.*, 2013; Xu *et al.*, 2015; Alessandrini *et al.*, 2019).

Day length and light intensity may also have an impact on fruit quality. According to Choudhury *et al.* (2008), sucrose synthesis in bananas (*Musa acuminata*, AAA group) increases with increasing light exposure. Other works show that solar radiation favours red coloration and a more developed yellow colour in apples suggesting there is an induction of the synthesis of anthocyanins at greater light intensity (Jakopic *et al.*, 2007; Drogoudi and Pantelidis, 2011; Baiamonte *et al.*, 2016; Ordoñez *et al.*, 2016).

Another interesting environmental parameter that may be related to apple quality is the photothermal unit (PTU), which is the product of the degree day (GDD favourable thermal unit for the occurrence or continuation of a biological process) times the sunshine hours. To the best of our knowledge, the effect of PTU on fruit quality has not been reported.

Therefore, to better understand how environmental factors affect apple quality, the present study characterized the environmental conditions of two different apple-producing zones, Washington (WA) and Chihuahua (CHIH), and evaluated the quality development of 'Golden Delicious' and 'Red Delicious' apples during the maturation of the fruits. Differences in flowering, internal ethylene concentration IEC, colour, firmness, and aroma volatiles of apples in the two zones were evaluated in relationship with temperature, photoperiod, solar radiation altitude, elevation and photothermal units, to elucidate whether these quality attributes may be related to environmental conditions.

## Materials and Methods

### *Plant material*

A three consecutive year study was simultaneously conducted on 'Golden Delicious' and 'Red Delicious' apples grown in Cuauhtémoc, Chihuahua (Mexico) and Pullman, Washington (USA). Characteristics of both regions (latitude, longitude, and elevation) are shown in Table 1. The study was carried out on commercial

orchards with general orchard management procedures. Apple trees on MM106 rootstocks 25 years old in Washington (WA) and 30 years old in Chihuahua (CHIH) were used for this experiment. A total of 20 trees per each variety were used. A total of 60 apples (3 apples per tree) were harvested, from the external western side of the tree canopy to avoid fruit quality variations due to fruit position on canopy. Fruit sampling was conducted on a weekly basis from August to October, corresponding to 130 to 176 days after full bloom (DAFB). Samples from immature to mature stages of development were collected, and evaluated for maturity parameters, IEC, acidity, total soluble solids, colour, firmness and volatile compounds.

**Table 1.** Apple orchards geographic data in Washington, USA (WA) and Chihuahua, Mexico (CHIH)

	WA	CHIH
Latitude	46°43'53.28" N	28°24'19.21" N
Longitude	117°10'13.21" W	106°50'03.11" W
Elevation	763 m	2062 m

WA: Pullman, WA, USA; CHIH: Cuauhtémoc, Chih., México

*Geographic environmental data, degree-days and photothermal units*

Weather data was collected from two weather stations: UNIFRUT weather station at Cuauhtémoc, Chihuahua, Mexico, and Paradise creek weather station at Pullman, Washington. Maximum, minimum, and average temperature were recorded. Meteorological indices, photoperiod, solar radiation, degree days and photothermal units, were calculated from flowering until apples reached  $\sim 1 \mu\text{L L}^{-1}$  IEC (Tables 2 and 3).

The total amount of heat required for an organism to develop from one developmental stage to another in its life cycle is calculated in units called growing degree-days (GDD), sometimes called heat units. GDD are the accumulated product of time and temperature between the developmental thresholds for each day. GDD were calculated according to the equation proposed by McMaster and Wilhelm (1997):  $\text{GDD} = [T_{\max} + T_{\min}]/2 - T_b$ , where  $T_{\max}$  and  $T_{\min}$  are daily maximum air temperatures, respectively, and  $T_b$  is base temperature, a threshold value below which no temperature contribution is effective. The  $T_b$  used was 7 °C, as proposed by Kronenberg (1983) for apple trees. Photothermal units (PTU) were calculated by using the equation given by Wilsie (1962)  $\text{PTU} = \text{GDD} * L$ ; Where: GDD= Growing degree days; L= Maximum possible sunshine hours.

**Table 2.** Average temperatures in Washington, USA (WA) and Chihuahua, Mexico (CHIH), five weeks before bloom (an average of the three seasons)

	Max T (°C)	Min T (°C)	Average T (°C)	Fluctuation (°C)
WA	11.6	2.0	6.8	9.7
CHIH	21.8	2.1	11.9	19.7

**Table 3.** Days after full bloom required for attaining  $\sim 1 \mu\text{L L}^{-1}$  IEC, cumulative photoperiod and solar radiation, from flowering to  $\sim 1 \mu\text{L L}^{-1}$  IEC, for Golden Delicious and Red Delicious apples grown in Washington, USA (WA) and Chihuahua (CHIH)

Cultivar	Zone	DAFB (IEC $\sim 1 \mu\text{L L}^{-1}$ )	Photoperiod <sup>1</sup> (light hours summation)	Solar radiation (Mj/m <sup>2</sup> ) <sup>3</sup>	Photothermal units <sup>1</sup>
'Golden Delicious'	CHIH	169	2264 (16.5) a	2050.0	24973 (655.9) a
	WA	151	2209 (34.6) a	1819.6	18027 (500.1) b
'Red Delicious'	CHIH	169	2279 (7.6) a	2052.4	24963 (658.2) a
	WA	144	2139 (54.3) a	1535.7	17630 (192.9) b

<sup>1</sup>Photoperiod and photothermal units were submitted to statistical analysis (standard error is presented in parenthesis).

Different upper-case letters among zone for each cultivar indicate statistically significant differences ( $p \leq 0.05$ )

*Internal ethylene concentration (IEC)*

Internal ethylene assays were performed by withdrawing a 0.2-0.5 mL gas sample from the core-space of a whole apple fruit through a 1 mL 23-gauge hypodermic needle that had been pushed through the calyx end of the fruit into the core and sealed to the fruit with silicon grease. The needle was pushed into the calyx of the apples submerged in water and the needle was sealed with a rubber stopper. Gas samples were analysed using a gas chromatograph (Varian 3800; Walnut Creek, CA, USA) in CHIH and a HP-5890 (Agilent Technologies; Avondale, PA, USA) in WA equipped with a Haysep Q Chrompack packed column (1.8 m, 3/8 ID, 20 mesh Silica) in (CHIH), and a 15 m × 0.53 mm, 40 µm HP-PLOT-Q in (WA) and a flame ionization detector. The chromatographic conditions were as follows, initial oven temperature 60 °C, held for 2 min, increased to 120 °C at 60 °C/min, and held for 1.5 min in (CHIH), and the oven temperature in (WA) was isothermal at 100 °C. The carrier gas flow of helium was 30 mL/min in (CHIH), and the column carrier gas in (WA) was 8.0 mL/min. Ethylene values reported were the average of 10-15 measurements.

*Colour and firmness measurements*

Ten fruits per treatment were used to evaluate skin colour and firmness. Two measurements per sample at two opposite locations on the equatorial area were assessed. Colour was measured with a Minolta CR-300 colorimeter (Konica Minolta; New Jersey, USA). Values were obtained using the Hunter Lab scale (L, a, b), and Hue angle was calculated. Apple firmness was determined with a Texture Analyser TA-XT2i (Stable Micro Systems; YL, England) in CHIH, and using a Topping penetrometer (Topping 1981) in WA. The tip used was an 11 mm diameter penetrometer tip. The maximum force needed to penetrate the peeled flesh, over a distance of 10 mm at a speed of 10 mm seg<sup>-1</sup> was measured.

*Acid and soluble solids contents*

Apple juice samples were obtained from 10 apples and titrated with 0.1 N NaOH to a pH 8.2 endpoint, then the acidity was expressed as % of malic acid. Total soluble solids (TSS) in the juice were determined with a refractometer ATC-1E (Atago Ltd; Tokyo, Japan) in CHIH, and a Reichert ABBE Mark II refractometer (AO Scientific Instruments; Keene, NH, USA) in WA.

*Aroma volatiles*

Concentration of volatiles in apples was determined by gas chromatography-mass spectrometry using the solid phase micro extraction (SPME) technique. Apple juice from 10 apples was obtained with a food processor (Turmix; Mexico) in CHIH, and with a Champion Juicer (Lodi; CA, USA) in WA. A 20 mL juice sample was placed in a 20 mL plastic scintillation vial, frozen in liquid nitrogen, and kept at -70 °C until analysis. An aliquot of 2 mL of thawed apple juice was placed in a 4 mL vial containing 0.7 g of sodium chloride and stirred while a SPME fiber (65 µm, PDMS-DVB, Supelco USA) was exposed to the headspace of the sample for 1 h at room temperature (25 °C). The fiber was desorbed by splitless injection, using a SPME liner, for 5 min at 200 °C into a GC-MS system (Varian Saturn 2100D GC/MS; Walnut Creek, CA, USA) equipped with an Equity-1 column (60 m × 0.25 mm ID × 0.25 µm film thickness, Supelco USA). Chromatography conditions were as follows: initial oven temperature of 33 °C held for 5 min, increased to 50 °C at 2 °C/min, increased to 250 °C at 5 °C min<sup>-1</sup>, and held for 6.5 min. Helium was used as the carrier gas with a linear velocity of 30 cm s<sup>-1</sup>. The GC/MS and column used in WA and CHIH to analyse volatiles from apple juice was similar to Olivas *et al.* (2007). Mass spectra were obtained by electron impact ionization at 70 eV. Transfer line and ion source temperatures were 250 and 180 °C, respectively. Spectra were recorded with a Saturn GC/MS workstation (Varian). Identification of the volatile organic compounds (VOCs) of interest was made by matching their spectra with those of the NIST 98 MS library and by comparing the retention times against those of high purity standards (Sigma-Aldrich and ChemService). Quantification was accomplished by external standard calibration curves integrating the peak areas. All values represent the average of 3 pooled samples from 10 apples.

### *Statistical analysis*

Two independent studies were conducted, one studying 'Red Delicious' apples, and one studying 'Golden Delicious' apples. On each one of the experiments, "geographical region" was studied as the relevant factor (WA vs. CHIH). The effect of geographical region on slopes, means and specific values at the commercially accepted ripening point ( $\sim 1$  ppm IEC) were compared. Three consecutive years were studied as replications. Statistical analysis was performed using SAS<sup>TM</sup> (Statistical analysis System, version 8.0). A one-way ANOVA was conducted.

## **Results and Discussion**

The present study compares the differences in 'Golden Delicious' (GD) and 'Red Delicious' (RD) apples produced in Washington (WA) and in Chihuahua (CHIH), and evaluates how environmental conditions, such as temperature, daylight hours, and altitude, among others, may influence the quality characteristics of the fruit. According to Faust (2000), WA and CHIH have different climates that may influence the general physiology of the apple trees. While Washington State offers an arid climate with warm days and cool nights, Chihuahua State is characterized by a climate with warm days and nights with moderate temperatures. Both regions receive about 400 mm of rain per year. Results show that WA trees took 34-42 days longer than CHIH trees to bloom. This delay in the flowering of WA trees may be due to lower temperatures in that area. Table 2 shows higher temperatures before flowering in CHIH compared to WA. According to Guédon and Legave (2008), higher temperatures cause early flowering; this may be explained by the completion of heat requirements in trees.

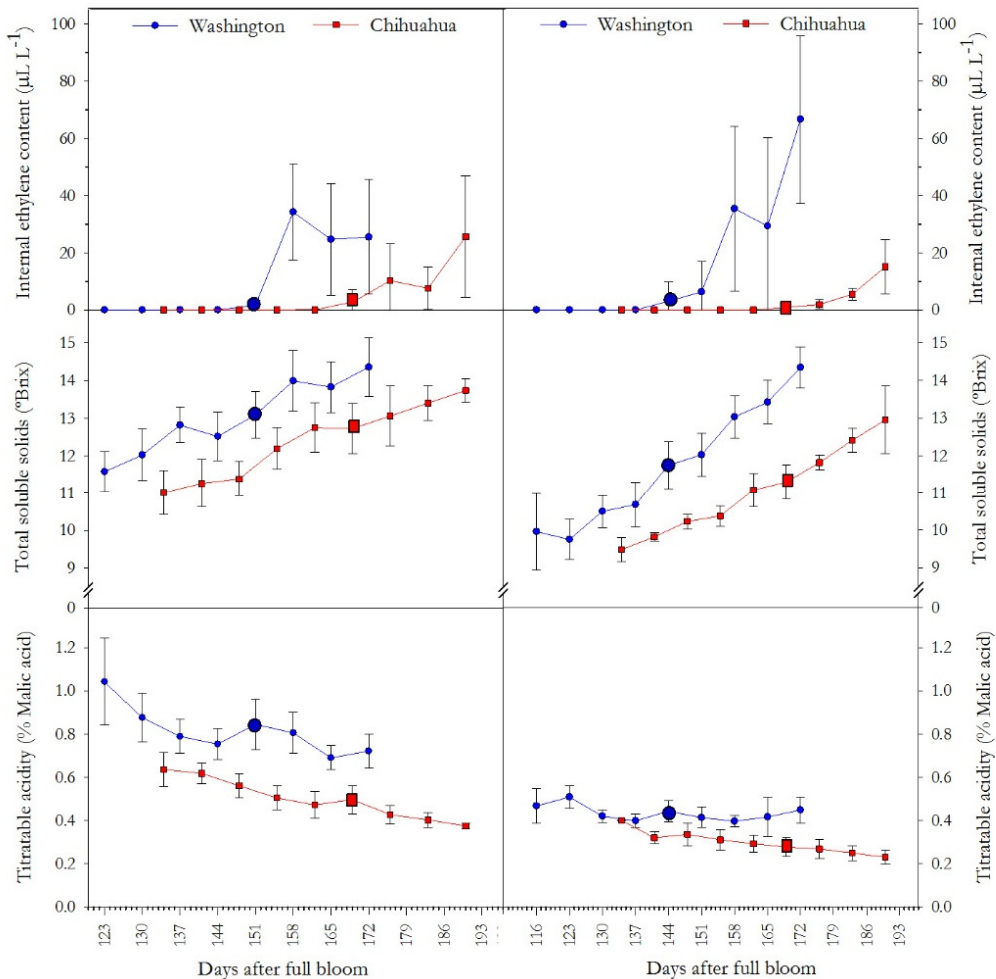
### *Internal Ethylene Concentration IEC*

Apples are climacteric fruits whose ripening is associated with an increase in ethylene production (Reid *et al.*, 1973). The optimal harvest date coincides with the beginning of the climacteric rise, followed by the rise in ethylene beyond a threshold of  $1 \mu\text{L L}^{-1}$  in 'Delicious' apples (Fellman *et al.*, 2003). Overripe fruit has short storage life, while fruit harvested at an immature stage has low quality and can be more susceptible to physiological disorders (Bai *et al.*, 2009). Therefore, picking apple fruit at an optimum maturity is essential. In the present study, quality comparisons between zones were performed at  $1 \mu\text{L L}^{-1}$  IEC, which is considered an ethylene threshold, beyond what a marked rise in IEC is observed. According to Fellman *et al.* (2003), apples harvested at  $1 \mu\text{L L}^{-1}$  IEC presented the highest overall rating by an untrained taste panel (considering one week of postharvest storage under  $25^\circ\text{C}$ ).

Figure 1 shows the ethylene production of GD and RD apples grown in WA and CHIH. The onset in ethylene production ( $\sim 1 \mu\text{L L}^{-1}$ ) was first observed in WA apples (152 DAFB for GD and 144 DAFB for RD) followed by CHIH apples (169 DAFB for GD and 169 DAFB for RD). The longer time needed by CHIH apples to achieve the onset in ethylene production could be due to the difference in elevation of the studied locations (2062 vs. 763 masl, CHIH and WA, respectively). According to Dhanaraj *et al.* (1986), 'Royal Delicious' and 'Red Delicious' apples at 2062 masl required more time to reach the same maturity stage than apples at 1675 masl. Danesin *et al.* (2004) found that 'Golden Delicious' apples cultivated at 900 masl showed a delay of ethylene biosynthesis and MACO2 and MCTR1 expression when compared to apples cultivated at 500 masl. Also, Dal Cin *et al.* (2007) found that the optimal apple harvest time was delayed at higher elevations (950 vs. 533 masl).

In the present study, higher ethylene values were observed in WA apples in comparison with CHIH apples (Figure 1). WA RD apples showed ethylene values up to  $67 \mu\text{L L}^{-1}$  at 173 DAFB while CHIH apples barely reached  $15 \mu\text{L L}^{-1}$  at 190 DAFB. WA GD showed values up to  $34 \mu\text{L L}^{-1}$  at 158 DAFB while CHIH apples only reached  $25 \mu\text{L L}^{-1}$  at 190 DAFB. Dal Cin *et al.* (2007) found that the amount of ethylene produced at harvest was much higher at lower elevations (533 masl) when compared to higher elevation sites (950 masl).

The intensity of the environmental light could affect the amount of ethylene produced by apples. Nilsson and Gustavsson (2007) studied the effect of the apple position inside the canopy. They discovered that the ethylene peak was considerably higher in the shaded apples positioned inside the canopy with lower light intensity. In the present work, less solar radiation was observed in WA (Table 3).



**Figure 1.** Internal ethylene concentration ( $\mu\text{L L}^{-1}$ ), total soluble solids ( $^{\circ}\text{Brix}$ ), and titratable acidity (% Malic acid) during maturation of 'Golden Delicious' apples (left) and 'Red Delicious' apples (right) grown in Chihuahua, Mexico (CHIH) and Washington, USA (WA). Vertical bars represent one standard deviation.

The ripening delay observed on CHIH apples (Figure 1) could be related to the photoperiod. Table 3 shows how apples from both zones needed the same photoperiod,  $\sim 2,222$  h, to reach the beginning of the ripening stage ( $1 \mu\text{L L}^{-1}$  IEC). However, while WA attained this photoperiod at 144 and 151 DAFB for RD and GD apples, respectively, CHIH required longer time (169 DAFB for both varieties RD and GD) (Table 3). Figure 3 shows how CHIH presented less accumulated light hours, so longer time was required for CHIH apples to obtain the same photoperiod as WA. According to Dwyer and Stewart (1987), barley's phenological development rate is proportional to the photoperiod. According to Keating *et al.* (1998), temperate leguminous species mature earlier at more extended photoperiod. In this case WA apples have the longest photoperiod averaging of  $14.74$  h/day, compared with  $13.44$  h/day for CHIH apples.

### *Acidity and soluble solids contents*

Total organic acid content, measured as titratable acidity (TA), and the concentration of total soluble solids (TSS), expressed as °Brix, are useful maturity indices measured in apples, in addition to the internal ethylene concentration, to determine optimum harvest date when long term storage is intended (Kingston, 1992; Bai *et al.*, 2009). The acidity (sour taste) in the apple is given by organic acids such as malic, citric, and tartaric acids (Kader 2008; Wu *et al.*, 2007). Malic acid, accumulated in the vacuoles (Khan *et al.*, 2013), comprises about 90% of the acid content (Wu *et al.*, 2007; Nour *et al.*, 2010). Organic acids are used as substrates for the respiratory process, and the percentage of malic acid may decrease up to 40% in pome fruits during ripening (Ackermann *et al.*, 1992; Knee, 1993). The TA varies with the cultivar, the season, and the orchard handling. Trad *et al.* (2013) found that in mountainous zones, fruits ripened earlier and were less acid.

The present work shows how titratable acidity is lower in CHIH apples when compared to WA apples. Malic acid at  $\sim 1 \mu\text{L L}^{-1}$  IEC was 0.84% and 0.5% for GD apples and 0.4% and 0.3% for RD apples, for WA and CHIH, respectively (Figure 1). Lower acidity on CHIH apples may be due to the higher solar radiation presented in CHIH compared to WA (Table 3). Nilsson and Gustavsson (2007) observed that apples outside of the tree, which received higher light intensity, presented lower titratable acidity when compared with fruit inside of the canopy. Altitude may also influence apple acidity. According to Timilsina and Tripathi (2019), titratable acidity lowers on mandarins as elevation increases. The present work shows how acidity was lower in the highest altitude area, CHIH (2062 masl) (Figure 1).

During apple senescence a decrease in starch is observed due to the activity of enzymes  $\alpha$ -amylase,  $\beta$ -amylase, and starch phosphorylase, which cause an increase in the sugar content (Azcon-Bieto and Talon, 1996). The sweetness of apple fruit is due to soluble solids, mainly fructose, glucose, and sucrose (Li *et al.*, 2012). In the present study, the total soluble solids (TSS) increased over time in both varieties at both zones. No significant differences were found on TSS on WA and CHIH RD apples (11.7 and 11.3°Bx, respectively) and GD apples (13.1 and 12.7°Bx, respectively) at the onset of ethylene production ( $1 \mu\text{L L}^{-1}$  IEC). Although there is no significant difference in TSS values at the same maturity stage, Figure 1 shows a lag in TSS development in CHIH. This probably happened because WA accumulates light hours faster than CHIH (Table 3). According to Choudhury *et al.* (2008), sucrose synthesis in bananas increases by increasing light exposure. Also, a high TSS content has been found on more sun-exposed apples (Drogoudi and Pantelidis, 2011) and peaches (*Prunus persica* L.) (Marini *et al.*, 1991).

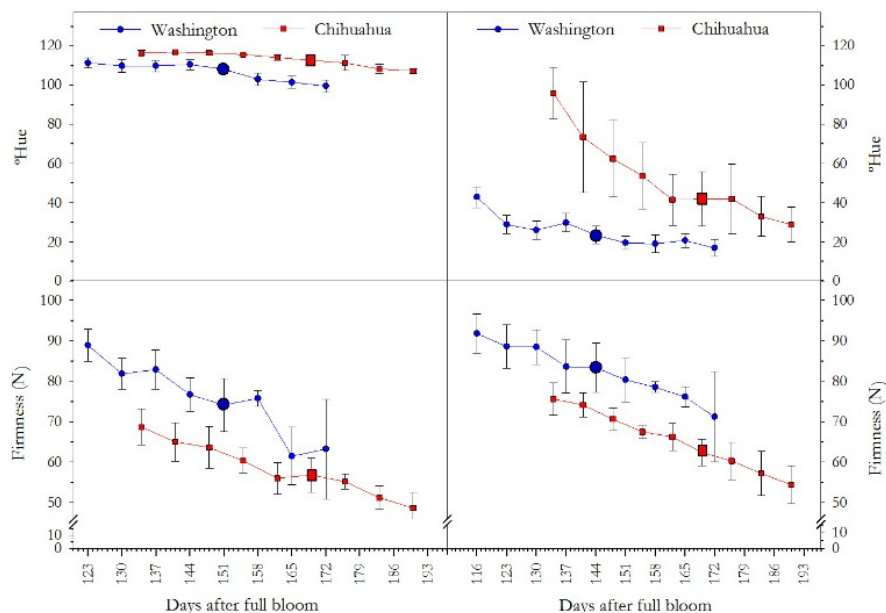
### *Color and firmness*

Skin color and fruit firmness are two of the most important apple attributes to determine fruit maturity (Wills *et al.*, 1981). Color changes are associated with changes in apples' metabolism during ripening, accompanied by changes in the chlorophyll content (Gross, 1987). The anthocyanins, which are water-soluble and accumulate in vacuoles, give the red colour to the apple skin (Lancaster and Dougall, 1992; Umemura, 2013); they are synthesized via the flavonoid pathway, a branch of the phenylpropanoid pathway (Takos *et al.*, 2006). In apple skin, the formation of anthocyanin is sunlight-dependent, mainly UV-B (280-320 nm) (Arakawa *et al.*, 1985; Arakawa, 1988; Saure, 1990; Steyn *et al.*, 2009). This process is enhanced by light exposure, cool nights and warm sunny days before harvest (Kingston, 1992).

No significant differences were found in the color of WA and CHIH apples (GD and RD) (Figure 2, Table 4), in contradiction with previous studies that show that solar radiation favors red coloration (Jakopic *et al.*, 2007; Drogoudi and Pantelidis, 2011; Baiamonte *et al.*, 2016) and a more developed yellow color (Ordoñez *et al.*, 2016). According to Jakopic *et al.* (2007 and 2010), greater light intensity induces anthocyanin synthesis. The present study did not show that higher solar radiation would induce red coloration in RD apples grown in CHIH (area with the highest solar radiation, Table 3). According to Dussi *et al.* (2005), if the solar radiation is not low enough, colour development may be achieved.

Firmness is an important indicator of apple quality (Huxsoll *et al.*, 1989). A texture difference of at least 6 N can be perceived by a trained panel (Harker *et al.*, 2002). Firmness could be affected by pre-harvest factors

related to the crop area, such as temperature, daylight hours, and solar radiation, among others (Sams, 1999). During maturation, there was a decrease in firmness of apples from both studied regions with no differences in the reduction rate (Figure 2, Table 4), which, according to Johnston *et al.* (2002), is due to the disruption of the middle lamella, responsible of cell adhesion and cell packing.



**Figure 2.** Hue angle (°Hue), and Firmness (N) during maturation of ‘Golden Delicious’ apples (left) and ‘Red Delicious’ apples (right) grown in Chihuahua, Mexico (CHIH) and Washington, USA (WA). Vertical bars represent one standard deviation.

**Table 4.** Quality parameters for ‘Golden Delicious’ and ‘Red Delicious’ apples grown in Washington, USA (WA) and Chihuahua, México (CHIH)

Cultivar	Zone	Firmness			TSS			Color			Malic acid		
		Slope <sup>1</sup>	Mean <sup>2</sup>	N <sup>3</sup>	Slope <sup>1</sup>	Mean <sup>2</sup>	Bx <sup>3</sup>	Slope <sup>1</sup>	Mean <sup>2</sup>	Hue <sup>3</sup>	Slope <sup>1</sup>	Mean <sup>2</sup>	% <sup>3</sup>
‘Golden Delicious’	WA	-0.509 <sup>a</sup>	73.7 <sup>a</sup>	74.15 <sup>a</sup>	0.0535 <sup>a</sup>	13.1 <sup>a</sup>	13.09 <sup>a</sup>	-0.2817 <sup>a</sup>	105.91 <sup>a</sup>	107.74 <sup>a</sup>	-0.0031 <sup>a</sup>	0.78 <sup>a</sup>	0.84 <sup>a</sup>
	CHIH	-0.329 <sup>a</sup>	60.8 <sup>b</sup>	56.84 <sup>b</sup>	0.0533 <sup>a</sup>	12.7 <sup>a</sup>	12.73 <sup>a</sup>	-0.1317 <sup>b</sup>	114.70 <sup>a</sup>	112.63 <sup>a</sup>	-0.0049 <sup>a</sup>	0.53 <sup>b</sup>	0.50 <sup>b</sup>
‘Red Delicious’	WA	-0.337 <sup>a</sup>	83.01 <sup>a</sup>	80.0 <sup>a</sup>	0.0907 <sup>a</sup>	11.7 <sup>a</sup>	11.74 <sup>a</sup>	-0.2726 <sup>a</sup>	22.80 <sup>a</sup>	23.48 <sup>a</sup>	-0.0003 <sup>a</sup>	0.41 <sup>a</sup>	0.44 <sup>a</sup>
	CHIH	-0.388 <sup>a</sup>	65.87 <sup>b</sup>	62.4 <sup>b</sup>	0.0558 <sup>b</sup>	11.3 <sup>a</sup>	11.30 <sup>a</sup>	-1.0044 <sup>a</sup>	55.73 <sup>a</sup>	41.88 <sup>a</sup>	-0.0022 <sup>a</sup>	0.31 <sup>a</sup>	0.28 <sup>a</sup>

<sup>1</sup>Slopes corresponding to data ranging from 130 to 173 DAFB for WA and from 134 to 176 DAFB for CHIH.

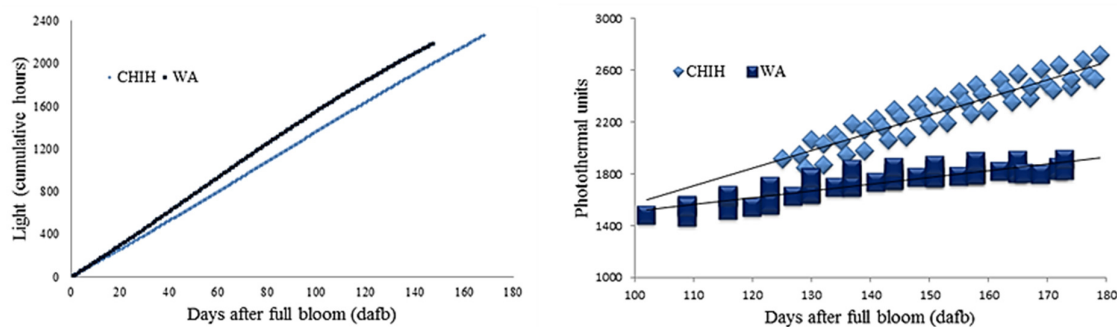
<sup>2</sup>Means corresponding to data ranging from 130 to 173 DAFB for WA and from 134 to 176 DAFB for CHIH.

<sup>3</sup>Values obtained when internal ethylene concentration (IEC) in apples was  $\sim 1 \mu\text{L L}^{-1}$ . Golden Delicious: 169 and 151 DAFB for CHIH and WA, respectively. Red Delicious: 169 and 144 DAFB for CHIH and WA, respectively.

Different upper-case letters among zone for each cultivar indicate statistically significant differences ( $p \leq 0.05$ ).

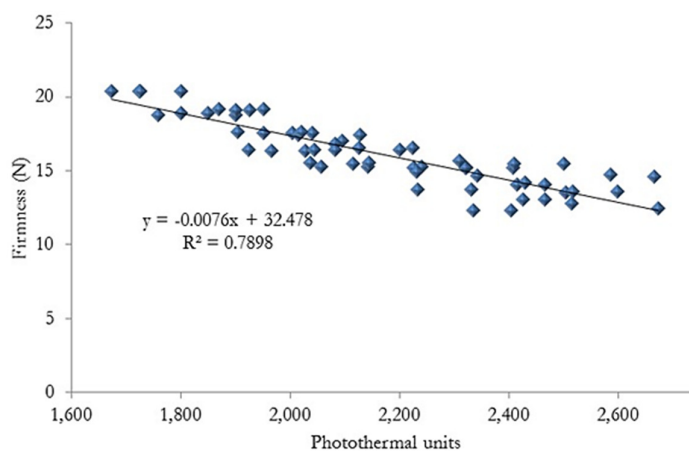
For both cultivars, RD and GD, WA apples showed considerably higher firmness values during the study when compared to CHIH apples (Figure 2, Table 4), for both cultivars. To better understand the possible effects of the environment in the two studied regions, photothermal units were calculated, and their correlation with firmness was analysed. Under a specific temperature, a given plant cannot develop; above a specific temperature, plants slow down their growth and eventually stop. A heat unit accumulates, when the average temperature of the day exceeds the lower critical point of development of the plant by one degree. It has been shown that photothermal units correlate better with plant development than heat units. So, photothermal units are obtained multiplying the light hours by the day degrees. Figure 3 shows how the photothermal units accumulated as the days after full bloom progressed. It can be seen that CHIH accumulated higher photothermal unit values when compared to WA.





**Figure 3.** Photoperiod (cumulative light hours), and photothermal units (cumulative), from bloom to harvest. Data pooled from two varieties, and three seasons

According to Dwyer and Stewart (1987), barley's phenological development (*Hordeum vulgare* Bruce) varies as a function of heat units. A high correlation between the apples' firmness and the photothermal units was observed in this study (Figure 4), with a strong correlation coefficient ( $r = 0.89$ ). Figure 4 shows three-year data on GD and RD apples grown in both areas, WA and CHI. This figure shows that photothermal units correlate with apple firmness, meaning that day-light and temperature are the main factors affecting apple firmness. The present work shows that photothermal units could be an excellent predictor of firmness in apples and that when manipulating photothermal units (temperature and light hours), the apple firmness could be controlled. More studies are needed to confirm this hypothesis.



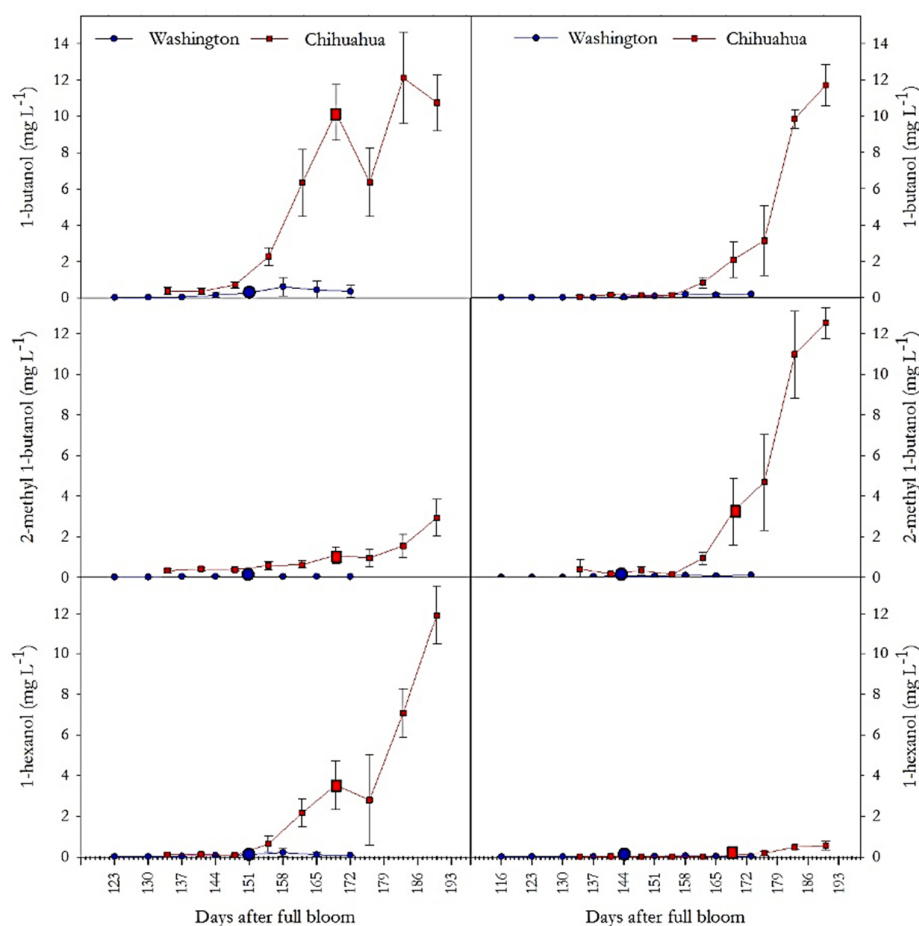
**Figure 4.** Apple firmness dependence on photothermal units

Firmness values include 'Golden Delicious' and 'Red Delicious' apples from Chihuahua, Mexico (CHI) and Washington, USA (WA), on three consecutive years.

#### *Aroma volatiles*

More than 300 volatile compounds have been identified in apples, being the main volatiles aldehydes, alcohols, and esters. Aldehyde synthesis is considered the first step in the production of volatiles, followed by alcohols, being esters the last ones synthesized (Pérez *et al.*, 2008; Espino-Díaz *et al.*, 2016). Esters are the most important compounds that contribute to ripe apples' aroma, followed by alcohols (Dimick *et al.*, 1983; Dunemann *et al.*, 2012; Espino-Díaz *et al.*, 2016). In the present study three alcohols (1-butanol, 2-methyl-1-butanol, and 1-hexanol) and three esters (butyl acetate, 2-methyl butyl acetate, and hexyl acetate) were analysed, as they are considered among the most important in apples due to their high content and impact on apple

aroma. The results of volatile aroma compounds are shown in Figures 5 and 6. As apple maturity develops, an increase in volatile compounds is observed, following the ethylene pattern. An increase in volatile production was observed at the IEC onset for WA apples (Figures 5 and 6). The same behaviour was observed on esters for CHIH apples. According to Song and Bangerth (1996), an increase in autocatalytic ethylene production may be essential for increasing aroma volatile production. According to Fellman *et al.* (2003), major volatile production is closely linked to the onset of climacteric ripening.

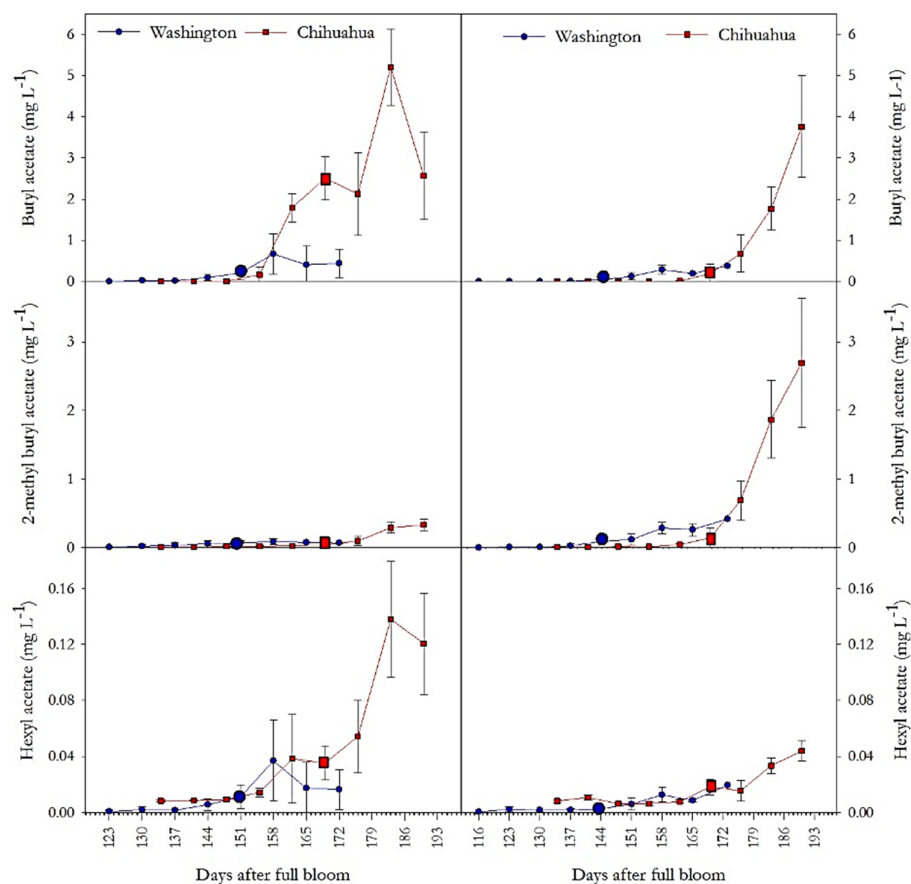


**Figure 5.** Production of alcohols 1-butanol, 2-methyl 1-butanol, and 1-hexanol, (mg L<sup>-1</sup>) during maturation of 'Golden Delicious' apples (left) and 'Red Delicious' apples (right) grown in Chihuahua, Mexico (CHIH) and Washington, USA (WA). Vertical bars represent one standard deviation.

Regardless of the production area, a higher concentration of 1-butanol and butyl acetate was found in GD apples (Figures 5 and 6). Similarly, other authors have found that the most abundant volatile aroma compounds in GD apples are 1-butanol and butyl acetate (Song and Bangerth, 1996; López *et al.*, 1998; Kondo *et al.*, 2005; Salas *et al.*, 2011). A higher concentration of 2-methyl-1-butanol and 2-methyl butyl acetate was found in RD apples (Figure 5). According to Rowan *et al.* (1999), high production of 2-methyl butyl acetate seems to be characteristic of the RD apples. These results suggest different enzymatic activities between cultivars contribute to the variation in volatile esters' accumulation, as reported by Rowan *et al.* (1999) and Holland *et al.* (2005).

At the onset in ethylene production ( $\sim 1 \mu\text{L L}^{-1}$  IEC), higher concentrations of 1-butanol, butyl acetate, 1-hexanol, butyl acetate, 2-methyl-1-butanol were observed in CHIH apples ( $p \leq 0.05$ ) when compared to WA

apples (Figures 5 and 6). No statistical difference was observed in 2-methyl butyl acetate of CHIH and WA apples ( $p \leq 0.05$ ). The concentration of 1-butanol and butyl acetate compounds in CHIH apples was 30 and 10-fold higher than in WA apples when the IEC reached  $1 \mu\text{L L}^{-1}$ . In the case of 1-hexanol, GD apples from CHIH showed a 12-fold concentration increment in comparison with WA apples, while in RD apples from both areas, the content of this alcohol remained very low. These results show that CHIH apples have a greater capacity to produce volatile aroma compounds than WA apples. The variety, the characteristics of the zone (light intensity, precipitation, temperature differences, soil conditions), and the effect of altitude (variation in temperature, humidity, and solar radiation), seem to affect the regulation of the volatile compound biosynthesis (Dhanaraj *et al.*, 1986; Jiang *et al.*, 2013; Jin *et al.*, 2017).



**Figure 6.** Production of esters hexyl acetate, 2-methyl butyl acetate, and butyl acetate ( $\text{mg L}^{-1}$ ) during maturation of 'Golden Delicious' apples (left) and 'Red Delicious' apples (right) grown in Chihuahua, Mexico (CHIH) and Washington, USA (WA). Vertical bars represent one standard deviation.

After the onset in ethylene production, volatiles increased markedly, with a considerably higher volatile production rate and volatile concentration on CHIH apples (Figures 5 and 6). Variations in the composition and content of volatile compounds have been attributed to the climate, elevation, or geographic location, for its effect on the activity of some enzymes (Qin *et al.*, 2017). The higher concentration in volatile compounds found in CHIH apples may be due to the considerably higher altitude in CHIH compared to WA (2062 vs. 763 masl). Some studies report that altitude has a marked influence on grape aroma production; low-altitude sites induce lower amounts of aroma compounds in grapes and wines (Jiang *et al.*, 2013; Xu *et al.*, 2015;

Alessandrini *et al.*, 2019). In bananas, the ‘Robusta’ cultivar showed a higher content of ketones and esters at high altitudes (Brat *et al.*, 2004). Production of aroma in tea leaf (*Camellia sinensis* L.) was higher in high altitudes (Bhattacharya and Sen-Mandis, 2011). Pomegranate (*Punica granatum* L.) fruit showed higher concentrations of volatile compounds at higher altitudes (Mphahlele *et al.*, 2016).

Additionally, the higher production of volatile compounds in CHIH apples may also be caused by the stronger solar radiation in this area (Table 3). According to Song *et al.* (2015), the incidence of light increases the concentration of volatile compounds in grapes. Volatile compound formation increases together with increasing light exposure of the fruit (Miller *et al.*, 1998). According to Ordoñez *et al.* (2016), lower photosynthetically available radiation caused by black hail nets in comparison with white hail nets, causes a decrease in the concentration of the main aroma compounds in GD apples. Baiamonte *et al.* (2016) also reported that the use of hail net reduced the concentration of compounds such as butyl acetate and hexenyl acetate.

## Conclusions

Apples from two different regions, WA and CHIH, showed variations in the development of quality characteristics during their maturation in the tree. Environmental variables and their meteorological indices could account for these quality differences. Lower temperatures before bloom may be the reason for WA to bloom later than CHIH. Apples from CHIH took longer to achieve the onset in ethylene production, which could be explained by the higher elevation when compared to WA. Apples from both zones reached the beginning of ripening after achieving the same photoperiod. The main quality differences between WA and CHIH apples were aroma volatiles, and texture. CHIH apples showed a higher aroma concentration which may be related to their higher altitude and solar radiation incidence. WA apples showed higher firmness compared to CHIH, possibly due to a lower accumulation of photothermal units in Washington. Our results show a strong correlation coefficient ( $R=0.89$ ) between changes in apples’ firmness and the accumulated photothermal units. To the extent of our knowledge this observation is reported here for the first time. More studies dealing with this correlation are necessary to better understand the nature of this relationship.

## Authors’ Contributions

JM-C: data acquisition, data analysis, formal analysis, writing and editing; M E-D: data analysis, data curation, writing and editing; JLJ-C: formal analysis, investigation; SM: design of methodology, data acquisition, data curation; JKF: conceptualization, supervision, design of methodology; DS: investigation, writing, and editing; GG-A: Design of methodology, investigation; NS-S: data acquisition, methodology, data analysis; GIO: conceptualization, visualization, supervision, design of methodology, writing and editing.

All authors read and approved the final manuscript.

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## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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