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RETRASO DE LA COSECHA DE CAFÉ (*Coffea arabica* L.) POR COMPUESTOS QUÍMICOS EXÓGENOS Y TRATAMIENTOS POSTCOSECHA DE FRUTOS

CELERINO VASQUEZ SANTIAGO

TESIS

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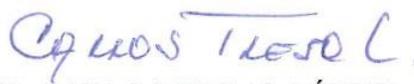ASESORA   DRA. CECILIA BEATRIZ PEÑA VALDIVIA

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COFFEE (*Coffea arabica* L.) HARVEST DELAY BY EXOGENOUS CHEMICAL
COMPOUNDS AND POSTHARVEST FRUIT TREATMENTS

Celerino Vasquez Santiago, Dr.
Colegio de Postgraduados, 2018

ABSTRACT

The aim of this research was to evaluate the harvest delay of coffee (*Coffea arabica* L.) fruit in the field 26 and 29 weeks after flowering. Coffee fruit was treated with salicylic acid (SA, 0.1 and 1.0 mM), gibberellic acid (GA$_3$, 57 and 115 µM), aminoethoxyvinylglycine (AVG, 0.6 and 0.9 µM) and chitosan at 1 % concentration. In an additional experiment, the effect of SA and GA$_3$ (at the same concentrations as above) on fruit harvested at two stages of maturity, green and yellow-green, was investigated. A final experiment investigated green fruit stored at three temperatures (4 ± 1, 8 ± 1 and 24 ± 3 °C) in biodegradable plastic bags. The hypotheses were that one compound would delay coffee fruit ripening and that one storage temperature would induce ripening. The measured response variables were: colour, equatorial diameter, firmness, chlorophyll content, respiration, ethylene production, weight loss and TSS content. Glucose, fructose, sucrose, longitudinal diameter and equatorial diameter were evaluated in dried fruit. Statistical analyses were carried out using an unbalanced repeated measures design for the first seven variables and a completely random design for the remaining variables. SA (0.1 mM) had the most ideal effect for delaying coffee fruit ripening in the field. In the laboratory, both SA (0.1 mM) and GA$_3$ (57 µM) delayed fruit ripening. Lastly, the fruit reached maturity when stored at 24 ± 3 °C.

Key words: coffee, ripening, salicylic acid, biodegradable bag.

RETRASO DE LA COSECHA DE CAFÉ (*Coffea arabica* L.) POR COMPUESTOS QUÍMICOS EXÓGENOS Y TRATAMIENTOS POSTCOSECHA DE FRUTOS

Celerino Vasquez Santiago, Dr.
Colegio de Postgraduados, 2018

RESUMEN

El objetivo de este trabajo fue evaluar el retraso de la cosecha de frutos de café (*Coffea arabica* L.) en campo a las 26 y 29 semanas después de la floración. Los frutos de café fueron tratados con ácido salicílico (SA, 0.1 y 1.0 mM), ácido giberélico (GA₃, 57 and 115 µM), aminoetoxivinilglicina (AVG, 0.6 and 0.9 µM) y quitosano al 1 %. En un experimento adicional, se evaluó el efecto del SA y GA₃ (a las mismas concentraciones) en frutos cosechados en dos estados de madurez, verde y verde-amarillo. En un último experimento se evaluó el proceso de maduración de los frutos almacenados a tres temperaturas (4 ± 1, 8 ± 1 y 24 ± 3 °C) en bolsas plásticas biodegradables. Las hipótesis fueron un compuesto retrasaría la maduración de los frutos de café y que una temperatura de almacenamiento la induciría. Las variables respuesta evaluadas fueron: color, diámetro ecuatorial, firmeza, contenido de clorofila, respiración, producción de etileno, pérdida de peso y contenido de sólidos solubles totales. En semillas secas se evaluó el contenido de glucosa, fructosa, sacarosa, diámetro longitudinal y diámetro ecuatorial. Los análisis estadísticos se llevaron a cabo usando un diseño de medidas repetidas desbalanceado para las primeras siete variables y un diseño completamente al azar para las demás variables. El SA (0.1 mM) tuvo el efecto ideal en retrasar la maduración de los frutos de café en campo. En el laboratorio, tanto el SA (0.1 mM) como GA₃ (57 µM) retrasaron la maduración de los frutos. Finalmente, los frutos maduraron cuando se almacenaron a 24 ± 3 °C.

Palabras clave: café, maduración, ácido salicílico, bolsa biodegradable.

DEDICATORIA

A mi esposa e hijos

AGRADECIMIENTOS

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GENERAL INTRODUCTION

Coffee is one of the exported agricultural products with the highest economic value in the world (Lashermes *et al.*, 2008). The main exporters are Brazil, Vietnam and Colombia, with 2.0, 1.4 and 0.6 million t and the countries that lead the imports are the United States of America, Germany and Italy with 1.5, 1.2 and 0.5 million t. The per capita consumption of coffee in Mexico is 1.13 kg; in contrast, in Finland, which is the main consumer in the world, it is 12.2 kg (ICO, 2017). The value of coffee exports in Mexico in 2013 was 466.247 million dollars (FAO, 2017).

Mexico coffee production has remained constant in the last six years, with an average of 212,640 t of dry coffee (ICO, 2017). The main producers are Chiapas, Veracruz, Puebla, Oaxaca and Guerrero, with 434 916, 311 376, 154 452, 105 188 and 45 411 t of cherry coffee (SIAP, 2017).

DaMatta *et al.* (2008) found that the development of the coffee fruit consists of five stages. The first comprises 6 to 10 weeks after flowering and in which growth is insignificant because only cell division and not cell expansion occurs. The second stage comprises from week 6 to 17 with the greatest growth in size and fresh matter. The third stage lasts 2 weeks and is when the fruit reaches the final size but not the maximum content of dry matter. This is reached in the fourth stage, which can range from week 17 to week 28, although the fruit is still green. The last stage is that of ripening, that can range from week 24 to week 34, and it is the period in which the fruit changes from green to red or yellow colour depending on the variety.

The harvest of coffee can be manual or mechanical. When the fruits are not harvested in their state of optimal ripening (fruits of red colour) they fall to the ground by effect of the rain. The fruits that are collected from the ground generate a coffee of low quality due to the microbiological fermentation (Marín *et al.*, 2003; Farah, 2012).

The ripening of the fruits is coupled to the ethylene biosynthesis and accompanied by the colour change in the plastids. The transition from green to another colour is due to the degradation of chlorophyll and synthesis of other pigments (Belitz *et al.,* 2009 and Pareek, 2016). As the fruits ripe, it increases the rate of ethylene biosynthesis as a consequence of the increase in the activity of the enzymes (*ACC synthase* and *ACC oxidase*) involved in its biosynthesis (Taiz and Zeiger, 2003).

Fruits are classified as climacteric and non-climacteric, the first ones show an increase in respiration rate and ethylene production during ripening and the second ones show a constant decrease (Pareek, 2016). Coffee fruits are classified as climacteric fruits (Pereira, 2005).

Covering a fruit with a film changes its internal atmosphere, therefore, applying chitosan to the fruits reduces the availability of O_2 which causes the *ACO* can no longer exert its action in the conversion of *ACC* to ethylene (Grierson, 2014 and Petriccione *et al.*, 2015). GA_3 delays fruit ripening by inhibiting the expression of the *chlorophyllase* (*Chl*) gene thus delaying the chlorophyll loss (Jacob-Wilk *et al.*, 1999).

Likewise, modified atmospheres can be used to increase the postharvest life of horticultural products. The modification can be active or passive. The first one consists of replacing the air with a mixture of gases before sealing the container of the product and the second one occurs as a result of respiration and metabolism of the product, complemented by the gases permeation through the packaging material (Zhuang, 2011). In this regard, Majidi *et al.* (2014) found that polyethylene bags reduce the firmness loss and colour change more than refrigeration (13 °C) in tomato. Also, Hafeez *et al.* (2016), observed that there is a significant delay in firmness loss and colour change when mango fruits are stored in bags (Xtend®).

Farmers from the Sierra Norte of Oaxaca, Mexico, produce coffee (*Coffea arabica* L.) in the mountains and the harvest must be by hand. Effectiveness of the labour force in the harvest season is constrained due to fruit ripens in all production areas of the region. Consequently, approximately 30 % of the fruits fall to the ground and it is not economic feasible to pick them up.

The objectives of this research were:

a) To delay on plant the coffee fruit ripening by applying AVG, SA, GA_3 and chitosan.

b) Evaluate the effects that exogenous salicylic and gibberellic acid have on ripening during the two initial coffee fruit maturity stages: green and yellow-green.

c) Assess the ripening of green coffee fruit inside a biodegradable bag in cold storage at 4 ± 1, 8 ± 1 °C and at ambient laboratory temperatures of 24 ± 3 °C, for 28 days.

The hypotheses were:

a) At least one chemical compound delays the ripening of coffee fruits compared to the control.

b) Salicylic acid (0.1 mM) would more successfully delay the ripening of coffee fruit compared to the other treatments.

c) One specific storage temperature favours the ripening of coffee fruit.

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CHAPTER I. SALICYLIC ACID DELAYS RIPENING ON COFFEE FRUITS (*Coffea arabica* L.)

1.1 Abstract

Coffee (*Coffea arabica* L.) harvest is made mainly by hand in Mexico. Approximately 30 % of the grain could be lost because there is insufficient labour force. Hence, delay of ripening is of interest as climacteric fruit, ethylene greatly influences its ripening process. Application of an aminoethoxyvinylglycine (AVG), salicylic acid (SA), gibberellic acid (GA_3) and chitosan to delay the ripening process of coffee fruits was studied during the harvest season (December-March) in the years 2014 – 2017 at Tanetze de Zaragoza, Mexico. Colour (luminosity, chroma and hue angle), equatorial diameter, firmness, chlorophyll content, total soluble solids, glucose, fructose, sucrose, respiration and ethylene production during fruit ripening were evaluated. The SA at 0.1 mM delayed 19 days the coffee fruit ripening compared to the control treatment (63 days).

Key words: coffee, climacteric, aminoethoxyvinylglycine, gibberellic acid, chitosan

1.2 Introduction

Farmers from the Sierra Norte of Oaxaca, Mexico, produce coffee (*Coffea Arabica* L.) in the mountains and the harvest must be by hand. Effectiveness of the labour force in the harvest season is constrained due to fruit ripens in all production areas of the region. Consequently, approximately 30 % of the fruits fall to the ground and it is not economic feasible to pick them up.

Coffee is a climacteric fruit and its ripening is induced by ethylene (Pereira *et al.*, 2005; Ságio *et al.*, 2014). A visual ripening indicator is that the coffee fruit exocarp colour changes from green (unripe fruit) to red (ripe fruit) (De Castro and Marraccini, 2006). This colour change is due to the disappearance of chlorophyll pigments and anthocyanin (cyanidin 3-rutinoside) accumulation during the last stages of coffee fruit ripening (Marín *et al.*, 2003; Murthy *et al.*, 2012).

Sugars influence in coffee quality because they are precursors of several aromas and flavours and their content in coffee beans is affected by the cultivars used and by shading conditions, however, the patterns of shading are not consistent (Somporn *et al.*, 2012). However, Geromel *et al.* (2008) observed that shade induces a reduction in sucrose content and an increase in glucose and fructose.

Ethylene is synthesized from S-adenosylmethionine (SAM) via 1-aminocyclopropane-1-carboxylic acid (ACC) (Yang and Hoffman, 1984). The enzymes *ACC synthase (ACS)* and *ACC oxidase (ACO)* are the primary regulation points in ethylene biosynthesis (Xu and Zhang, 2015). In addition, the *ACO* genes are involved in the increase of ethylene production during coffee fruit ripening (Pereira *et al.*, 2005) and control ethylene biosynthesis in higher plants (Ruduś *et al.*, 2013).

Plant hormones and chemical compounds have been used to control ripening of different fruits (e.g. tomato, pear, apricot, peach, banana and kiwi fruit) by reducing respiration rate and ethylene production (Canli and Orhan, 2009; Malik, 2009; Shao *et al.*, 2012). Hossain and Iqbal (2016) reported that 1 % chitosan delayed banana ripening by reducing respiration activity.

Aminoethoxyvinylglycine (AVG) is an inhibitor of *ACS* used commercially to reduce pre-harvest fruit drop and also to delay fruit ripening (Pech *et al.*, 2012). In citrus fruit, Jacob-Wilk *et al.* (1999) found that *chlorophyllase (Chl)* gene expression is stimulated by ethylene and inhibited by gibberellic acid (GA_3).

Salicylic acid (SA) is linked with several plant processes such as growth, thermogenesis, flower induction and ion uptake. In addition, SA affects ethylene biosynthesis, stomatal movement, root growth and reverses the effects of ABA on leaf abscission (Gutiérrez-Coronado *et al.*, 1998; Yusuf *et al.*, 2013). Zhang *et al.* (2003) assessed the acetylsalicylic acid (ASA), a derivative of SA, which they showed suppressed activities of *ACC synthase*, *ACC oxidase* and ethylene production in kiwifruit. Likewise, the ethylene peak was delayed. Fan *et al.* (1996) showed that ASA inhibits respiration and ethylene production by inhibiting *ACC oxidase* activity in apple fruit discs.

The objective of the current study was to delay on plant the coffee fruit ripening by applying AVG, SA, GA_3 and chitosan. The hypothesis was that at least one chemical compound delays the ripening of coffee fruits compared to the control.

1.3 Material and methods

1.3.1 Plant Material

The study was carried out in a coffee plantation (seven years old) at Tanetze de Zaragoza, Oaxaca, Mexico (17° 23' 10.2" N, 96° 17' 52.9" W and altitude of 1311

masl). Green coffee fruits with full endosperm formation were used. The coffee trees height averaged 1.8 ± 0.2 m.

Three experiments were developed:

1.3.1.1 Experiment 1

Green coffee fruits (26 weeks after flowering) were sprayed (1 L per plant) on five coffee trees on December 2014 - March 2015. The treatments assessed were AVG (0.6 and 0.9 µM), SA (0.1 and 1.0 mM), GA₃ (57 and 115 µM), 1 % chitosan and water as control. Silwet L-77 a surfactant (0.05 % v/v) was added. Measurements of the response variables were made every 6 days on four fruits (sub-replicates) of five trees (replicates) for each treatment.

1.3.1.1.1 Response variables

Colour, equatorial diameter, firmness, chlorophyll content and total soluble solids were measured.

Fruit colour was evaluated using a colorimeter (APOLLINAIRE AEROSPACE AND INSTRUMENTS (U.K.)). Red, green and blue values were obtained and transformed to CIELAB (L*, a*, b*) scale with an algorithm used by OpenRGB version 2.20.40712 software. Hue angle (h°) and chroma (C*) were calculated using the equations proposed by McGuire (1992):

$$C^* = (a^{*2} + b^{*2})^{1/2}$$

$$h° = tan^{-1}\left(\frac{b*}{a*}\right)$$

Fruit equatorial diameter (mm) was measured with a vernier (Stainless Hardened).

Fruit firmness was determined on the equatorial region of the fruit by a portable texturometer (Fruit Pressure Tester, mod. FT 327) with an 8 mm tip.

Fruit chlorophyll content (SPAD value) was quantified by a SPAD (SPAD-502, Minolta, Japan) in 16 mm^2 tissue (exo and mesocarp) discs.

Total soluble solids (TSS) of red fruits were measured on fruit juice using a digital refractometer (ATAGO, PAL-1, Japan).

1.3.1.2 Experiment 2

To confirm the results of the first experiment the treatments were replicated from December 2015 to March, 2016. The AVG and chitosan treatments were excluded due to the former one did not delay fruit ripening and the latter damage the fruits.

1.3.1.2.1 Response variables

Fruit colour, dry seed glucose, fructose and sucrose content

Photographs were taken of the coffee fruits with a digital camera (Casio®, 12.1 MP) at 10 o´clock and at 1 m distance to evaluate their colour. These images were analyzed with Photoshop CS5® software to obtain colour space CIE L*a*b. Measurements of the response variables were made every 14 days to four fruits (sub-replicates) of five trees (replicates) for each treatment.

Glucose, fructose and sucrose were analyzed by an enzymatic method (Viola and Davies, 1992; Bernal *et al*., 2005). Sample of ground dry coffee (20 mg) of each treatment were homogenized in 700 µL 80 % ethanol at 80 °C for 1 h. Then they were centrifuged at 16 000 g for 5 min. Ethanol extract (10 µL) was added to 210 µL buffer reaction (25 mM Tris-HCl (pH 8.0), 50 mM KCl, 1 mM ATP, 3 mM $MgCl_2$, 0.3 mM NAD^+ and 1 U mL^1 yeast hexokinase (EC 2.7.1.1)) and its absorbance measured at 340 nm. Glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49) (10 µL) was added to the mixture and incubated 1.5 h at 37 °C. Its absorbance at 340 nm was recorded. Glucose concentration was calculated using the difference between the two above absorbances. For fructose concentration, 10 µL phosphoglucose isomerase (EC 5.3.1.9) were added to the preceding mixture and incubated 1.5 h at 37 °C. Its absorbance was measured at 340 nm and used for calculate the difference between this and previous absorbance. Sucrose concentration was calculated by the difference between the absorbance obtained previously and that recorded after adding a pinch of invertase (EC 3.2.1.26) to the preceding mixture and incubating for 1.5 h at 37 °C. Absorbances were measured in a Microplate reader (Multiskan FC microplate, Thermo Scientific, USA) and sugars concentration was calculated from standard curve constructed with glucose.

1.3.1.3 Experiment 3

The third experiment assessed 0.1 mM SA, which was the treatment that delayed fruit ripening and did not damage them in the previous experiments, and a control treatment with water.

1.3.1.3.1 Response variables

Respiration rate (mL CO_2 kg^{-1} h^{-1}) and ethylene production (μL C_2H_4 kg^{-1} h^{-1}) were the response variables measured in coffee fruits *in situ* on plants.

Cylinders of polyethylene terephthalate (PET) of 230 mL were built to measure respiration and ethylene production by coffee fruits *in situ* on plants (Figure 1.1). The cylinders enclosed 16 ± 2 coffee fruits. Samples of 6 mL were taken after 1.3 h (Vacutainer®, Becton Dickinson Co.). After that, the tubes were refrigerated (6 ± 1 °C) until evaluation. CO_2 and ethylene were assessed by a chromatograph (Hewlett Packard 5890, series II).

Figure 1.1. Cylinders (h = 8.1 cm and r = 3 cm) used to take samples of ethylene and CO_2 produced by coffee fruits on plant in Tanetze de Zaragoza, Mexico.

1.3.2 Statistical analysis

An unbalanced repeated measurement design (RMD) was used to analyze the effects of treatments on colour, equatorial diameter, firmness and chlorophyll content. The evaluations were made each 6 days. A t-Student test ($\alpha = 0.05$) was established beforehand among treatments (day 1 and final) when the interaction of treatments with time was significant.

To analyze total soluble solids, glucose, fructose, sucrose, CO_2 and ethylene production, a completely random design and Tukey test ($\alpha = 0.05$) was employed. SAS® software (version 9.3) was used for the statistical analysis.

1.4 Results

1.4.1 Experiment 1

1.4.1.1 Colour

The interaction between treatment and time was significant in the luminosity, chroma and hue angle. The AVG (0.9 µM), GA_3 (115 µM) and control treatments maintained the luminosity values during the experiment ($p < 0.05$) (Figure 1.2A). Chitosan injured the exocarp and mesocarp, browning the coffee fruits, and the luminosity diminished from 31 to 9 in 24 days. Luminosity decreased slower in fruits treated with 0.1 mM SA than in those with control treatment.

In chroma, the effect of treatments was not significant over the experimental time ($p < 0.05$). The average chroma at the end of the experiment was around 15 which indicates that colours were not pure but they were mixed with gray (Figure

1.2B). All the treatments induced a diminished in chroma over time. Chitosan treatment damaged the fruits.

The hue angle values decreased in the fruit exocarp as the colour changed from a light green to a red colour. Salicylic acid (0.1 mM) treatment delayed exocarp colour change for 19 days compared to the control treatment. Chitosan damaged coffee fruits (Figure 1.2C).

1.4.1.2 Diameter

There was a significant interaction between treatment and time. The *t-Student* test showed significant differences in fruit diameter between the initial and the final day of the experiment (Figure 1.3). The AVG (0.6 µM) and 0.1 mM SA produced the biggest diameter of coffee fruits 13.3 and 13.02 mm, respectively compared with the average at the start of the treatments (12.0 mm).

1.4.1.3 Chlorophyll content

Chlorophyll content in the coffee fruit exocarp decreased in the treatments as a consequence of the fruit ripening. The *t-Student* test showed significant difference between the chlorophyll of the initial and final day of the experiment. Chitosan treatment caused a total loss of chlorophyll by 24 days because this compound caused darkening on the fruit exocarp (Figure 1.4). The interaction treatment and time was significant. Chlorophyll loss was slower in fruits sprayed with SA (0.1 mM) than in the other treatments. Salicylic acid retained chlorophyll 19 days more than control treatment.
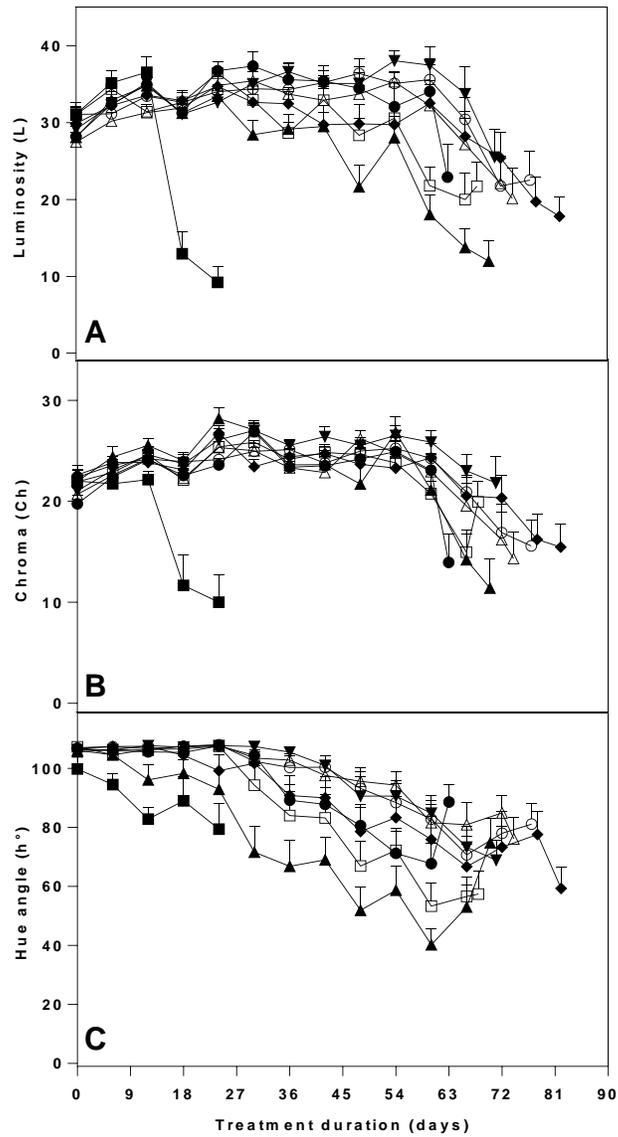
Figure 1.2. Coffee fruits luminosity (A), chroma (B) and hue angle (C) during ripening after applications of (●) water (control), (■) 1 % chitosan, (▲) 0.6 and (▼) 0.9 μM aminoethoxyvinylglycine (AVG), (◆) 0.1 and (○) 1 mM salicylic acid (SA), (□) 57 and (△) 115 μM gibberellic acid (GA$_3$). Values are the average of 20 replicates + SE (Tukey, $p < 0.05$).

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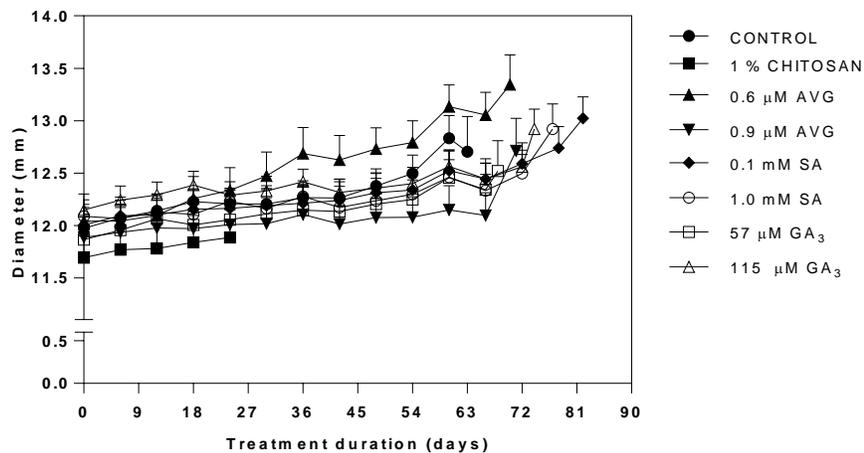
Figure 1.3. Diameter of coffee fruits during ripening after applications of water (control), 1 % chitosan, 0.6 and 0.9 µM aminoethoxyvinylglycine (AVG), 0.1 and 1 mM salicylic acid (SA) and 57 and 115 µM gibberellic acid (GA_3). Values are the average of 20 replicates + SE (Tukey, $p < 0.05$).
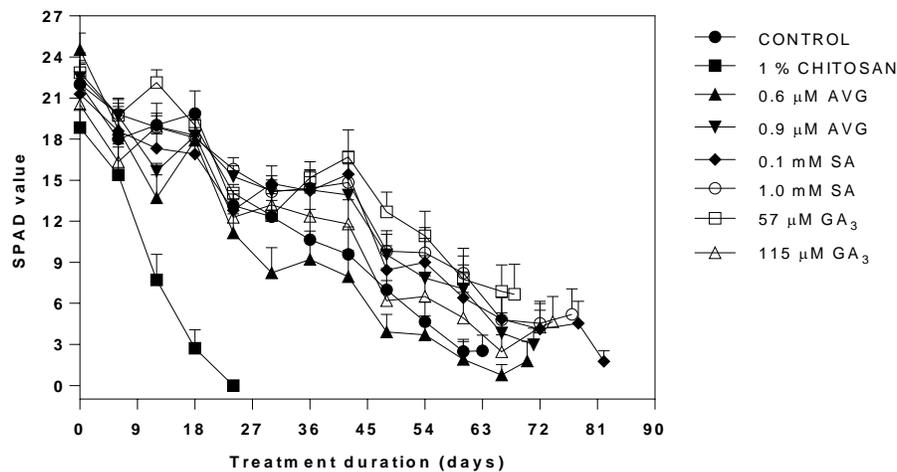
Figure 1.4. Chlorophyll content in the exocarp of coffee fruits during ripening after applications of water (control), 1 % chitosan, 0.6 and 0.9 µM aminoethoxyvinylglycine (AVG), 0.1 and 1 mM salicylic acid (SA) and 57 and 115 µM gibberellic acid (GA_3). Values are the average of 20 replicates + SE (Tukey, $p < 0.05$).

1.4.1.4 Firmness

The firmness of the fruits decreased as they ripened (Figure 1.5). The analysis of variance showed a significant interaction between treatment and time. *t-Student* test revealed a significant difference between the initial and final day of evaluation, indicating that the fruits lost their firmness in the ripening process. Coffee fruits treated with chitosan (1 %) accelerated the loss of fruit firmness to 24 days compared with the best treatment (0.1 mM SA) which took 82 days for traits to lose firmness.
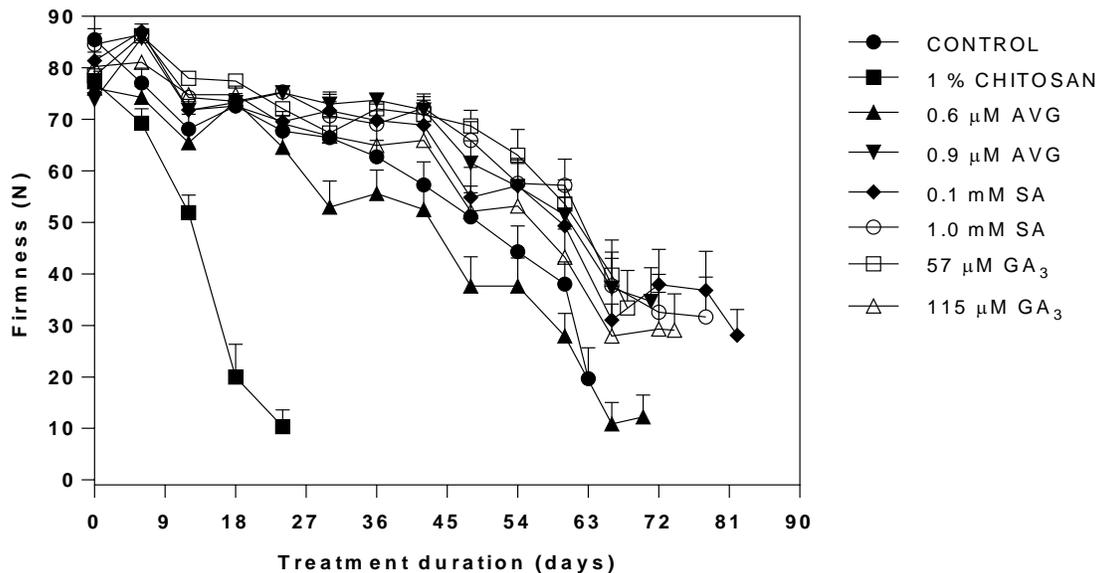
Figure 1.5. Firmness in coffee fruits during ripening after applications of water (control), 1 % chitosan, 0.6 and 0.9 µM aminoethoxyvinylglycine (AVG), 0.1 and 1 mM salicylic acid (SA), 57 and 115 µM gibberellic acid (GA_3). Values are the average of 20 replicates + SE (Tukey, $p < 0.05$).

1.4.1.5 Total soluble solids

Red fruits showed no significant differences on the amount of total soluble solids (TSS) between treatments at the end of the experiment. TSS ranged from 11.5 to 13.3 %. Fruits treated with 0.1 mM SA had on average 11.68 % TSS (data no presented).

1.4.2 Experiment 2

1.4.2.1 Colour

Luminosity, chroma and hue angle showed a significant interaction between treatments and time, therefore the data of initial and final day of the experiment were compared with a t-Student test.

For luminosity there was no significant difference in any of the treatments evaluated. Fruit luminosity of the control treatment on day 42 was the highest then it decreased as the other treatments. GA_3 treatment did not show significant changes across time (Figure 1.6A). Salicylic acid (0.1 mM) showed later increase of luminosity during ripening and a decrease at final stage.

Chroma values did not differ in any of the treatments between day 0 and final day. However, the highest average value was recorded for on 0.1 mM SA on day 70 but then diminished by the end of the experiment (Figure 1.6B).

For hue angle a significant difference was found between the initial and final readings using a *t-Student* test. The values decreased in all treatments with the exception of 1 mM SA from the beginning to the day 42 (except control) the hue

angle was constant and then diminished to the end of the experiment, most slowly for 0.1 mM SA. In contrast, the control treatment showed a constant decrease from day 0 to the final measurement (Figure 1.6C).
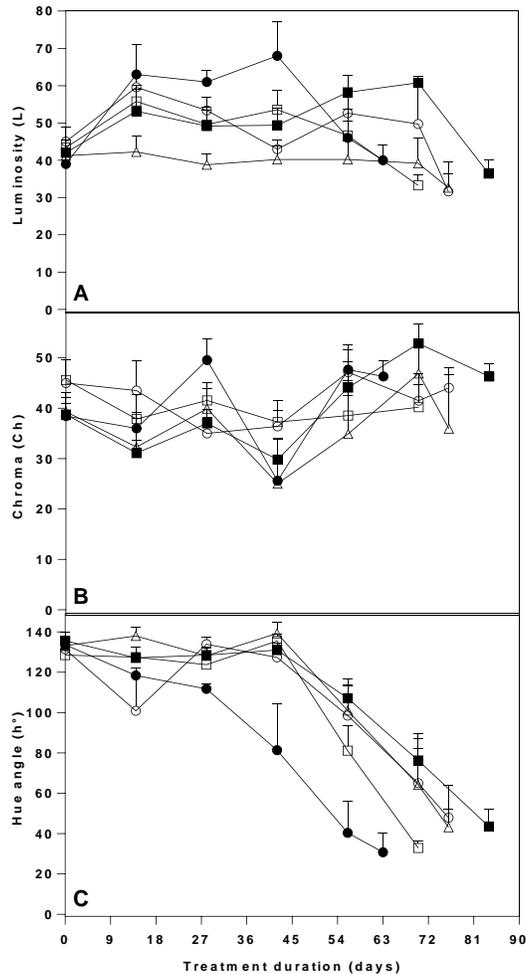
Figure 1.6. Coffee fruit luminosity (A), chroma (B) and hue angle (C) during ripening after applications of (●—) water (control), (■—) 0.1 and (—○—) 1 mM salicylic acid (SA), (—□—) 57 and (—△—) 115 µM gibberellic acid (GA₃). Values are the average of 20 replicates + SE (Tukey, p < 0.05).

1.4.2.2 Glucose, fructose and sucrose in dry coffee grains

Significant differences were found among treatments for glucose and sucrose content in dry coffee grains. The highest glucose and sucrose content was found in 115 µM GA_3 and 1.0 mM SA, respectively. On the other hand, the lowest glucose and sucrose content was found in 57 µM GA_3 and 115 µM GA_3 (Figure 1.7). Fructose content was not detected.
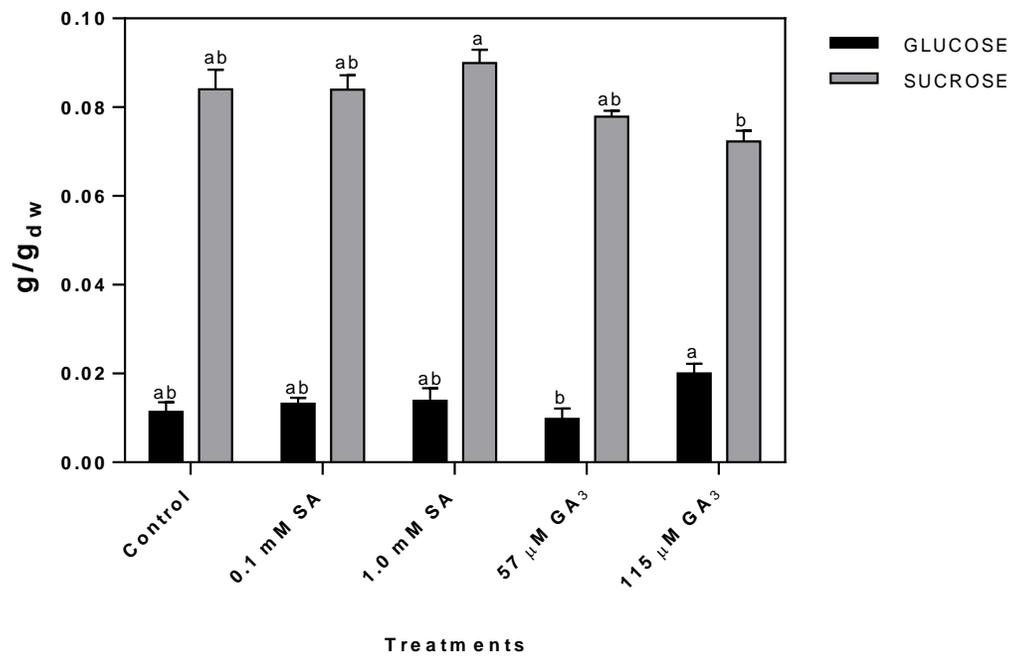
Figure 1.7. Glucose and sucrose content in dry coffee fruits which ripened on the plant and after applications of 0.1 and 1 mM salicylic acid (SA), 57 and 115 µM gibberellic acid (GA_3) and water (control). Values are the average of 20 replicates + SE (Tukey, $p < 0.05$).

1.4.3 Experiment 3

There was no difference between the 0.1 mM SA and control for ethylene (8.44 µL C_2H_4 kg^{-1} h^{-1}) or respiration (7.29 mL CO_2 kg^{-1} h^{-1}).

1.5 Discussion

Delayed ripening of Robusta coffee fruits was achieved at concentrations of 10 to 500 ppm of gibberellic acid (GA_3), with 10 and 50 ppm as the best treatments. These reduced the percent ripening compared with control treatment by 14 and 7.9 %, respectively, after 51 days (Adenikinju, 1977). Our results were similar to Adenikinju but with 0.1 mM SA the ripening was delayed by 19 days more than that of the control treatment, which is reportedly due to these plant hormones delaying ethylene production (Zhang *et al.*, 2003; Zaliha *et al.,* 2016).

A reduction of the luminosity, chroma and hue angle was observed during coffee fruit ripening in our study which indicates a change of the fruit colour from green to red as consequence of a ripening normal process; these results agree with Radzevičius *et al*. (2009) and Radzevičius *et al*. (2014) who observed the same behavior in tomato fruit.

Equatorial diameter of the coffee fruits increased during the experiment because the fresh and dry weight of the pulp increases during ripening, or did the water content (Dancer, 1963; Wormer, 1964).

The applied compounds, mainly SA, delayed natural chlorophyll degradation. This result agrees with Martínez *et al.* (1994) who also found that gibberellic acid

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delayed the degradation of chlorophyll in strawberry fruits indicating a delay of the ripening process.

The decrease in firmness was slower in the fruits sprayed with plant hormones or chemical compounds compared to that of the control. This response is due to the delayed degradation of the pectins of the cell walls by the enzyme pectin methyl esterase (PME) (Cação *et al.*, 2012). Ben-Arie *et al.* (1996) reported that sprinkling persimmon fruits with GA_3 delayed fruit ripening and postharvest softening, indicating that this compound retarded or inhibited all cellular changes leading to fruit softening. In addition, Srivastava and Dwivedi (2000) found that the banana softening process was inhibited by subjecting the fruits to a treatment with SA at 500 and 1000 µM. This is because SA prevents the conversion of 1-aminocyclopropene-1-carboxylic acid (ACC) to ethylene by reducing the production and activity of ACC oxidase (Leslie and Romani, 1988).

Berlanga-Reyes *et al.* (2011) found that SA (0.01 and 1.0 µM) caused the same effect as AVG in delaying the Golden Delicious apple ripening process. In peach Amarante *et al.* (2005) observed that GA_3 and AVG delayed fruit ripening at harvest and during refrigerated storage. These compounds permit better retention of exocarp colour, firmness and a minimum increase in the total soluble solids content. This is due to the AVG inhibits ethylene production by inhibiting ACC synthase action avoiding ACC production (Capitani *et al.*, 2005).

Sucrose content found in our study agrees with data of Murkovic and Derler (2006), Knopp *et al.* (2006) and Farah (2012) for coffee beans. They found 73 mg/g_{dw} (0.073 g/g_{dw}), 7.07 % (0.07 g/g_{dw}), 6 - 9 $g/100\ g_{dw}$, respectively. However, glucose

content (> 1 %) found in the present study was higher than the 0.23 and 0.5-1 % reported by Knopp *et al*. (2006) and Herrera *et al*. (2011), respectively. The absence of fructose in our samples agrees with Murkovic and Derler (2006) who observed that fructose content can vary from 0 until 1.9 mg/g dw. The presence or absence of fructose in coffee beans depends on cultivar or quantification method used. Besides, the differences among sugars contents can be due to the different shading conditions used to produce the coffee beans (Geromel *et al.,* 2008; Somporn *et al.,* 2012).

Our data of respiration rate (7.29 mL CO_2 kg^{-1} h^{-1}) were similar in green than in ripe fruits which is not agree with those of Marín *et al.* (2003) and Ságio *et al*. (2013) who found CO_2 production rate higher in ripe fruits (9 mL CO_2 kg^{-1} h^{-1} and 12-22 mg CO_2 kg^{-1} h^{-1}, respectively) than in unripe fruits (5.67 mL CO_2 kg^{-1} h^{-1} and 8 -13 mg CO_2 kg^{-1} h^{-1}, respectively). The same behavior was observed on the data of ethylene production (8.44 µL C_2H_4 kg^{-1} h^{-1}) which do not agree with those of Pereira *et al.* (2005) and Ságio *et al*. (2013) who report that ethylene quantity was higher in ripe fruits (1.2 nL C_2H_4/g/h and 2.5-3.5 µL C_2H_4/kg/h, respectively) than in unripe ones (0.2 nL C_2H_4 kg^{-1} h^{-1} and 1.6-1.9 µL C_2H_4 kg^{-1} h^{-1}). These differences in respiration rate and ethylene production can be due to the different temperatures on which the evaluations were carried out, because the temperature is the factor most important affecting the rate of respiration and ethylene production (Saltveit, 2016; Shibuya and Ichimura, 2016). Besides, the cultivars and system used to quantify the CO_2 and ethylene production could influence in the results. If we want to observe the climacteric nature of the coffee fruits the samples must be taken in shorter periods.

1.6 Conclusions

SA was the best treatment in delay the ripening process of coffee fruits on plant. Chitosan had no inhibitory effect on the ripening of coffee fruits. The peak in respiration and ethylene production was not detected due to the time elapsed between the samples taken. Of interest, coffee chemical quality was not affected by the application of these compounds.

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CHAPTER II. POSTHARVEST EFFECTS OF EXOGENOUS SALICYLIC AND GIBBERELLIC ACID ON COFFEE (*Coffea arabica* L.) FRUIT RIPENING

2.1 Abstract

Salicylic acid (SA) and gibberellic acid (GA$_3$) delay ripening in climacteric fruits, including coffee (*Coffea arabica* L.). The objective of this research was to evaluate the effect of SA (at 0.1 and 1 mM) and GA$_3$ (at 57 and 115 μM) on the ripening process of coffee fruit harvested during the green and yellow-green maturity stages. The hypothesis was that, at a specific concentration, SA or GA$_3$ would delay ripening in coffee fruit. The experimental design was completely random (CRD) with three replications. The following fruit traits were evaluated at the end of a 19-day storage period: weight loss, initial and final moisture content, and maturity stage (proportion of green, yellow, yellow-green, red and grey fruit). The 100-seed dry weight, longitudinal diameter, and equatorial diameter of the dried fruit were also recorded. No significant differences in weight loss were found among treatments at the end of the 19-day storage period. An average of 25 % of the fruits that were harvested in the yellow-green maturity stage reached the red ripe stage. Yellow-green fruit had a lower moisture content than green fruit after 19 days of storage. When harvested during the initial yellow-green stage of maturity, fruit colour, 100-seed dry weight and the longitudinal and equatorial diameters of the dried fruit were similar to the ripe fruits harvested *in situ* at the farm. Green coffee fruit does not ripen at room temperature. The lowest percent of coffee fruit ripening was observed in the 0.1 mM SA treatment.

Key words: climacteric, delay, maturity, storage

2.2 Introduction

Ethylene regulates the ripening process of fruits, inducing changes in colour and texture (Barry and Giovannoni, 2007). Climacteric fruits can be harvested unripe and continue to mature off the plant (Kader and Barret, 2005). Ságio *et al.* (2014) and Pereira *et al.* (2005) reported that coffee (*Coffea arabica* L.) fruit is a climacteric fruit.

Gibberellic acid (GA_3) can delay the ripening of tomato (*Lyspersicon esculentum* L. var. Ailsa Craig), peach (*Prunus persica* L.) and mango (*Mangifera indica* L.) fruits during storage (Dostal and Leopold, 1967; Martínez *et al.,* 2000; Islam *et al.*, 2013). GA_3 delays the ripening of fruits and does not trigger some of the deleterious changes that ethylene does, such as increased respiration and *chlorophyllase (Chl)* gene expression (Dostal and Leopold, 1967; Jacob-Wilk *et al.*, 1999). Likewise, salicylic acid (SA) has been found to delay ripening by inhibiting the activity of 1-aminocyclopropane-1-carboxylate synthase (ACS) and 1-aminocyclopropane-1-carboxylate oxidase (ACO) during ethylene biosynthesis in kiwifruit (Zhang *et al.*, 2003) and by inhibiting enzymes that hydrolyse cell walls in banana fruit (Srivastava and Dwivedi, 2000).

Coffee fruit begins to ripen approximately 24 weeks after flowering (WAF) occurs and is accompanied by a colour change in the exocarp from green (unripe fruit) to red (ripe fruit) due to the disappearance of chlorophyll pigments and

anthocyanin accumulation (Marín *et al*., 2003; De Castro and Marraccini, 2006; DaMatta *et al*., 2008).

The aim of this research was to evaluate the effects that exogenous salicylic and gibberellic acid have on ripening during the two initial coffee fruit maturity stages: green and yellow-green. The hypothesis was that salicylic acid (0.1 mM) would more successfully delay the ripening of coffee fruit compared to the other treatments.

2.3 Materials and methods

2.3.1 Plant Material

The study was carried out in a *C. arabica* L. var. Typica plantation at Tanetze de Zaragoza, Oaxaca, Mexico (17° 23' 10.2´´ N, 96° 17' 52.9´´ W and altitude of 1311 masl). The coffee tree heights averaged 1.8 ± 0.2 m.

In March 2015, green (26 WAF) coffee fruit (6 kg) from eight plants and yellow-green (29 WAF) coffee fruit (4.5 kg) from 15 plants (Figure 2.1) was harvested at 8:00 a.m and transported to the laboratory in an ice cooler. Five samples each of 1.2 kg of green and 0.9 kg of yellow-green coffee fruit were weighed. The samples were treated by immersion in 0.1 mM SA, 1.0 mM SA, 57 mM GA_3, 115 mM GA_3, or water (control) for 5 min in beakers. The fruit was then divided into three groups (replicates) and stored in plastic trays (Figure 2.2) at room temperature (24 ± 3 ⁰C and 56 ± 7 % Relative Humidity (R.H.)). Evaluations were performed every 2 days.

A   B

Figure 2.1. Initial green (A) and yellow-green (B) maturity stages of coffee fruit treated with SA (0.1 or 1.0 mM) or GA$_3$ (57 or 115 μM).
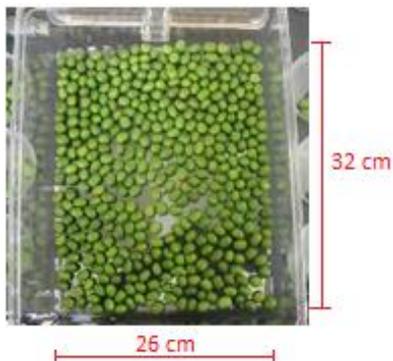
Figure 2.2. Plastic tray used for the storage of coffee fruit treated with SA (0.1 or 1.0 mM) or GA$_3$ (57 or 115 μM) and stored at 24 ± 3 °C and 56 ± 7 % R.H.

2.3.2 Fruit and seed response variables

The following response variables were evaluated: fresh weight loss, green fruit ripening progress, yellow-green fruit ripening progress at the end of the storage period (19 days), initial and final moisture content, 100-seed dry weight, and seed longitudinal and equatorial diameter.

The fresh fruit was weighed with a digital balance (Precisa, XB 2200C®). The weight data were expressed as the percent of the post-harvest weight loss to the original weight at harvest.

Green and yellow-green fruit ripening progress was assesed by the change in exocarp colour of the coffee fruit throughout the storage period (19 days) (De Castro and Marraccini, 2006).

To determine the initial and final coffee fruit moisture content, the samples were dried in an oven at 105 °C for 16 h until they reached a constant weight (ISO, 2001).

The 100-seed dry weight was recorded with a digital balance (Precisa, XB 2200C®). The seeds were obtained manually from the experimentally treated fruit. The 100-seed dry weight was also measured for samples of ripe coffee fruit harvested *in situ* at the farm, as a control.

Dry seed longitudinal and equatorial diameter (mm) were measured with a Vernier calliper (Stainless Hardened). Longitudinal and equatorial diameter (mm) were also measured in samples of ripe coffee fruit *in situ* at the farm, as a control.

2.3.3 Statistical analysis

The experiment was conducted with a completely random design. The experimental unit consisted of either 400 g of green coffee fruit or 300 g of yellow-green coffee fruit, with three replicates. A Tukey test ($\alpha = 0.05$) was used to compare the means. SAS® software (version 9.3) was used for all statistical analyses.

2.4 Results

2.4.1 Fresh weight loss

Cumulative fruit weight loss increased steadily during storage ($p < 0.5$) for both the green (Figure 2.3A) and yellow-green (Figure 2.3B) coffee fruit maturity stages. After 19 days of storage, the green coffee fruit lost 62.9 % of its fresh weight, while the yellow-green coffee fruit lost 57.5 %, relative to the control.

2.4.2 Green fruit ripening progress

Green coffee fruit treated with SA and GA_3 did not ripen. Only 2.4 % of the fruit treated with SA (0.1 mM) changed to yellow-green colour after 19 days of storage, and 7.3 % of the fruit in the control treatment became yellow-green in colour but did not ripen (Figure 2.4).

2.4.3 Yellow-green fruit ripening progress

In total, 22 % of the fruit harvested at the yellow-green stage and treated with SA (0.1 mM) reached maturity after 4 days of storage, compared to 30.7 % that ripened to red colour in the control. In all treatments, the change to red was followed by a change to grey (Figure 2.5).
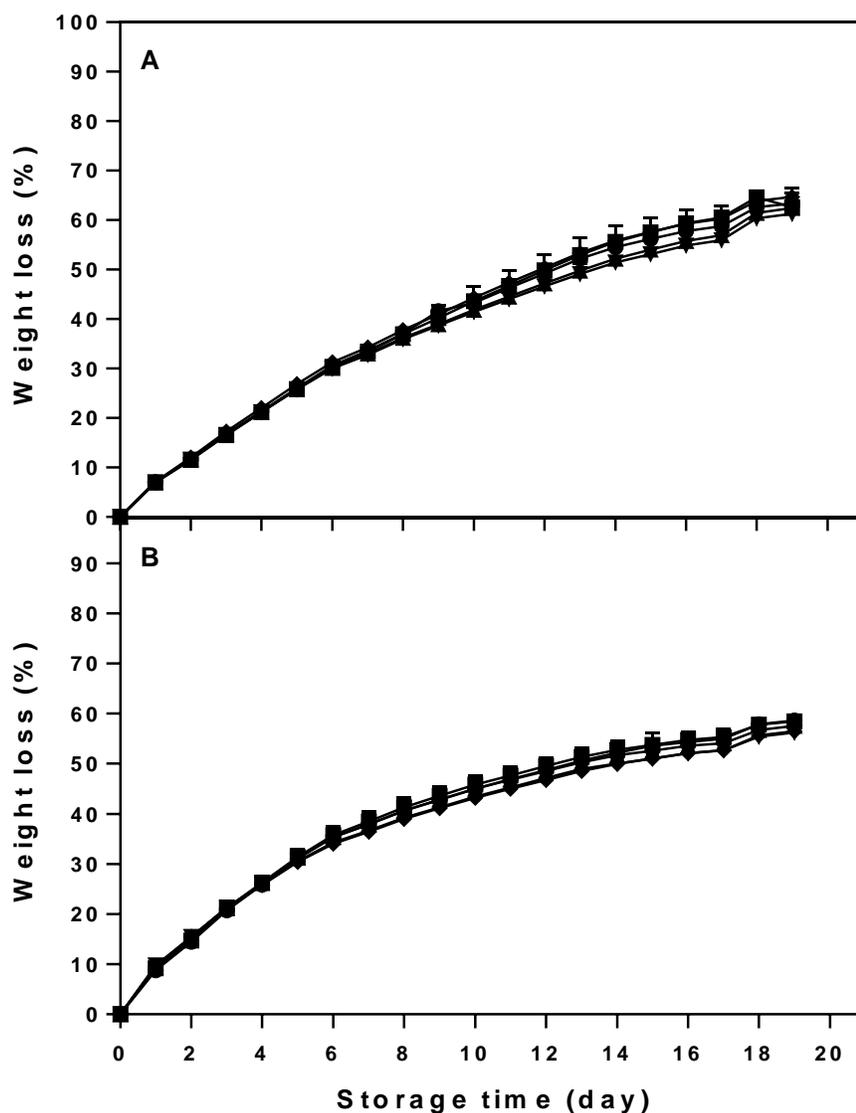
Figure 2.3. Cumulative fresh weight loss in green (A) and yellow-green (B) coffee fruit treated with (●—) water (control), (■—) 0.1 mM SA, (▲—) 1.0 mM SA, (▼—) 57 µM GA_3 or (◆—) 115 µM GA_3 and stored at 24 ± 3 °C and 56 ± 7 % R.H % for 19 days. Values are the average of three replicates + SE (Tukey, $p < 0.05$).
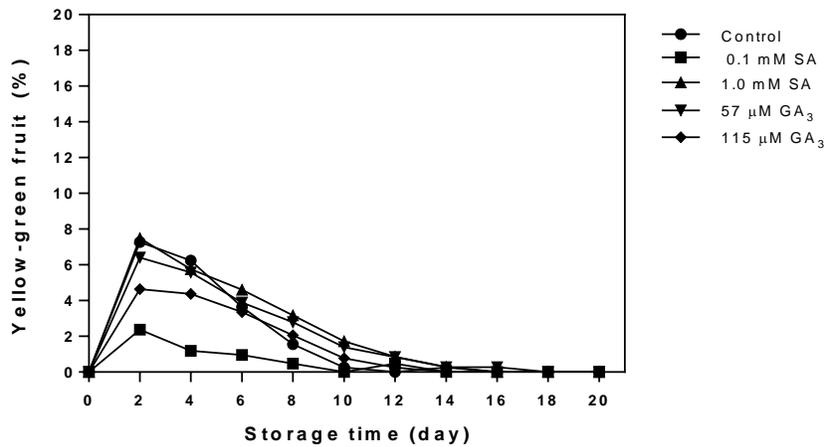
Figure 2.4. Percent of fruit harvested at the green stage that ripened to yellow-green after 19 days of storage at 24 ± 3 °C and 56 ± 7 % R.H. The fruit was treated with SA (0.1 or 1.0 mM) or GA_3 (57 or 115 µM). Values are the average of three replicates + SE (Tukey, $p < 0.05$).
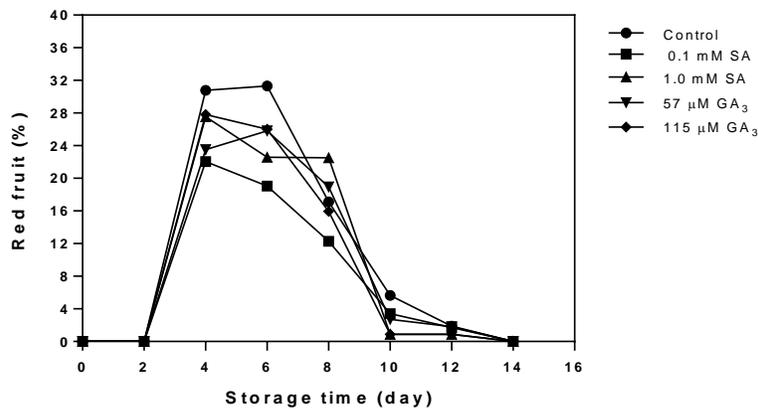
Figure 2.5. Percent of fruit harvested at the yellow-green stage that ripened to red after 19 days of storage at 24 ± 3 °C and 56 ± 7 % R.H. Fruit was treated with SA (0.1 or 1.0 mM) or GA_3 (57 or 115 µM). Values are the average of three replicates + SE (Tukey, $p < 0.05$).

2.4.4 Initial and final moisture content of the fruit in the control treatment

Fruit moisture content was measured at the start and end of the 19 days storage period for fruit in the control treatment. At the beginning of the storage period, the initial moisture content was higher for fruit at the green stage than for yellow-green fruit. Inversely, after 19 days of storage, the moisture content was higher in yellow-green fruit than in green fruit (Figure 2.6).
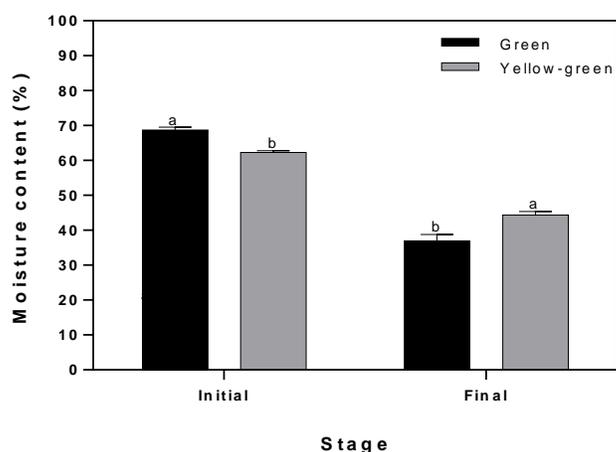
Figure 2.6. Moisture content for two maturity stages of coffee fruit at the beginning and end of a 19-day storage period at 24 ± 3 °C and 56 ± 7 % R.H. Values are the average of three replicates + SE (Tukey, $p < 0.05$).

2.4.5 100-seed dry weight at the green fruit stage

As a standard for comparison, the 100-seed dry weight was measured for ripe fruit at the farm and yielded the highest weight (18 g). The lowest 100-seed dry

weight value (12 g) was recorded in the green coffee fruit treated with 115 µM GA_3 and stored for 19 days (Figure 2.7).
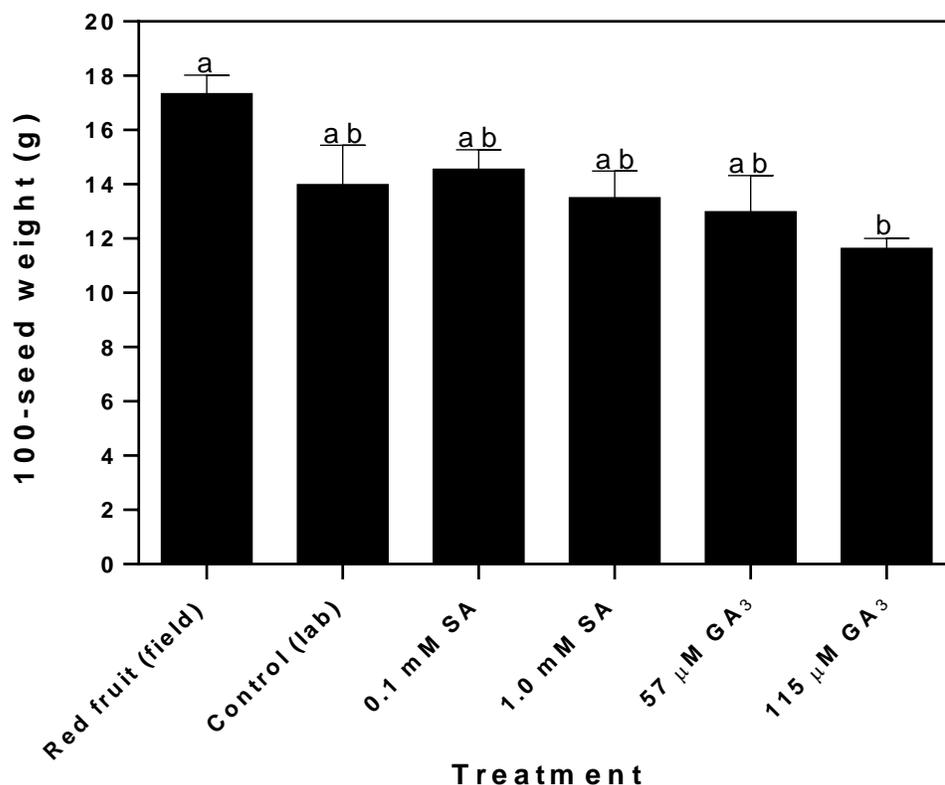
Figure 2.7. One-hundred dry seed weight of green coffee fruit treated with SA (0.1 or 1.0 mM) or GA_3 (57 or 115 µM), and stored for 19 days at 24 ± 3 °C and 56 ± 7 % R.H. Red ripened fruit from the farm was included in this analysis. Values are the average of three replicates + SE (Tukey, $p < 0.05$).

2.4.6 100-seed dry weight of fruit harvested in the yellow-green stage

After 19 days of storage, some of the fruit that was harvested at the yellow-green stage ripened to yellow or red colour, while some remained yellow-green; the

fruit that was treated with SA (0.1 mM) and ripened to red (after the storage period) had the highest 100-seed weight value. The lowest value was observed in fruit treated with 57 µM GA_3. The 100-seed dry weight of the fruit treated with 57 µM GA_3 changed little over the three final maturity stages (yellow-green, yellow and red) (Figure 2.8).
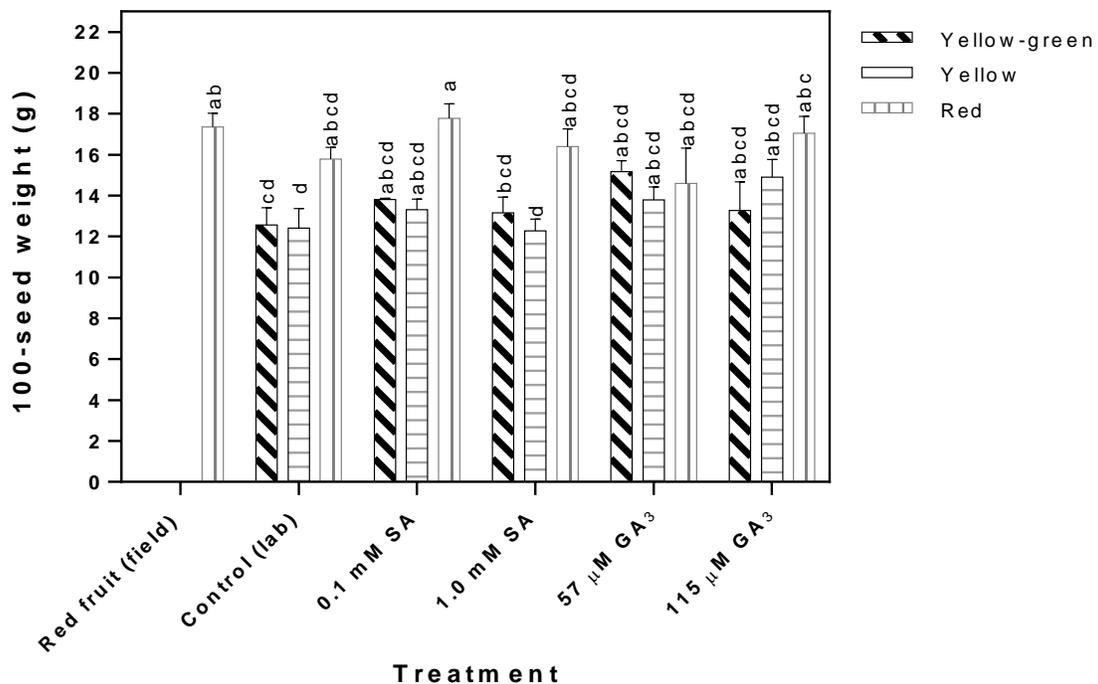
Figure 2.8. One-hundred dry seed weight of coffee fruit harvested at the yellow-green stage, treated with SA (0.1 or 1.0 mM) or GA_3 (57 or 115 µM), and stored for 19 days at 24 ± 3 °C and 56 ± 7 % R.H. Values are the average of three replicates + SE (Tukey, $p < 0.05$).

2.4.7 Seed longitudinal and equatorial diameter

Seed longitudinal diameter was significantly different between treatments. Seeds from red fruit had higher longitudinal diameter values than seeds from grey fruit in all treatments (Figure 2.9A).
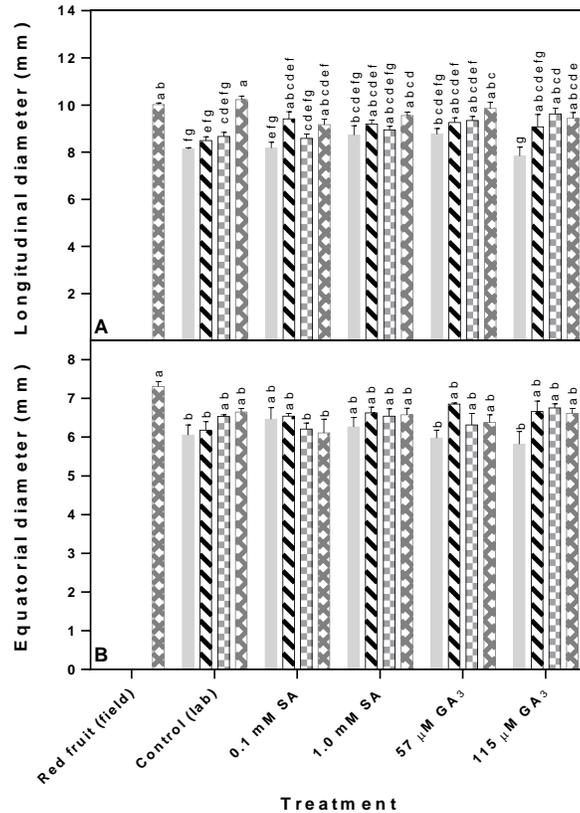
Figure 2.9. Coffee seed longitudinal (A) and equatorial (B) diameter when harvested at the green (grey bars) and yellow-green stage, treated with SA (0.1 or 1.0 mM) or GA$_3$ (57 or 115 μM), and stored for 19 days at 24 ± 3 °C and 56 ± 7 % R.H. Seeds were obtained from fruit with a final colour of grey (▬), yellow-green (◤◥), yellow (▦) and red (✕✕). Values are the average of three replicates + SE (Tukey, p < 0.05).

There were significant differences in equatorial diameter among treatments. The highest equatorial diameter was observed in ripe coffee fruit harvested *in situ*. All the treatments (except 0.1 mM SA) with coffee fruit harvested at the green stage showed the lowest equatorial diameter values (Figure 2.9B).

2.5 Discussion

The high weight loss observed in the stored coffee fruit was a result of the low relative humidity (56 ± 7 %) in the laboratory. Similarly, Tu *et al*. (2000) showed that at lower relative humidity increased fruit weight loss. After 19 days of storage, both the yellow-green and the green fruit lost the same amount of weight. These results are similar to the results of Moneruzzaman *et al*. (2008), in which weight losses were the same for tomatoes at three different stages of maturity after storage for 15 days at 30 °C. In contrast, for strawberries, Moshiur *et al*. (2016) found that weight loss is a function of the fruit's maturity stage.

The lower moisture content observed in the yellow-green stage is in contrast to Dancer (1963) and Herrera *et al*. (1993), who observed in *Robusta* and *Arabica* coffee species that moisture content increased as the coffee fruit matured. In this research, yellow-green fruit was found to have a lower moisture content than green fruit. However, after 19 days of storage, dry yellow-green fruit had a higher moisture content than dry green fruit. Therefore, green fruit contained more water at harvest, but lost water more rapidly during storage.

The 100-seed dry weight of fruit harvested in the green stage that ripened to the red colour, in this research, was similar to results obtained by Herrera *et al*.

(1993), who reported a 100-seed dry weight of 14.4 g for coffee harvested in the green stage and 16.4 for ripe fruit. This study also observed that the 100-seed weight increases with fruit maturity. Likewise, Dancer (1963) observed that dry matter content increases as the state of maturation progresses.

The ripe fruit harvested *in situ* and the fruit harvested during the yellow-green stage that ripened to red in the laboratory had higher longitudinal and equatorial diameters, which is in accordance with Álvarez *et al.* (1999) and Marín *et al.* (2003), who found that coffee fruit grew during maturity.

2.6 Conclusions

Coffee fruit harvested in the green stage did not ripen, while the fruit harvested in the yellow-green stage did ripen, but at a low percentage.

It was not possible to clearly observe the effects of treatment with SA and GA_3 because the fruit rapidly lost water.

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CHAPTER III. COFFEE FRUIT RIPENING INSIDE A BIODEGRADABLE PLASTIC BAG

3.1 Abstract

Coffee fruit maturity has a large influence on the quality of coffee products. Red ripe fruit is higher quality than green, unripe fruit. Farmers in Mexico sometimes harvest coffee fruit that is still immature and green. To address this situation, new technologies that induce fruit ripening are necessary. The objective of this research was to assess the ripening of green coffee fruit inside biodegradable bags at three storage temperatures, 4 ± 1, 8 ± 1 and 24 ± 3 ºC, over 28 days. The percent of ripe fruit, fresh weight loss, colour (luminosity, chroma and hue angle), total soluble solids (TSS), glucose, fructose, sucrose, respiration rate and ethylene production during fruit ripening were evaluated. A repeated measures design was used to analyse the effects of the treatments on the fresh weight loss, colour, respiration and ethylene production of the fruit, and a completely random design was used to analyse the percent of ripe fruit, TSS, glucose, fructose and sucrose. A bag with 500 g of coffee fruit was the experimental unit, and there were five replicates. The green coffee fruit in bags stored at 24 ± 3 ºC ripened. The cold storage treatment (4 ± 1 ºC) induced chilling injuries, and at 8 ± 1 ºC, fruits did not ripen. Biodegradable bags are a storage alternative that favours the ripening of green coffee fruit.

Key words: coffee, ripening, biodegradable bag, repeated measurements.

3.2 Introduction

Coffee (*Coffea arabica* L.) is a climacteric fruit and can therefore be harvested immature, during the green colour stage, and the ripening process will continue, leading to physiological maturity (Pereira, *et al.*, 2005; Kader and Barret, 2005).

In Mexico, coffee fruit is manually harvested, and it is common for some green fruit to be gathered along with the ripe red fruit. In addition, coffee crops may be far from towns or lack access to road infrastructure.

The quality of coffee products relies upon the fruit's maturity at harvest. When the coffee fruit is harvested in the red, ripe stage, it produces a higher-quality beverage when compared to overripe or green fruit (Preedy, 2015). Sugar content also plays a crucial role in coffee quality. The proportions of different sugars present in the coffee fruit (glucose, fructose and sucrose) are related to the type of cultivar grown and the shading conditions during cultivation (Somporn *et al.,* 2012). Geromel *et al.* (2008) observed that sucrose content decreases, and glucose and fructose content increases in coffee beans when they are cultivated in shady conditions.

Modified atmosphere packaging (MAP) can be used for intact and minimally processed fruits and vegetables (Yahia, 2009). MAP is used to modify O_2 and CO_2 concentrations inside a package containing actively respiring products; the modification of the atmosphere is a consequence of the respiration of the fruit and the package's permeability (Mangaraj *et al.*, 2009).

Modified atmospheres (MA) decrease or remove the O_2 inside the package and replace it with CO_2. As a result, fruit respiration, transpiration and weight loss

are reduced in comparison to fruits stored without packaging (Valero and Serrano, 2010).

There are a great variety of polymeric films used for MAP, but most of them are constructed of polyvinyl chloride (PVC), polyethylene terephthalate (PET), polypropylene (PP) and polyethylene (PE). Polymeric films are primarily flexible structures; however, there are also rigid and semi-rigid package structures (Mangaraj *et al.*, 2009).

Relative humidity (R.H.) has a great influence on the fruits stored in MAP; for example, low R.H. inside MAP can increase fruit transpiration rates, whereas a high R.H. can induce condensation on the film (Sandhya, 2009).

Light is not a crucial factor in the postharvest handling of most commodities but is very important for green vegetables because, with sufficient light, photosynthesis can continue (and therefore CO_2 consumption and O_2 production continue) (Sandhya, 2009).

The permeability of polymeric films to O_2 and CO_2 is related to temperature. The Q_{10}^P value indicates that for each 10 °C increase in temperature, the storage permeability increases, and CO_2 increases more than O_2 (Mangaraj *et al.*, 2009; Sandhya, 2009).

Product respiration rate, storage temperature, O_2 and CO_2 concentrations are all factors to be considered when selecting a type of polymeric film for MAP (Mangaraj *et al.*, 2009).

The objective of the current study was to assess the ripening of green coffee fruit inside a biodegradable bag in cold storage at 4 ± 1, 8 ± 1 ºC and at ambient laboratory temperatures of 24 ± 3 ºC, for 28 days. The hypothesis was that one specific storage temperature favours the ripening of coffee fruit.

3.3 Materials and methods

3.3.1 Plant material

The study was carried out in March 2015 in a seven year old *C. arabica* L. var. Typical plantation at Tanetze de Zaragoza, Oaxaca, Mexico (17° 23' 10.2´´ N, 96° 17' 52.9´´ W and altitude of 1311 masl). Green fruit with full endosperm formation was harvested at 8:00 a.m and transported to the laboratory in an ice cooler. The coffee tree heights averaged 1.8 ± 0.2 m.

500 g of coffee fruit was stored for 28 days inside biodegradable bags in cold storage at 4 ± 1 ºC, 8 ± 1 ºC / 90 ± 3 % R.H. and in the laboratory, as a control (24 ± 3 ºC / 56 ± 7 % R.H.). The biodegradable plastic bags had the following properties: thickness = 19 µm, density = 9.6 g m^{-2}, luminosity = 64, chroma = 19, and hue angle = 61°.

3.3.2 Fruit response variables

The percent of ripe fruit, fruit colour (luminosity, chroma, hue angle), fresh weight loss, total soluble solids (TSS), glucose, fructose, sucrose, respiration and ethylene production were evaluated during fruit ripening.

The percent of ripe fruit was assessed visually by the change in colour of the coffee fruit exocarp from green (unripe fruit) to red (ripe fruit) after 28 days of storage inside the biodegradable bags (De Castro and Marraccini, 2006).

The fresh fruit was weighed with a digital balance (Precisa, XB 2200C®). The results were expressed as the percent of the cumulative post-harvest weight loss to the original weight at harvest.

The fruit colour was evaluated by analysing digital images (Casio®, 12.1 MP) with Photoshop CS5® software. The colour space CIE L*a*b* (luminosity, chroma and hue angle, respectively) was used for the determinations. The photographs were taken at the same position, time of day (10 am) and distance (0.3 m) for all measurements. Based on the reading taken by the CIE L*a*b* model, chroma (C*) and hue (h⁰) were calculated using the following equations (McGuire (1992):

$$C^* = (a^{*2} + b^{*2})^{1/2}$$

$$h° = tan^{-1}\left(\frac{b*}{a*}\right)$$

Total soluble solids of the red fruit were measured from fruit juice using a digital refractometer (ATAGO, PAL-1, Japan) after 28 days of storage inside biodegradable bags. TSS was also measured in samples of green and ripe coffee fruit harvested at the farm as a comparison with fruit stored in biodegradable bags.

Glucose, fructose and sucrose were analysed by an enzymatic method (Viola and Davies, 1992; Bernal *et al.*, 2005). A sample of ground dry coffee (20 mg) from each treatment was homogenized in 700 µL 80 % ethanol at 80 °C for 1 h. The samples were then centrifuged at 16 000 g for 5 min. Ethanol extract (10 µL) was

added to the 210 µL buffer reaction (25 mM Tris-HCl (pH 8.0), 50 mM KCl, 1 mM ATP, 3 mM $MgCl_2$, 0.3 mM NAD^+ and 1 U mL^1 yeast hexokinase (EC 2.7.1.1)) and its absorbance was measured at 340 nm. Glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49) (10 µL) was added to the mixture and incubated for 1.5 h at 37 °C. Its absorbance at 340 nm was recorded. Glucose concentration was calculated using the difference between the two above absorbances. For fructose concentration, 10 µL of phosphoglucose isomerase (EC 5.3.1.9) was added to the preceding mixture and incubated for 1.5 h at 37 °C. Its absorbance was measured at 340 nm and used to calculate the difference between this and the previous absorbance. The sucrose concentration was calculated by the difference between the absorbance obtained previously and that recorded after adding a pinch of invertase (EC 3.2.1.26) to the preceding mixture and incubating it for 1.5 h at 37 °C. Absorbance was measured in a Microplate reader (Multiskan FC microplate photometer, Thermo Scientific, USA) and sugar concentration was calculated from a standard curve constructed with glucose. Glucose, fructose and sucrose content were also measured in samples of green and ripe coffee fruits harvested at the farm as a comparison with the experimental fruit that ripened inside the biodegradable bags at the laboratory.

Coffee fruits respiration (mL CO_2 kg^{-1} h^{-1}) and ethylene production (µL C_2H_4 kg^{-1} h^{-1}) were measured as described by Pereira *et al.* (2005). Airtight flasks (250 mL) were used to measure respiration and ethylene production by coffee fruit (Figure 3.1). The receiving flasks contained 24 coffee beans and samples of 6 mL were taken after 4 h (Vacutainer®, Becton Dickinson Co.). Then, the tubes were

refrigerated (6 ± 1 °C) until evaluation. CO_2 and ethylene levels were assessed by chromatography (Hewlett Packard 5890, series II).
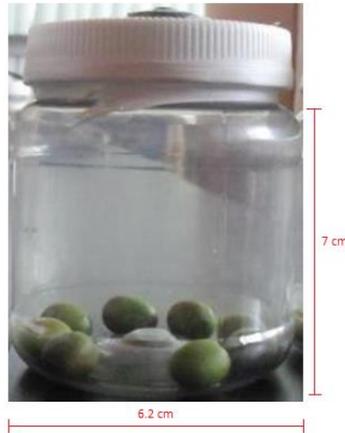
Figure 3.1. Flask used to evaluate respiration rate and ethylene production in coffee fruit during storage inside biodegradable bags at 4 ± 1, 8 ± 1 and 24 ± 3 °C for 28 days.

3.3.3 Statistical analysis

A repeated measures design was used to analyse the effects of the treatments on fresh weight loss, colour, respiration and ethylene production. The evaluations were made every 7 days. A t-Student test ($\alpha = 0.05$) was established beforehand among treatments (day 0 and 28) when the interaction of the treatments with time was significant.

The ripe fruit percentage, TSS, glucose, fructose and sucrose were analysed by a completely random design and a Tukey test ($\alpha = 0.05$). SAS® software (version 9.3) was used for the statistical analyses. Each bag was an experimental unit, and five replicates were included.

3.4 Results

3.4.1 Ripe fruit percentage

98 % of the fruit in the control treatment at 24 °C changed from green to yellow or yellow-green colour after 28 days of storage (Figure 3.2). Fruit had chilling injuries after 7 days in 4 ºC. Fruits stored at 8 °C did not ripen after 28 days and these fruit were moved to 24 °C; however, these samples became infested with fungus 2 days later.
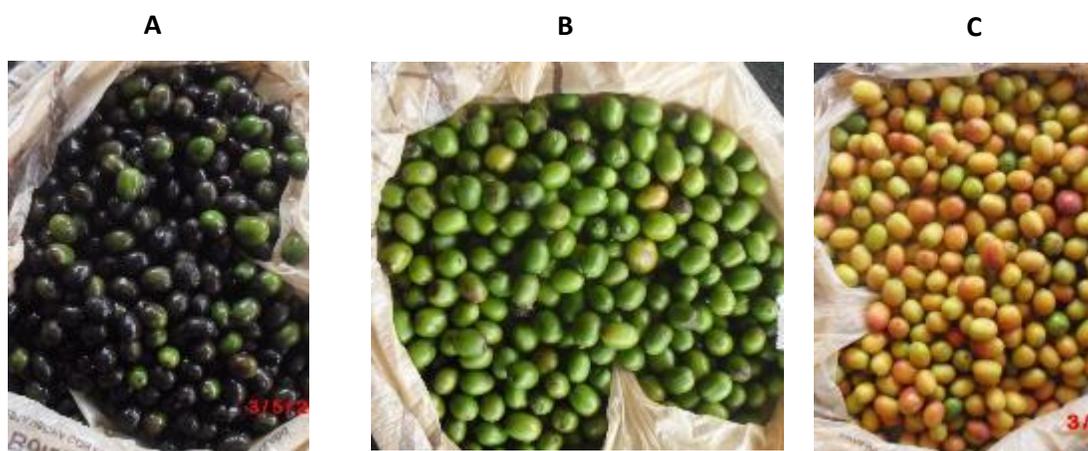

| A | B | C |

Figure 3.2. Coffee fruit colours inside biodegradable plastic bags at 4 ± 1 °C (A), 8 ± 1 °C (B) and 24 ± 3°C (C) after 28 days of storage.

3.4.2 Cumulative fresh weight loss

The interaction between treatment and time was significant for fresh weight loss. All treatments showed progressive cumulative fresh weight loss (Figure 3.3).

The control treatment (24 °C) at the end of storage (28 days), showed the highest cumulative fresh weight loss (averaging 7 %) and the coffee fruit was ripe. Coffee fruit stored at low temperatures did not ripen. There was a significant difference between 0 and 28 days in the three treatments and at the end of the experiment among the treatments.
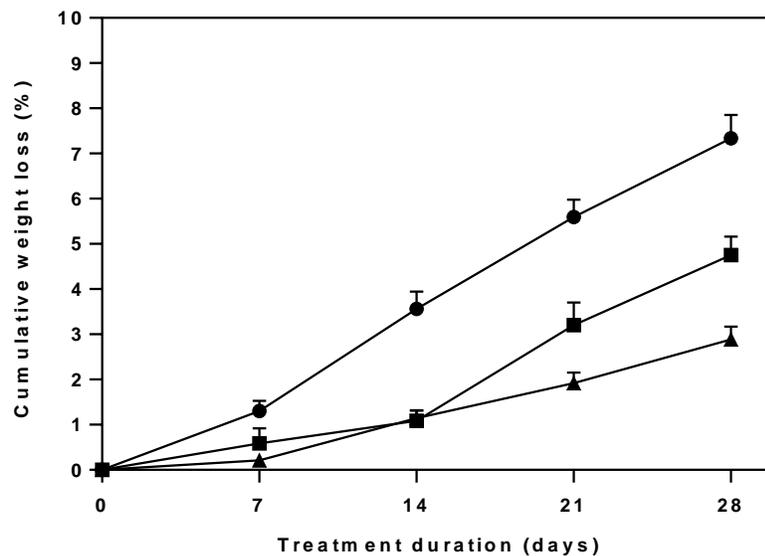

Figure 3.3. Cumulative fresh weight loss of coffee fruits packed in biodegradable bags and stored at (▲) 4 ± 1 °C, (■) 8 ± 1 °C and (●) 24 ± 3 °C. All fruit was green at day zero. Values are the average of five replicates + SE (Tukey, p < 0.05).

3.4.3 Colour

The interaction between treatment and time was significant for the luminosity, chroma and hue angle.

3.4.3.1 Luminosity

There was no significant luminosity difference between 0 and 28 days of storage at 24 °C, however, the decrease in luminosity at 21 days was attributed to the fruit colour change from orange to red. Statistical differences among treatments were observed at the final day of storage. Diminished luminosity was observed in fruit stored at 4 °C due to chilling injury (fruit darkening). In contrast, fruit stored at 8 °C had higher luminosity values due to the change from dark green to pale green 28 days from the start of the experiment ($p < 0.05$) (Figure 3.4A).

3.4.3.2 Chroma

There was no significant difference in chroma between 0 and 28 days of storage at 24 and 8 °C, but a significant difference was observed among treatments by the final day of storage. Fruit stored at 4 °C had a lower chroma value due to chilling injury (fruit darkening) (Figure 3.4B).

3.4.3.3 Hue angle

There was significant difference in hue angle between 0 and 28 days of storage at 4, 8 and 24 °C. Fruits stored at 8 °C showed almost the same hue angle values over time because the fruit colour did not change in this treatment. Fruits stored at 24 °C had a lower hue values due to fruit ripening (red colour) (Figure

3.4C). Hue values for fruits stored at 4 °C were lower due to chilling injury (fruit darkening) over time.

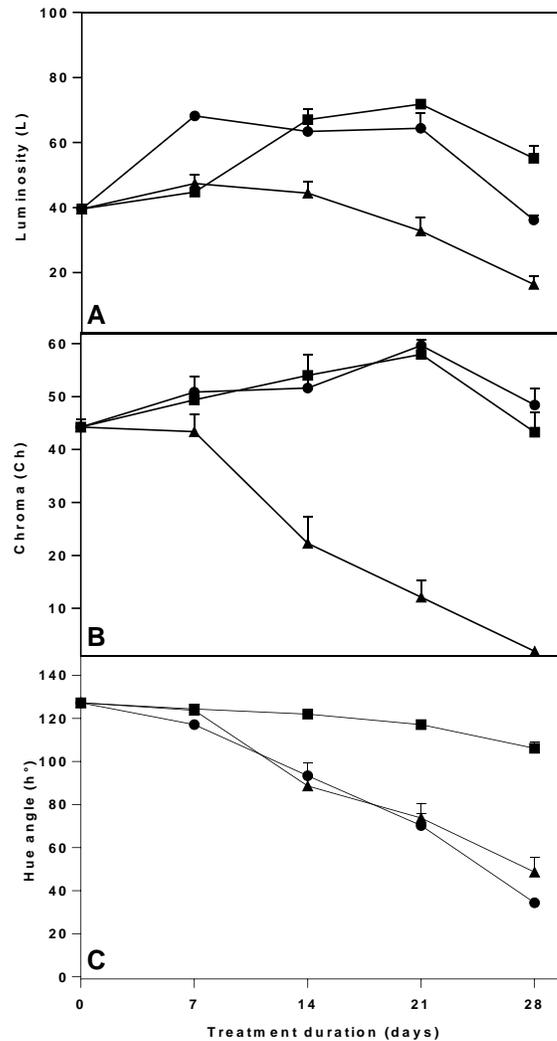**Figure 3.4**. Luminosity (A), chroma (B) and hue angle (C) of coffee fruit packed in biodegradable plastic bags and stored at (▲) 4 ± 1 ºC, (■) 8 ± 1 ºC and (●) 24 ± 3 ºC. All fruit was green at day zero. Values are the average of five replicates + SE (Tukey, p < 0.05).

3.4.4 Respiration

The interaction between treatment and time was significant. A significant difference in respiration between 0 and 28 days of storage was demonstrated at the 24 and 8 °C treatments. On final day of treatment, respiration in fruit stored at 4 °C was different from the 8 and 24 °C treatments. Respiration decreased in fruit stored at 4 °C between 0 and 14 days, followed by an increase that was caused by microbial pathogens (Figure 3.5). The same respiration rate (2.9 mL CO_2 $kg^{-1}h^{-1}$) was observed in green and red fruit at 24 and 8 °C, on the final day.
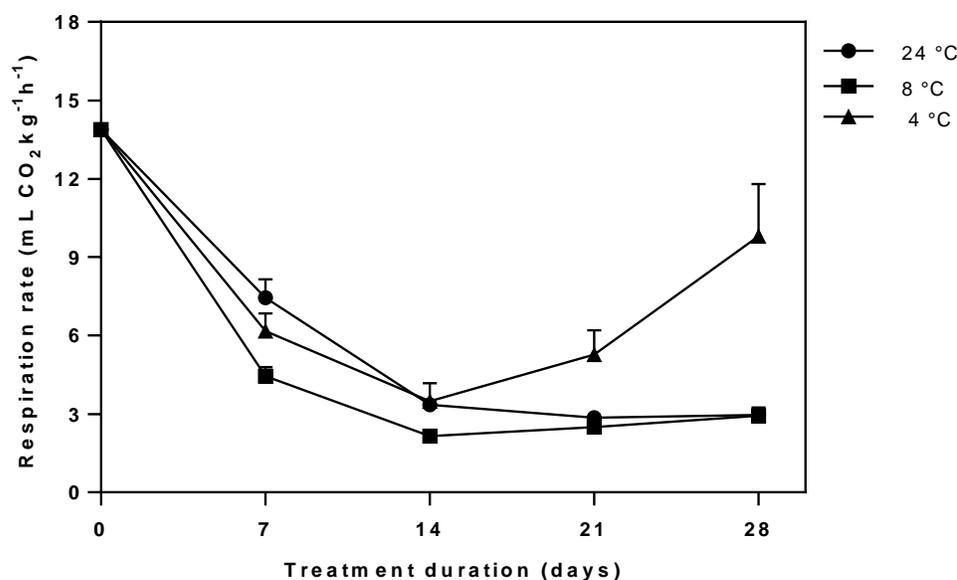
Figure 3.5. Respiration rate of coffee fruit packed in biodegradable plastic bags and stored at (▲) 4 ± 1 °C, (■) 8 ± 1 °C and (●) 24 ± 3 ºC. All fruit was green at day zero. Values are the average of five replicates + SE (Tukey, p < 0.05).

3.4.5 Ethylene production

The interaction between treatment and time was significant. Ethylene production in all the treatments was significantly different between 0 and 28 days. As a result of the modified atmosphere around the fruit, ethylene production was minimal (0.47 μL C_2H_4 $kg^{-1}h^{-1}$) (Figure 3.6).
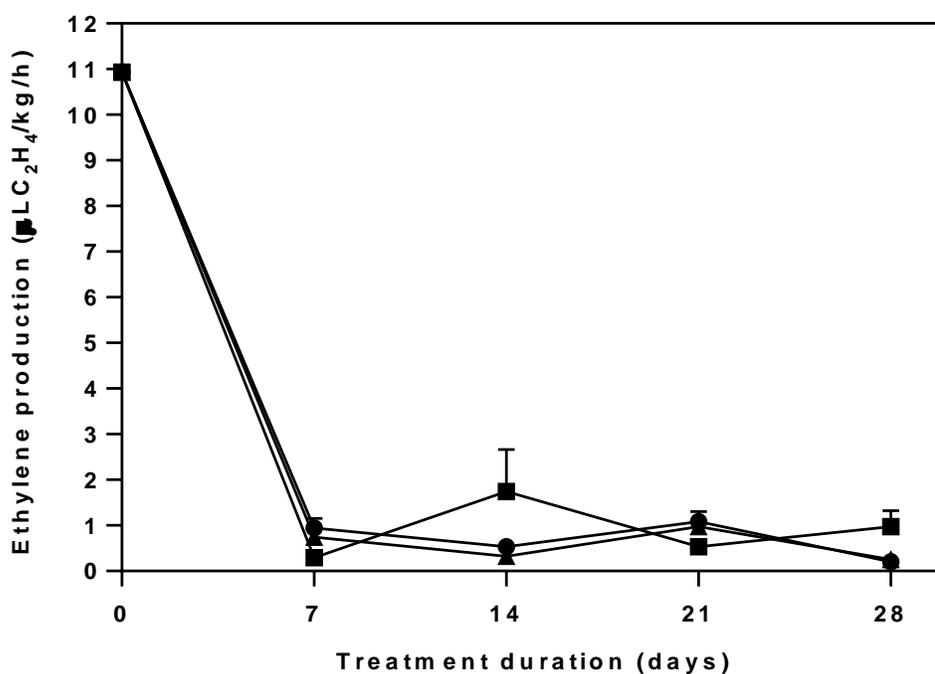
Figure 3.6. Ethylene production of coffee fruit packed in biodegradable plastic bags and stored at (—▲—) 4 ± 1 °C, (—■—) 8 ± 1 °C and (—●—) 24 ± 3 °C. All fruit was green at day zero. Values are the average of five replicates + SE (Tukey, $p < 0.05$).

3.4.6 Total soluble solids

Ripe coffee fruit stored inside biodegradable bags at 24 °C had the highest value for TSS (Figure 3.7) and the value was similar to the fruit that ripened on the plant at the farm.
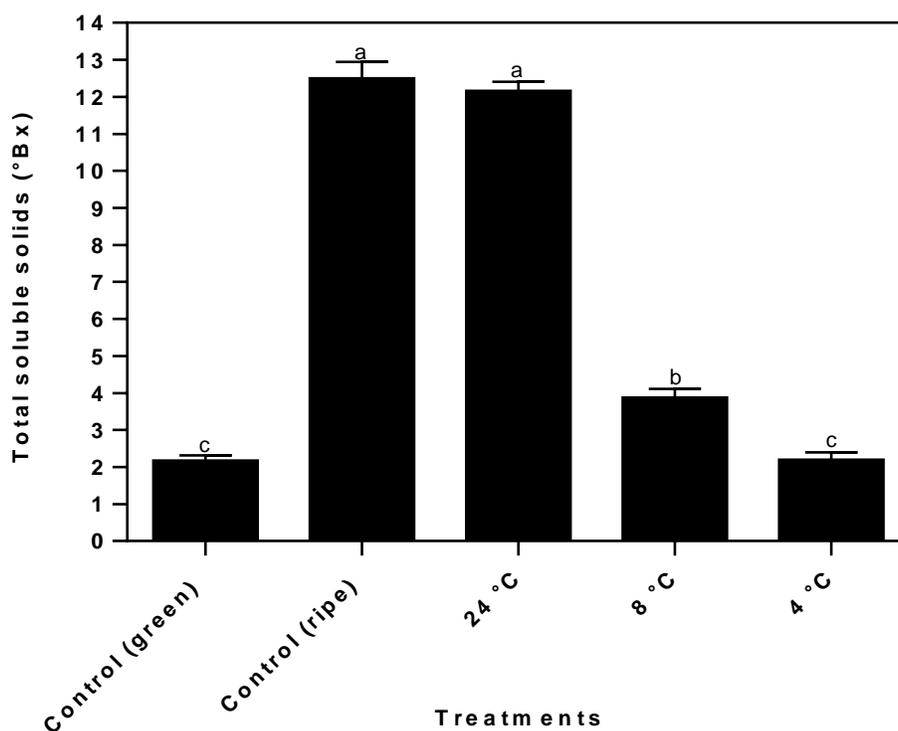

Figure 3.7. Total soluble solids of coffee fruit packaged inside biodegradable plastic bags and stored for 28 days at 4 ± 1 °C, 8 ± 1 °C and 24 ± 3 °C. Control green and ripe coffee fruit were harvested at the farm. All fruit was green at the start of the experiment (day zero). Values are the average of five replicates + SE (Tukey, $p <$ 0.05).

3.4.7 Glucose, fructose and sucrose content

Glucose content was low in coffee fruit stored at 24 °C and was similar to green fruit and fruit harvested ripe at the farm ($p < 0.05$). Glucose content was high in coffee fruit stored at 4 °C. Fructose was not detected.

The highest sucrose content was recorded in ripe coffee fruit harvested at the farm, and was similar to the levels in ripe fruit stored at either 24 or 8 °C for 28 days. In contrast, fruit stored at 4 °C had the lowest sucrose value (Figure 3.8).
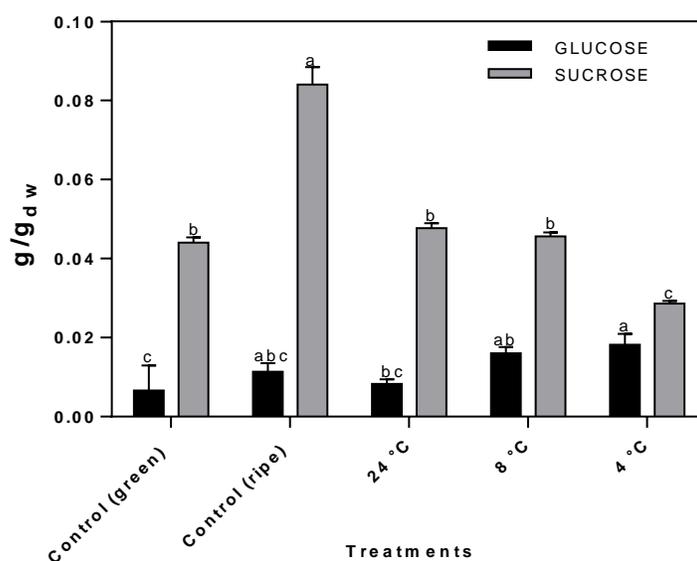

Figure 3.8. Glucose and sucrose content in coffee fruit packaged inside biodegradable bags and stored for 28 days at 4 ± 1 °C, 8 ± 1 °C and 24 ± 3 °C. Control green and ripe coffee fruit was harvested at the farm. All fruit was green at the start of the experiment (day zero). Values are the average of five replicates + SE (Tukey, $p < 0.05$).

3.5 Discussion

The atmosphere inside the biodegradable bags in this experiment reduced coffee fruit weight loss in the 4 and 8 °C treatments. Aglar *et al.* (2017) found higher weight loss (6.05 %) in cherries stored without MA than with MA (1.03 %); the cherries were stored for 21 days at 0 °C / 90 ± 5 % R.H. Tomato and banana fruits showed similar results (Kantola and Helén, 2001; Caron et al., 2013; Sabir and Agar, 2011; Kudachikar *et al.*, 2011). In this experiment, fruit stored outside the biodegradable bags at 24 °C lost 51% of its weight over 14 days (data not shown). This high weight loss was due to the lower R.H. compared with other reports (Pinto *et al.*, 2015). The change in colour of the coffee fruit is similar to the results obtained by Aglar *et al. (*2017) and Sabir and Agar (2011) in cherries and tomatoes, respectively. Luminosity, chroma and hue values diminished over 21 days of storage. However, L*, C* and h° were higher in treatments with MA compared with the control treatment. Suparlan and Itoh (2003) found that MA delayed colour change in tomatoes. However, once the MA was withdrawn, the fruit ripened. Green coffee fruit ripened inside biodegradable bags stored at 24 ± 3 °C for 28 days.

Coffee fruit respiration decreased during storage (28 days). Suparlan and Itoh (2003) showed that during storage for 14 days at 10 °C, CO_2 production in tomatoes was constant for 3 days and then diminished to 5 %. Briano *et al.* (2017) demonstrated that CO_2 production increases during storage as the temperature increases, because the respiratory activity of the fruit is faster than the gas diffusion through the packaging.

The coffee fruit inside a biodegradable bag at 4 °C became infested with fungus at 28 days of storage as a result of the high R.H. and condensation of water on the fruits.

Ethylene production by the coffee fruit diminished over time; similarly Caron *et al.* (2013) found that when tomatoes were stored at 20 ºC ± 1 ºC in either a low density polyethylene or a biodegradable bag, ethylene production diminished, probably because it was inhibited by an increase in CO_2 concentration. Suparlan and Itoh (2003) also observed an increase in ethylene production during the first 4 h of storage (23.1 ppm), followed by a reduction to 14.6 ppm after 14 days. In addition, an increase in TSS was recorded. Sabir and Agar (2011) and Kudachikar *et al.* (2011) also found an increase in TSS in tomatoes and banana fruits, respectively. In contrast, Briano *et al.* (2017) recorded a decrease in TSS in strawberry fruit during storage in a MA.

Glucose content was 0.82 % in coffee fruit stored at 24 °C, in this study. These data are in line with Herrera *et al.* (2011), who found a glucose content of 0.5 - 1.0 % and Knopp *et al.* (2006) who reported a value of 0.23 %. We found 0.0477 g/g$_{dw}$ of sucrose in coffee fruit stored at 24 and 8 °C. Knopp *et al.* (2006) and Farah (2012) found higher values for sucrose; 7.07 % (0.07 g/g$_{dw}$) and 6 - 9 g/100 g$_{dw}$, respectively, in ripe coffee fruit on the plant. The differences can be attributed to the cultivars studied and to the shady conditions used in the production system (Geromel *et al.,* 2008; Somporn *et al.,* 2012).

3.6 Conclusion

The atmosphere inside the biodegradable plastic bag allows coffee fruit to ripen at 24 °C. Coffee fruit does not tolerate cold storage (4 °C).

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GENERAL CONCLUSIONS

It is possible to alter the ripening process of the coffee fruits on plant with direct aspersion with salicylic acid to the fruits. Chitosan does not delay the ripening of coffee fruits and damages fruit exocarp and mesocarp.

Coffee fruits did not show characteristics that allow us to classify them as climacteric fruits, as documented in the literature.

Coffee fruits ripening depends on the storage conditions. The closer the saturation point to the relative humidity and the higher the temperature, without exceeding 27 ° C, a higher percentage of ripe fruits is obtained.

The use of biodegradable plastic bags is an alternative to induce the ripening of coffee fruits because they generate an adequate atmosphere for this process.