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Effect of modified atmospheres storage on physicochemical and biological parameters of arabica Mexican green coffee

Efecto del almacenamiento en atmósferas modificadas sobre los paramétros fisicoquímicos y biológicos de café verde arabiga mexicano

C.Trujillo-Carretero[1], O. González-Ríos[1] , C.Y. Figueroa-Hernández[2], M.L. Suárez-Quiroz[1*]

[1] *Tecnológico Nacional de México/Instituto Tecnológico de Veracruz, Unidad de Investigación y Desarrollo en Alimentos, Av. M.A. de Quevedo 2779, CP 91897 Veracruz, Ver. México.*

[2] *CONACYT-Tecnológico Nacional de México/Instituto Tecnológico de Veracruz, Unidad de Investigación y Desarrollo en Alimentos, M. A. de Quevedo 2779, Veracruz, Ver. C.P. 91897, Mexico.*

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Abstract

Green coffee (GC) is commonly stored in jute bags. Moreover, like all food, this product deteriorates over time, as shown by its quality indicators, so it is essential to preserve its original attributes during storage. The work aim was to evaluate the effect of the use of modified atmospheres packaging at two storage conditions (environmental and 18 ºC) on physicochemical (moisture content, water activity, color and reducing sugar content), biological (viability) parameters, fungal infection, and ochratoxin A (OTA) content on GC for one-year storage. The GC used in this study is from Comalapa, Chiapas, México. For one year, storage in modified atmospheres could preserve GC moisture content (11.30-8.87 %) and a_w (0.63-0.55). The color of GC samples was maintained without significant changes for six months. Viability in GC stored for one year under all conditions was less than 25 %. Fungal infection was less than 40 % in all stored GC samples. Ochratoxin A (OTA) content in GC samples showed no significant differences during storage.

Keywords: green coffee, modified atmospheres storage, viability, physicochemical parameters, fungal infection.

Resumen

El café verde (CV) se almacena habitualmente en sacos de yute. Además, como todo alimento, este producto se deteriora con el paso del tiempo, como muestran sus indicadores de calidad, por lo que es fundamental preservar sus atributos originales durante el almacenamiento. El objetivo de este trabajo era evaluar el efecto del uso de empaques de atmósferas modificadas en dos condiciones de almacenamiento (ambiental y 18 ºC) sobre los parámetros fisicoquímicos (contenido de humedad, actividad de agua, color, y el contenido de azúcares reductores), biológicos (viabilidad), infección fúngica y el contenido de ocratoxina A (OTA) en CV durante un año de almacenamiento. El CV usado es de Comalapa, Chiapas. El almacenamiento en empaques de atmósferas modificadas pudo conservar el contenido de humedad de las muestras del CV (11.30- 8.87 %) y la a_w (0.63-0.55) durante un año. El color en las muestras de CV se mantuvo sin cambios significativos por seis meses. La viabilidad del CV almacenado durante un año en todas las condiciones fue inferior al 25 %. La infección fúngica fue menor al 40 % en todas las muestras de CV almacenado. El contenido de Ocratoxina A en las muestras de CV no mostró tener diferencias significativas durante el almacenamiento.

Palabras clave: café verde, almacenamiento en atmósferas modificadas, viabilidad, parámetros fisicoquímicos, infección fúngica.

* *Corresponding author. E-mail*: mirna.sq@veracruz.tecnm.mx
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1 Introduction

Coffee (*Coffea arabica* L.) is a highly traded commodity worldwide, only surpassed by petroleum (Alvarado-Ambriz *et al*., 2020, Ramírez-Velasco *et al*., 2016) and an important source of income for the countries producing it (Muñoz-Neira *et al*., 2018). The quality of coffee beans is related to the edaphic and climatic conditions, cultivation practices, post-harvest processing, and other technological factors (Ferreira *et al*., 2021, Hernandez-Aguirre *et al*., 2019, Virgen-Navarro *et al*., 2016; Worku *et al*., 2018). To guarantee quality coffee beans, its necessary to preserve the proper conditions during the harvest, post-harvest, and processing. Drying, storage, and roasting are the most critical stages to increase its profit during coffee bean marketing. Therefore, the coffee industry is obligated to invest in technological innovations that maintain and increase the coffee bean's quality attributes to ensure its economic profitability.

During roasting, key flavor compounds are formed through browning reactions determined during the green coffee beans (GC) (Borém *et al*., 2013). Therefore, preserving quality parameters in green coffee is key to maintaining its quality and final price. Most Mexican GC is stored using the conventional system, stored in woven jute bags that provide limited protection to the beans against physical, chemical, or microbiological damage, mainly due to temperature and relative humidity (RH). It is needed that during storage, the physicochemical, microbiological, and biological characteristics and their commercial value are preserved as much as possible (Bonazzi and Dumoulin, 2011; Rojas, 2004), so it is necessary to monitor various quality indicators during storage.

Environmental temperature and RH during storage must be controlled to maintain the quality of the coffee without losing its intrinsic sensory characteristics and physicochemical properties. It has been observed that when green coffee is stored at RH above 60 % and temperatures above 25 °C, there can be cell degradation, leading to oil leakage and a loss in the chemical composition of coffee beans (Sedana and Astawa 2016). Moisture migration between green coffee and the atmosphere is one of the leading causes of quality deterioration, as it causes sucrose molecules to polymerize into glucose, which has been associated with "stale crop" flavor (Williams *et al*., 2017). Whereas moisture is a key indicator of the quality of coffee beans that must be monitored during storage, water activity is also a critical parameter for their conservation. Water activity is the coefficient between the vapor pressure of the food product and the vapor pressure of pure water in a closed system (Donovan *et al*., 2019, Fontana Jr., 2001).

Color changes in green coffee are evidence of oxidative processes and natural enzymatic biochemical transformations that will result in a loss of quality in the bean. Changes in the color of the bean can also occur in storage, where there may exist changes in the coloration of the coffee bean from bluish-green to light brown and whitish colorations, where the wet processing beans are more susceptible to mechanical damage. Coffee bean discoloration can be reflected by taking colorimetric measures such as L* (lightness) and C* (chromaticity) coordinate, where increasing L* value is associated with bean bleaching and decreasing C* with color loss (Ferreira *et al*., 2018).

Free amino acids and soluble carbohydrates are considered the most prominent precursors of numerous volatile and non-volatile roasted coffee flavors (Rendón *et al*., 2014). Joët *et al*. (2010) analyzed reducing sugars along the coffee production chain. A significant correlation was found between the content of reducing sugars and temperature during coffee development. A decrease in reducing sugars was found during fermentation, followed by an increase during storage due to sucrose hydrolysis. The chemical composition changes have also been related to the loss of viability in the bean that impacts cup quality (Selmar *et al*., 2008). Although some investigators have analyzed the time course for a loss of germination capacity, only limited data for changes in stored coffee beans' composition are available. They observed a decrease in the coffee viability during the storage time.

The influence of environmental conditions, such as moisture content, temperature, incubation time, and the substrate's nature, can play an essential role in fungal colonization and Ochratoxin A (OTA) production, which could affect the microbiological and sanitary quality of coffee and other crops (Hernández-Díaz *et al*., 2013, Maman *et al*., 2021, Martínez-Moreno *et al*., 2021). Some reports have confirmed that filamentous fungi are the most common contaminants in coffee beans after harvest and drying, increasing their biomass during storage (Gil-Serna *et al*., 2014; Iamanaka *et al*., 2014a; Khalesi and Khatib, 2011). Broissin-Vargas *et al*. (2018) studied the effects of warehouse storage conditions on the fungal community composition of GC stored in jute sacks for

one year. After six months of storage, they reported that the GC showed changes in fungal population dynamics, decreased chromaticity in GC by bleaching, and other quality changes. They suggested that the jute sacks should be used to store GC for only a few months.

The modified atmosphere is formed in an airtight container created around the product due to the impermeability of the packaging and the product's respiration and other living organisms present in it, but without controlling the concentrations of the gases formed or existing (Meneses and Valenzuela, 2008). The fundament of MAP is based on modifying the concentration of gases in the natural atmosphere, which makes the products present a drastic reduction in metabolic processes, so they maintain their initial characteristics for more extended periods (Moreno *et al*., 2000; Borém *et al*., 2013).

According to Ribeiro *et al*. (2011), the packaging of coffee beans in containers enriched with 60 % CO_2 presented the best results in relation to the preservation of chemical composition, color, and sensory characteristics. Almeida and Morais (1997) observed that the type of packaging interferes with coffee beans' longevity and physiological quality during storage. According to these authors, modified atmosphere packaging (MAP) is superior to semi-impermeable and permeable packaging. Bredmose and Nielsen (2009) concluded that using a controlled atmosphere of CO_2 and N_2 did not alter the technological characteristics of the grains, such as water content, water absorption capacity, and color index. Tripetch and Borompichaichartkul (2019) concluded that the use of packaging such as high-density polyethylene (HDPE) bags has better results on the moisture content, color, phenolic content, chlorogenic acid content, and antioxidant activity of the Arabica green coffee bean for 15 months of storage compared to the use of jute bags. However, the dissemination and application of new technologies for GC storage at a commercial scale require the validation of laboratory results. Therefore, this research aimed to investigate the effects of storage in modified atmospheres with vacuum and nitrogen gas on quality markers on the moisture content, water activity, color, bean viability, respiratory activity, reducing sugars, fungal infection, and ochratoxin A production under environmental conditions and controlled temperature of 18 °C for 12 months (Table 1).

2 Methodology

2.1 *Green coffee storage conditions*

One hundred fifty kilograms of green coffee (GC) (*Coffea arabica* L.) were obtained from Comalapa, Chiapas, México, located at an altitude of 1231 masl. The GC was transported to the laboratory of the Instituto Tecnológico de Veracruz in Veracruz, México, and then were packed and stored. The green coffee was classified according to ISO 4149:2005 as High Grown Europrep. The bean size was retained by sieve 18 and only presented a minimum of 12 defects in 300g. Subsequently, the defects in the coffee beans were classified as genetic (triangle, elephant, and snail), agronomic (brocade and cold- damage), and due to processing (sour and mechanically damaged). The number of coffees with defects was multiplied by a coefficient assigned to the type of defect according to ISO 10470:2004. The GC was packaged in two modified atmospheres: vacuum and nitrogen gas (99.99 %). As a result, 72 packs of 1 kg of coffee were obtained under vacuum and 72 packs under nitrogen gas atmosphere. The coffee packaging was carried out by high vacuum equipment (model EOS ATM, Beeser Vacum®, Mexico). The level of vacuum used for all bags was 200 Pa (± 5).

A vacuum was first performed for the nitrogen gas samples, and then a nitrogen gas injection was applied for 10 seconds. The packaging material used was three coextruded layers of Bynel, Polyamide, and Metallocene manufactured by MultiFilm S.A. de C.V. (México), recommended for modified atmosphere packaging. Finally, the coffee was distributed in four treatments, 36 bags for green coffee vacuum at 18 °C (GCV18), and 36 bags for environmental conditions (GCV), 36 bags for green coffee packed with nitrogen gas at 18 °C (GCN18), and 36 bags environmental conditions (GCN). The remaining coffee was used as control; the sample represented fresh coffee without storing evaluated in this study. The treatments corresponding to environmental conditions were stored in one area simulating industrial storage conditions, with a concrete floor, natural ventilation, mechanical. Environmental temperature and relative humidity were measured daily for one year of storage (November 2017-December 2018). The treatments stored at 18 °C were stored in one warehouse with controlled temperature by air conditioning (Table 1). Each month, samples were taken in triplicate, taking a representative sample of 1.5 kg from each of the treatments for their subsequent study.

Table 1. Average Temperature (T) and Relativity Humidity (RH) for GCV and GCN treatments stored for 12 months **

Storage time	Month	Year	T (°C)	RH (%)
0	December	2017	16.03 ± 0.34	94.00 ± 0.58
1	January	2018	24.01 ± 2.56	46.00 ± 4.16
2	February	2018	26.83 ± 0.43	64.00 ± 4.91
3	March	2018	29.53 ± 0.43	75.00 ± 4.04
4	April	2018	31.23 ± 0.79	87.67 ± 3.48
5	May	2018	32.60 ± 0.21	96.33 ± 1.33
6	June	2018	33.50 ± 0.78	98.33 ± 0.88
7	July	2018	32.40 ± 0.14	97.33 ± 0.33
8	August	2018	34.47 ± 0.15	98.33 ± 0.88
9	September	2018	31.60 ± 0.15	99.33 ± 0.89
10	October	2018	30.97 ± 0.28	90.33 ± 3.75
11	November	2018	28.67 ± 0.33	76.00 ± 4.16
12	December	2018	27.33 ± 0.33	62.33 ± 3.75

** Means (n=3) ± standard error

2.2 Determination of moisture content of GC samples during modified atmospheres packaging at two different condition storage

According to ISO 6673 (2012), moisture content determination used the drying method to estimate the mass loss. First, 10 g of green coffee beans were dried at 105 ± 1 °C for one h and subsequently allowed to cool down at environmental temperature in a desiccator, then the samples were placed in capsules and dried at 105 ± 1 °C for 16 ± 0.5 h. Thus, the data obtained represents the average of simultaneous experiments performed in triplicate. The moisture content present in the GC samples was calculated with the following Eq. 1:

$$\omega = \frac{m1 - m2}{m1 - m0} \times 100\% \quad (1)$$

where m0 is the mass in grams of the dish and lid; m1 is the mass in grams of the dish, GC sample, and lid before drying, and m2 is the mass in grams of the dish, GC sample, and lid after drying.

2.3 Determination of water activity of GC samples during modified atmospheres packaging storage

Water activity (a_w) was determined by weighing 5 grams of each of the GC samples. The analysis was performed in triplicate using a Decagon AquaLab 3TE Series water activity meter (Pullman, WA) at 25°C ± 1°C.

2.4 Determination of color of GC samples during modified atmospheres packaging storage

A 5 g sample of green coffee beans of each storage condition was used to determine the color. The color of coffee beans was measured using a Konica® Minolta colorimeter (model CR-410, Hunter Lab, USA). The coffee bean color is significant for coffee quality as part of visual characteristics and often determines the acceptance or rejection during marketing. The loss of GC beans' coloration during storage is known as bleaching, causing the product to lose its value. The quantitative color evaluation results of green coffee beans during storage at modified atmospheres were expressed in terms of the L* and C* coordinates of the L* a* b* C* space. The L* coordinate represents the coffee bean's lightness, and the chromaticity parameter is defined as a change in color intensity. The color determination was performed, measuring the colorimetric coordinates L*, a*, and b* to the CIELab system. The reflectance spectra were recorded using the standardized CIE L*a*b* chromaticity system as a wavelength (BYK-Gardner catalog). The CIEL*a*b* color system estimates the value of three variables: coordinate L* for lightness, representing the position on the black-white axis (L* = 0 for black, L* = 100 for white), coordinate a* for the position on the red-green axis (+100 = positive values for red, -80 negative

values for green), and coordinate b* for the position on the yellow-blue axis (+70= positive values for yellow, -80 = negative values for blue) (Broisin-Vargas *et al*., 2018).

2.5 *Determination of viability of GC samples during modified atmospheres packaging storage*

Several researchers suggest a relationship between the loss of viability and a decrease in cup quality (Selmar *et al*., 2008; Deepak *et al*., 2012); therefore, it is relevant to determine this parameter. The coffee bean's viability determination was made by the method established by Selmar *et al*. (2008). Fifty GC beans were immersed in water and placed in filter paper, then incubated for 16 h at 25 °C. The green coffee samples of each storage condition were placed in desiccators with water to prevent moisture loss, and later were immersed in the tetrazolium solution (0.075 %) and then incubated in the dark at 40 ± 1 °C for 180 min. When the coffee bean is immersed in tetrazolium, a reduction reaction occurs with the living cells present therein. As a result, triphenyl formazan is formed, a red, stable compound that causes selective coloration of viable beans. The number of viable GC beans was calculated with Eq. 2:

$$\text{GC viability} = \frac{\text{number of viable beans}}{\text{number of total beans}} \times 100 \quad (2)$$

2.6 *Determination of the respiratory activity of GC samples during modified atmospheres packaging storage*

Green coffee bean respiration was quantified based on the reaction capacity of basic solutions (KOH 1N) with carbon dioxide and the reaction of this solution in the form of carbonate ion (CO_3^{2-}) (Natywa and Selwet, 2011). The green coffee samples of all storage conditions' initial and final time were placed in a vacuum respirometer proposed by Carmona *et al*. 2006 (Figure 1) for 41 days. The CO_2 produced by breathing the bean sample was collected in tubes with 25 mL of KOH (0.15 N) with a methyl orange indicator. A test was also carried out with no coffee sample to determine that leaks existed in the equipment. A 5 mL aliquot was taken from each collected KOH tube, and 4 mL of barium carbonate solution (0.03 N), three drops of phenolphthalein, and 75 mL of H_2O were added. This solution was titrated with HCl (0.03 N).

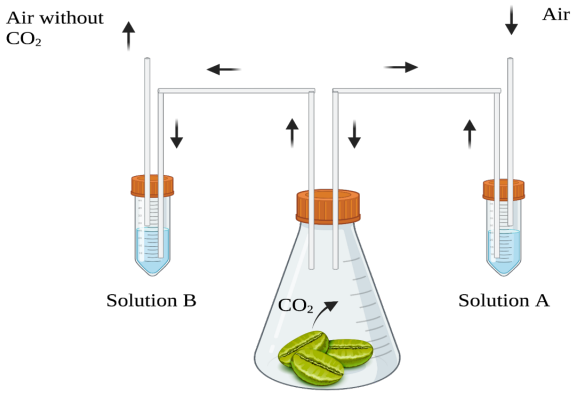

Figure 1. Respirometer used. The GC sample receives CO_2 free air, and the CO_2 produced by GC respiration is collected in solution B. The amount of CO_3^{2-} formed is estimated from the amount of OH^- remaining, determined by titration with HCl. Solution A: KOH 1N and Solution B: 25 mL KOH 0.15 N.

The respiration rate per unit dry mass was reported as (mg CO_2/100 g).

2.7 *Quantification of reducing sugars of GC extracts during modified atmospheres packaging storage*

Reducing sugar in GC extracts was determined by the 3, 5-dinitrosalicylic acid (DNS) according to Miller (1959) with modifications. The DNS reagent solution consisted of 10 g DNS, 0.5 g sodium sulfate, 2 g phenol, and 10 g sodium hydroxide in 1000 mL of distilled water. The extracts of the green coffee samples from all storage conditions were prepared in the following manner. First, ten g of the ground green coffee samples were weighed and diluted in 100 mL of distilled water. Next, the mixture was heated to 50 ºC and agitated at 600 rpm for 15 minutes, heating in a hotplate stirrer. Subsequently, the volume was adjusted to 125 mL. The extraction was performed three times to maximize the extraction of the sugars. Then, green coffee extracts solutions containing 1.0 mg/mL were prepared using distilled water. After that, 0.5 mL of each extract solution and 0.5 ml of the DNS reagent solution were mixed and heated in boiling water at 100 °C for 5 min. The concentration of reducing sugars was measured at 540 nm after the addition of 5.0 mL of distilled water to each sample. Finally, the reducing sugar content of the GC extracts was measured at 540 nm using a spectrophotometer (VE-5600UV, VELAB, México) and calculated using standard curves prepared with D-glucose (Broissin-Vargas *et al*., 2018) and the results were reported as

g / 100 g d.m.

2.8 Determination of fungal infection of GC samples during modified atmospheres packaging storage

The fungal infection was determined by the direct plating method (Pitt and Hocking, 2009). Coffee bean surfaces were disinfected with a 0.4 % chlorine solution for 1 min and rinsed once with sterile water. A total of 100 beans were plated directly onto Dichloran 18 % (W/V) agar containing 100 mg/L chloramphenicol and 50 mg/L chlortetracycline to inhibit bacterial growth. The plates were incubated at 25 °C for five days and were visually analyzed for colony growth. Finally, the fungal infection percentage, FI (%), was calculated with Eq. 3.

$$FI(\%) = \frac{\text{number of beans infected}}{100 \text{ beans}} \times 100 \quad (3)$$

2.9 Fungal isolation of GC samples during modified atmospheres packaging storage

Green coffee's fungal isolation was incubated at 25 °C for seven days in Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA). The identification of the isolates was carried out according to macroscopic and microscopic morphological characteristics. They were visually analyzed in an optical microscope (BA200, Motic, USA) with 1% lactofuchsin (W/V) staining using taxonomic keys for *Nigri*, *Circumdati*, and *Flavi* sections (Pitt and Hocking, 2009).

2.10 Quantification of Ochratoxin A (OTA) of GC samples during modified atmospheres

GC samples were stored frozen at -80 °C, then ground to pass through a 0.5 mm sieve, and subsequently, the OTA concentration in the samples was determined using the methodology proposed by Nakajima *et al*. (1997). It was only used the samples corresponding to GC beans stored at 18 ºC (GCV18 and GCN18) because these samples showed the highest percentage of fungal infection. The samples were extracted for 30 min with a methanol/ 3 % sodium bicarbonate solution (50:50 V/V). The extracts were filtered and diluted with phosphate-buffered saline and cleaned through an immunoaffinity column (Ochraprep®, Rhône Diagnostics). OTA was eluted with 3 mL HPLC grade methanol. Eluate was evaporated to dryness under a nitrogen stream at 70 °C, and the residue was redissolved in 1 mL of HPLC OTA was detected and quantified by HPLC with a fluorescence detector (Shimadzu LC-10ADVP, Shimadzu Corporation, Japan). The mobile phase consisted of distilled water/ acetonitrile/glacial acetic acid (51:48:1). The injection volume was 100 μL, and the flow rate was 1 mL/min. OTA was detected by absorption at 333 nm excitation and 460 nm emission and a 13.3-13.5 min retention time. Standard OTA curves were established with an ochratoxin standard (1000 ng/mL; ref PD 226 R. Biopharm Rhône Ltd., Scotland): the detection limit was 0.075 ng/mL.

2.11 Statistical analysis

The mean value and standard deviation were calculated using NCSS 11 Statistical Software (2016) (NCSS, LLC. Kaysville, Utah, USA). The resultant data were evaluated by ANOVA, and mean values were compared by Tukey-Kramer Multiple comparison test with a confidence level of 95 %.

3 Results and discussion

3.1 Monitoring of temperature and RH parameters during environmental storage conditions

Temperature and RH changed naturally due to climatic variations; GC treatments were stored under environmental conditions (GCV) and (GCN) were monitored as shown in Table 1. The months with higher values of temperature and RH; were from May to September 2018 with an average of 32.91 °C and 98.05 % RH, respectively, and from September to March 2018, the average temperature was 29.64 °C and 82 % RH. For the treatments (GCV18) and (GCN18), the temperature and RH for one year were 18 ± 0.29 °C and 65 ± 0.43 % RH.

3.2 Moisture content and water activity in GC samples during modified atmospheres packaging storage

Moisture content is an important parameter to consider during the storage of coffee beans. Table 2 showed the variations in the moisture content of green coffee bean samples during storage in modified atmospheres

Table 2. Moisture content of GC beans stored under modified atmosphere packaging at two conditions storage **

Storage Time (months)	Moisture content (%) GCV18	GCV	GCN18	GCN
0	11.05 ± 2.17^{ab}	11.05 ± 2.17^{a}	11.05 ± 2.17	11.05 ± 2.17^{a}
1	10.42 ± 0.03^{abcd}	10.46 ± 0.22^{a}	10.91 ± 0.22	11.11 ± 0.26^{ab}
2	11.31 ± 0.07^{a}	11.05 ± 0.40^{ab}	10.91 ± 0.22	10.92 ± 0.21^{ab}
3	9.26 ± 0.09^{cd}	9.05 ± 0.08^{cd}	10.76 ± 4.53	9.63 ± 0.05^{ab}
4	9.50 ± 0.04^{cd}	9.91 ± 0.06^{cd}	9.25 ± 0.13	9.63 ± 0.06^{abc}
5	9.83 ± 0.08^{bcd}	9.94 ± 0.13^{bc}	9.74 ± 0.07	9.88 ± 0.16^{cd}
6	10.53 ± 0.05^{abcd}	10.58 ± 0.35^{abc}	10.20 ± 0.04	10.80 ± 0.09^{cd}
7	9.96 ± 0.17^{bcd}	10.09 ± 0.82^{bc}	9.83 ± 0.28	10.41 ± 0.25^{cd}
8	9.57 ± 0.50^{cd}	9.66 ± 0.12^{de}	9.26 ± 0.70	9.63 ± 0.30^{cde}
9	9.83 ± 0.36^{bcd}	9.63 ± 0.01^{bc}	8.87 ± 1.73	9.99 ± 0.06^{cde}
10	9.50 ± 0.04^{cd}	9.91 ± 0.06^{cd}	9.25 ± 0.13	9.63 ± 0.06^{cde}
11	9.48 ± 0.03^{cd}	10.10 ± 0.24^{bcd}	9.99 ± 0.19	9.78 ± 0.09^{cde}
12	9.63 ± 1.44^{cd}	9.57 ± 0.17^{bcd}	9.26 ± 0.24	9.65 ± 0.54^{cde}

** Means (n=3) ± standard error followed by different letter within a same row (lower) and/or within a same column (upper) are significantly different (Tukey, $P < 0.05$). GCN: Green coffee with nitrogen environmental conditions, GCN18: Green coffee with nitrogen at 18 °C, GCV: Green coffee vacuum in environmental conditions, GCV18: Green coffee vacuum at 18 °C.

(GCV, GCN, GCV18, and GCN18). The ANOVA analysis does not show a significant effect ($p < 0.05$) between treatments. That is, all the treatments showed the same behavior in the moisture content throughout the storage. At the same time, the storage time had a significant effect ($p < 0.05$), which determined that the longer the storage time would influence the moisture content. The initial sample (without storage) corresponding to the sample stored in November 2017 showed a moisture content of 11.05 %. In the first six months of storage, the bean's moisture content of all treatments ranged between 11.30 and 9.05 %, and six months later, the values were between 8.87-10.41 %. The storage in modified atmospheres can preserve the green coffee moisture content (8.87-11.30 %). Moisture contents found in green coffee beans stored in modified atmospheres at two different conditions for 12 months never exceeded the maximum moisture content for green coffee beans of 12.5 %, which is an essential requirement for its commercialization, not exceeding 12.5 %, according to the technical evaluation of the identity and quality of green coffee beans (Bicho *et al*., 2014). Moisture content in green coffee beans higher than 14 % during storage can generate the development of off-flavors, fungal growth, and undesirable aromatic profiles, which adversely affect the quality of the bean (Donovan *et al*., 2020).

These moisture contents found in this study are probably related to the packaging material used to store GC beans, such as polyamide and metallocene. Polyamide has a high vapor permeability 6-22 g/m^2 per day. This material is used for its high elasticity in the food industry due to its amorphous structure for vacuum packaging. On the other hand, metallocene has a low permeability to water vapor 0.5-0.7 g/m^2 per day that allows a negligible water absorption from the environment (Osswald *et al*., 2012). Ribeiro *et al*. (2011) verified that coffee beans stored in GrainPro sealed bags (high strength Polyethylene (PE) with barrier layer) with CO_2 injection kept a stable moisture content (10 %) for one year. In contrast to the moisture content found in green coffee beans stored in jute bags for one year (from 10.93 to 12.50 %), which may affect their quality, as found by Broissin-Vargas *et al*. (2018).

Another critical parameter to be considered during green coffee storage is a_w, as shown in Table 3. At the beginning of storage, the a_w of the green coffee samples was 0.63; for all treatments, this a_w value was maintained without significant differences until the ninth month; therefore, it is advantageous for the storage of green coffee since this a_w value is preserved within an optimal range for this parameter. The a_w values for green coffee samples stored in modified atmospheres at two temperatures for 12 months ranged from 0.63-0.55. Harris and Miller (2008) determined that a_w for GC during storage should be between 0.50 and 0.70.

Table 3. Water activity (a_w) of GC beans stored under modified atmosphere packaging at two conditions storage **

Storage Time (months)	Water activity (aw) GCV18	GCV	GCN18	GCN
0	0.63 ± 0.00^{bc}	0.63 ± 0.00^{ef}	0.63 ± 0.00^{abc}	0.63 ± 0.00^{d}
1	0.63 ± 0.00^{bcAB}	0.65 ± 0.00^{bcdA}	0.63 ± 0.01^{abcAB}	0.62 ± 0.01^{dB}
2	0.63 ± 0.00^{bAB}	0.65 ± 0.00^{bcA}	0.62 ± 0.00^{abcB}	0.64 ± 0.00^{bcdAB}
3	0.66 ± 0.01^{aA}	0.66 ± 0.00^{abA}	0.62 ± 0.00^{abcB}	0.66 ± 0.00^{abcA}
4	0.61 ± 0.00^{cdB}	0.64 ± 0.00^{bcdA}	0.60 ± 0.00^{fC}	0.65 ± 0.00^{abcdA}
5	0.62 ± 0.00^{bcC}	0.64 ± 0.00^{deB}	0.62 ± 0.00^{bcdC}	0.65 ± 0.00^{abcdA}
6	0.62 ± 0.00^{bcC}	0.65 ± 0.00^{bcB}	0.61 ± 0.00^{bce}	0.67 ± 0.00^{ab}
7	0.61 ± 0.00^{cd}	0.65 ± 0.01^{bcd}	0.63 ± 0.01^{abcC}	0.62 ± 0.00^{dA}
8	0.59 ± 0.00^{eB}	0.62 ± 0.00^{efA}	0.59 ± 0.00^{fAB}	0.65 ± 0.01^{abcdB}
9	0.58 ± 0.00^{efB}	0.63 ± 0.00^{efA}	0.57 ± 0.00^{gB}	0.63 ± 0.01^{cdA}
10	0.66 ± 0.00^{aB}	0.67 ± 0.00^{aA}	0.64 ± 0.00^{abA}	0.67 ± 0.00^{aB}
11	0.62 ± 0.00^{bcB}	0.64 ± 0.00^{cdA}	0.64 ± 0.00^{abA}	0.65 ± 0.00^{abcdA}
12	0.56 ± 0.00^{fgC}	0.61 ± 0.00^{fgA}	0.55 ± 0.00^{hD}	0.59 ± 0.00^{eB}

** Means (n=3) ± standard error followed by different letter within a same row (lower) and/or within a same column (upper) are significantly different (Tukey, P < 0.05). GCN: Green coffee with nitrogen environmental conditions, GCN18: Green coffee with nitrogen at 18 °C, GCV: Green coffee vacuum in environmental conditions, GCV18: Green coffee vacuum at 18 °C.

Table 4. Coloration (lightness value, L*) of GC beans stored under modified atmosphere packaging at two conditions storage **

Storage Time (months)	Lightness of the color, L* GCV18	GCV	GCN18	GCN
0	62.89 ± 0.00^{aE}	62.89 ± 0.00^{aD}	62.89 ± 0.00^{aF}	62.89 ± 0.00^{aE}
1	62.8 ± 0.06^{aE}	62.9 ± 0.00^{aD}	62.8 ± 0.06^{aF}	62.9 ± 0.00^{aE}
2	62.8 ± 0.00^{aE}	62.9 ± 0.00^{aD}	62.8 ± 0.06^{aF}	62.8 ± 0.05^{aE}
3	62.8 ± 0.02^{aE}	62.9 ± 0.02^{aD}	62.8 ± 0.06^{aF}	62.9 ± 0.33^{aE}
4	63.0 ± 0.06^{aE}	63.5 ± 0.00^{aD}	62.8 ± 0.06^{aF}	62.9 ± 0.33^{aE}
5	63.0 ± 0.00^{bE}	63.6 ± 0.00^{aD}	62.9 ± 0.09^{bF}	63.4 ± 0.03^{aE}
6	63.1 ± 0.03^{cE}	63.6 ± 0.01^{aD}	62.9 ± 0.03^{dF}	63.3 ± 0.01^{bE}
7	74.2 ± 1.82^{bcD}	81.5 ± 0.01^{aC}	71.0 ± 0.02^{cE}	77.0 ± 0.89^{abD}
8	74.7 ± 2.00^{aD}	81.9 ± 2.39^{aC}	76.4 ± 0.73^{aD}	81.0 ± 0.72^{aC}
9	77.7 ± 1.62^{cCD}	83.0 ± 0.72^{abBC}	79.2 ± 0.76^{bcC}	84.9 ± 0.59^{aB}
10	89.5 ± 0.93^{abA}	88.7 ± 0.04^{abA}	86.9 ± 0.46^{bA}	90.1 ± 0.87^{aA}
11	86.2 ± 0.47^{bAB}	89.1 ± 0.31^{aA}	85.7 ± 0.24^{bA}	89.6 ± 0.36^{aA}
12	81.2 ± 1.06^{cBC}	86.0 ± 0.44^{abAB}	83.0 ± 0.68^{bcB}	88.0 ± 0.91^{aA}

** Means (n=3) ± standard error followed by different letter within a same row (lower) and/or within a same column (upper) are significantly different (Tukey, P < 0.05). GCN: Green coffee with nitrogen environmental conditions, GCN18: Green coffee with nitrogen at 18 °C, GCV: Green coffee vacuum in environmental conditions, GCV18: Green coffee vacuum at 18 °C.

It was found by Broissin *et al*. (2018) that the a_w of GC increased from 0.51 to 0.70 in five months of storage in jute bags and that the a_w at the end of the year was 0.79, which is a higher value than the one found in this study. Therefore, it is observed that in this study, a_w is preserved at values lower than 0.65 in green coffee beans for up to nine months, which is relevant for the coffee industry since a_w is the cause of changes in color, fungal growth, and OTA production.

3.3 Color evaluation in GC samples during modified atmospheres packaging storage

As shown in Table 4, the L* coordinate represents the GC bean's lightness; the initial GC samples

had a value of 62.89. The lightness value (L*) for green coffee beans showed no significant differences during the first six months of storage in modified atmospheres at environmental temperature and at 18 ºC. After twelve months of storage, green coffee stored by vacuum packaging at 18 ºC showed a lower lightness value (81.2) than the other storage conditions studied. Interestingly, no whitish coffee beans were observed in any coffee beans samples during any storage conditions. Statistical analysis (Two-way ANOVA) showed that the storage time and the four storage conditions studied have a significant effect ($p < 0.05$) on the lightness (L*) of the green coffee beans. This effect is consistent with the findings of Ismail *et al*. (2013). They studied the effect of the physicochemical properties on Liberica green coffee during storage in jute bags at temperatures and RH ranging between 28-30.6 ºC and 66.1-75.2 %, respectively. They observed a color change in the beans, where the parameters L*, a*, and b* showed a significant increase from the first month of storage. Significant differences in the L* parameter suggesting that the bean's discoloration could indicate that biochemical changes, as chlorophyll degradation that have occurred in the beans during storage; and, therefore, be a marker of their quality.

On the other hand, the chromaticity (C*) parameter is defined as a change in color intensity and showed a similar trend to the lightness parameters, Table 5. It was observed that the two treatments at two storage conditions (environmental and 18 ºC) remain stable until six months of storage. After six months of storage, a change in the intensity of the color is observed in all storage conditions in room temperature where the treatments GCV (17.97) and GCN (18.60) showed the most significant differences in coloration of the green coffee bean, compared to the initial sample, which had a value of 11.83. Conversely, the treatments stored at 18 °C GCV18 and GCN18 exhibited the slightest significant differences in coloration regarding non-stored green coffee, 13.6 and 14.7, respectively. It was also observed that the values of chromaticity (C*) in green coffee beans stored at controlled temperatures (GCV18 and GCN18) were significantly lower than those found in green coffee stored at environmental temperature (GCV and GCN). It suggests that a lower storage temperature reduces the color change in green coffee storage Previously, it had been observed that GC storage during eight months at 10 °C showed more stable chromaticity values (C*) than coffees stored at 25 °C (Abreu *et al*., 2015). Another study showed that the storage of green coffee beans at 10 °C and RH of 52-67 % do not produce color variations even after 192 days (Abreu *et al*., 2017).

Table 5. Coloration (Chromaticity value, C*) of GC beans stored under modified atmosphere packaging at two conditions storage **

Storage time (months)	Chromaticity value (C*) GCV18	GCV	GCN18	GCN
0	11.83 ± 0.01^{aC}	11.83 ± 0.01^{aD}	11.83 ± 0.01^{aC}	11.83 ± 0.01^{aD}
1	11.83 ± 0.03^{aC}	11.83 ± 0.02^{aD}	11.80 ± 0.01^{aC}	11.80 ± 0.00^{aD}
2	11.83 ± 0.01^{aC}	11.81 ± 0.01^{aD}	11.82 ± 0.00^{aC}	11.83 ± 0.01^{aD}
3	11.81 ± 0.00^{aC}	11.80 ± 0.00^{bD}	11.80 ± 0.01^{abC}	11.81 ± 0.00^{aD}
4	11.81 ± 0.01^{aC}	11.80 ± 0.00^{aD}	11.81 ± 0.01^{aC}	11.81 ± 0.00^{aD}
6	11.81 ± 0.01^{aC}	11.80 ± 0.00^{aD}	11.81 ± 0.01^{aC}	11.81 ± 0.00^{aD}
7	11.45 ± 0.01^{bCD}	11.00 ± 0.00^{dD}	11.66 ± 0.01^{aC}	11.27 ± 0.01^{cD}
8	12.31 ± 0.97^{bBC}	15.59 ± 1.00^{aC}	12.81 ± 0.12^{bB}	15.19 ± 0.59^{aC}
9	13.31 ± 0.73^{bBC}	16.31 ± 0.42^{aC}	13.48 ± 0.66^{bB}	17.34 ± 0.19^{aAB}
10	17.57 ± 0.57^{aA}	16.43 ± 0.13^{abC}	15.11 ± 0.65^{bA}	18.49 ± 1.01^{aA}
11	15.86 ± 0.65^{bA}	19.42 ± 0.23^{aA}	15.33 ± 0.22^{bA}	18.90 ± 0.52^{aA}
12	13.6 ± 1.41^{bB}	17.97 ± 0.18^{aB}	14.70 ± 0.37^{bA}	18.60 ± 0.61^{aA}

**Means (n=3) ± standard error followed by different letter within a same row (lower) and/or within a same column (upper) are significantly different (Tukey, $P < 0.05$). GCN: Green coffee with nitrogen environmental conditions, GCN18: Green coffee with nitrogen at 18 °C, GCV: Green coffee vacuum in environmental conditions, GCV18: Green coffee vacuum at 18 °C.

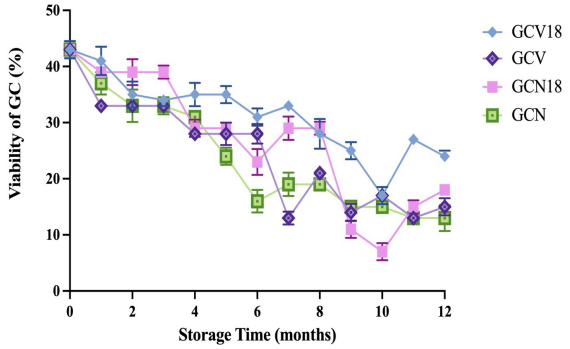

Figure 2. Viability percentage of GC beans stored under modified atmosphere packaging at two conditions storage for 12 months. Values presented as the average ± SD. GCN: Green coffee with nitrogen environmental conditions, GCN18: Green coffee with nitrogen at 18 °C, GCV: Green coffee vacuum in environmental conditions, GCV18: Green coffee vacuum at 18 °C.

Differences observed in the chromaticity value (C*) of coffee beans stored in modified atmospheres (GCV and GCN) represent oxidation reactions and enzymatic transformations in the coffee bean. These chemical and biochemical processes that occur inside the green coffee bean could modify its chemical composition. Some of the compounds that undergo significant modifications are the flavor and aroma precursors, reducing the quality of the beverage produced with these beans (Borém *et al*., 2013). At the end of storage, it was observed that the coffee cuticle called silverskin that surrounds the coffee bean underwent an oxidation process, which caused it to bean acquired a reddish coloration, increasing the values the coordinate a* (values not shown) resulting in increased chromaticity. The samples of green coffee stored at 18 °C (GCV18 and GCN18) did not present a silverskin with reddish coloration.

3.4 Viability and respiration index of the GC samples during modified atmospheres packaging storage

The changes in the coffee samples' viability under the different storage treatments were shown in Figure 2. The initial sample showed 43 % viability. The viability of green coffee beans stored in modified atmospheres at different temperatures was significantly decreased in all storage conditions throughout the storage time. This loss of viability in coffee beans was higher during storage in modified atmospheres at environmental temperature (GCN and GVN treatments), with approximate viability of 14 % for both cases at the end of storage (12 months) compared to the viability found in the treatments with controlled temperature, 18 % (GCN18) and 24 % (GCV18).

Loss of viability may be related to coffee beans respiratory activity (Selmar *et al*., 2014); therefore, the respiratory rate value was obtained. As a result, it was shown that there were no significant differences p (<0.05) in the variations to the GC respiratory rate during the 12 months of storage, where the initial sample had a concentration of 4.18 mg CO_2/100 g for 41 days and the respiration rate at 12 months was 4.236 mg of CO_2/100 g for 41 days for all treatments. This coffee respiration index is lower than the fresh food index. Duffus and Slaughter (1985) mentioned the relation between the capacity of O_2 absorption by the bean and its germination ability (measured as CO_2 produced/volume of O_2 absorbed ratio). High values of this ratio are indicative of bean deterioration. The impact of respiration on bean deterioration can be highlighted by the fact that every 24 h, an average of 4.4 mg of CO_2 is produced per 100 g of coffee beans, and 96 Cal of heat produced by 44 mg of CO_2 will raise the temperature of the bean by 0.25 °C (Rojas, 2004).

Coffee beans damaged during processing can affect the beans viability as high metabolic or respiratory activity significantly affects the quality of the bean (Deepak *et al*., 2012). Selmar *et al*. (2008) analyzed the decrease in viability of green coffee and the relationship of viability loss with a change in the chemical composition of coffee beans during storage. They found coffee viability of 50 % during the first three months of storage, and after six months of storage, the viable fraction only was 10 %; finally, there was no viability after one year of storage. Viability is maintained for a longer time if the green coffee is stored at temperatures of 5-15 °C and relative humidity of 35-55 % (Rojas, 2004). The germinative capacity of the coffee beans can also be preserved if the beans are stored as parchment or cherries with a moisture content between 15 to 18 % (Selmar *et al*., 2008). However, these conditions are only used when the beans are used as propagation seeds. Therefore, it is concluded that the GV analyzed in this study cannot be used as a seed.

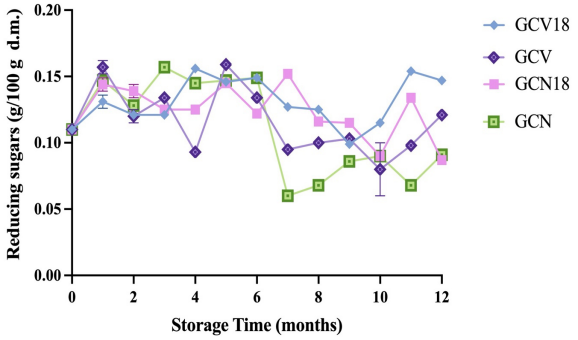

Figure 3. Concentration of reducing sugars of GC beans extracts stored under modified atmosphere packaging at two conditions storage for 12 months. Values presented as the average ± SD. GCN: Green coffee with nitrogen environmental conditions, GCN18: Green coffee with nitrogen at 18 °C, GCV: Green coffee vacuum in environmental conditions, GCV18: Green coffee vacuum at 18 °C.

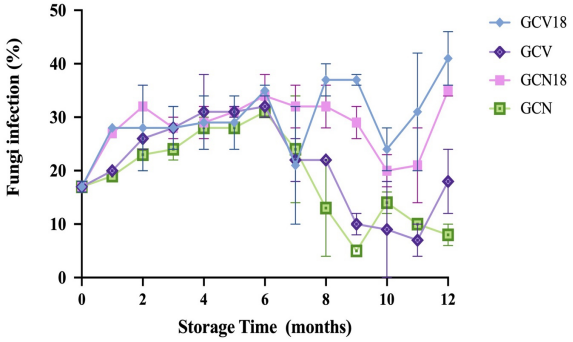

Figure 4. Percentage of fungal infection of GC beans stored under modified atmosphere packaging at two conditions storage for 12 months. Values presented as the average ± SD. GCN: Green coffee with nitrogen environmental conditions, GCN18: Green coffee with nitrogen at 18 °C, GCV: Green coffee vacuum in environmental conditions, GCV18: Green coffee vacuum at 18 °C.

3.5 Reducing sugars content in the GC extracts during modified atmospheres packaging storage

During storage, significant differences were observed in the concentration of reducing sugars in the green coffee extracts of all treatments, Figure 3. The initial concentration of reducing sugars in the GC extracts was 0.110 ± 0.04 g / 100 g d.m. However, it was found that after 12 months of storage, none of the extracts analyzed exceeded the concentration of 0.15 g / 100 g d.m. Thus, the concentration of reducing sugars in the GC extracts found in this work was like reported by Selmar *et al*. 2008. The increased concentration of glucose (reducing sugars) is an important marker of GC quality since glucose participates in Maillard reactions, forming unpleasant compounds known as off-flavors. Knopp *et al*. (2006) showed that the mode of processing influences concentration glucose and fructose concentrations, the concentration of these two sugars is significantly higher than in the beans processed for the dry method than by the wet method.

On the other hand, sucrose is the most abundant disaccharide in green coffee and is susceptible to hydrolyzing during storage to produce glucose and fructose (reducing sugar) (Sedana and Astawa 2016; Selmar *et al*., 2014). Therefore, when green coffee is stored in jute bags and RH equal to or above 85 %, it is also highly probable that the sucrose present in the beans is hydrolyzed, increasing the concentration of reducing sugars (Bucheli *et al*., 1998). Broissin-Vargas *et al*. (2018) observed a change in the content of reducing sugars during storage in wet-processed GC jute bags. They found that initially, the concentration of reducing sugars increased when a_w reached a value of 0.75, causing sucrose hydrolysis. Subsequently, after six months of storage, a decrease in reducing sugar content was observed. The authors simultaneously noted an increase in the fungal population. Therefore, they established that reducing sugar content could be explained by the fungi's ability to use sugars as carbon to maintain their viability. This phenomenon has been observed in other crops was attributed to the utilization of disaccharides and polysaccharides by fungi strains (Rao *et al*., 2014; Ahmed *et al*., 2016).

3.6 Fungal infection and quantification of Ochratoxin A in GC samples during modified atmospheres packaging storage

The initial percentage of fungal infection in green coffee was 17 % (Figure 4). During the green coffee storage, all storage conditions showed fluctuations in fungal infection levels. The green coffee samples stored at 18 °C for one year (GCV18 and GCN18) showed the highest percentages of fungal infection with 41 % and 35 %, respectively. In the case of green coffee samples stored under environmental conditions after one year, a decrease in fungal infection for GCV and GCN (18 and 8 %, respectively) was observed.

During storage, the predominant fungal strains

found in GC were isolated and identified using taxonomic (Pitt and Hocking, 2009). However, only two strains remained viable throughout the storage, *Aspergillus carbonarius* and *Aspergillus niger*. Various studies have established that filamentous fungi can develop in coffee under certain conditions and cause a general loss of its quality; due they produce defects in taste and, in some cases producing mycotoxins such as Ochratoxin A, which can turn coffee into a product not suitable for human consumption (Abreu *et al*., 2017; Gil-Serna *et al*., 2014; Iamanaka *et al*., 2014b; Leduc *et al*., 2012; Maman *et al*., 2021; Ribeiro *et al*., 2011; Suárez-Quiroz *et al*., 2004). The infection percentages have been associated with OTA's presence; however, the OTA concentration remained unchanged throughout the storage period. The results showed that there is no significant difference ($p < 0.05$); the initial concentration was 0.942 μg kg^{-1} and after 12 months of storage 1.11 μg kg^{-1} for treatment GCV18 that showed the highest percentages of fungal infection (41 %). The above results demonstrate that green coffee storage conditions in modified atmospheres did not allow toxin production by the identified species, even though both are recognized as OTA producers.

A study by Akbar *et al*. (2016) showed that water activity, temperature, and CO_2 play an essential role in ochratoxin A production in *Aspergillus* from *Circumdati* and *Nigri* sections, where these species showed higher growth at 30 °C, and lower growth at 35 °C. Most of the fungal strains exhibited optimal growth at 30 °C and a water activity of 0.98.

Broissin-Vargas *et al*. (2018) determined the development of fungal infection on green coffee stored in jute bags, found after six months of storage, the fungal infection was 50 %, and at 12 months, it was 100 %. Thus, green coffee's most significant fungal contamination is present in two stages in its production during drying and storage. However, this study's percentage of fungal infection is lower than found in the green coffee storage with jute bags. It has been shown that fungi overgrow at oxygen concentrations below 2 % and develop in the presence of high concentrations of carbon dioxide. Therefore, when fungi are under conditions of lack of nutrients or at conditions that exceed the growth limits, they produce stabilized cells to survive (Wyatt *et al*., 2013). This stabilization is characterized by a decrease in metabolic activity, the absence of cell multiplication, and more resistance to stress conditions, in which the protection of cellular constituents and biomolecules play a determining role. Thus, fungi's most notable survival strategy to overcome adverse periods is forming spores that disperse in the environment. These spores can germinate from a period of stopped development or inactivity (Dijksterhuis, 2017), which could explain that even under storage conditions in a modified atmosphere, there was fungal growth due to the germination spores where a temperature of 18 °C was better for this.

The GC bean stored in jute sack is susceptible to fungal contamination by *Aspergillus ochraceus*, *Aspergillus westerdijikiae*, *Aspergillus niger*, and *Aspergillus carbonarius* (Waters *et al*., 2017). In this work, only two strains remain viable during the whole storage, *Aspergillus carbonarius*, and *Aspergillus niger*. Some fungal genera such as *Aspergillus* and *Penicillium* have a great capacity to form spores to the point that they can be found everywhere (Mullins *et al*., 2011), which allows them to be distributed in many foods. The high spore-producing capacity of these fungal genera allows them to compete with and often dominate other fungal communities in various environments (Medina *et al*., 2015). Also, these fungal genera could produce a "battery" of extracellular hydrolytic enzymes and the production of secondary metabolites and volatile compounds, making it possible for these strains to remain even under modified atmospheric conditions such as during the storage of green coffee in modified atmospheres.

Coffee, being one of the most consumed beverages globally, the presence of ochratoxin A (OTA) in coffee has raised significant concerns in the international coffee trade market (Paterson *et al*., 2014; Poltronieri *et al*., 2016). Three mold species have been found concerning coffee: *A. ochraceus*, *A. carbonarius*, and *A. niger* (Waters *et al*., 2017). Other species like *A. westerdijkiae* and *A. steyn* are recognized as an OTA producers by DNA sequence identification (Velmourougane *et al*., 2011), but *A. ochraceus* has been recognized as the primary source of OTA in green coffee (Waters *et al*., 2017). Ochratoxin A production can occur during post-harvest processing, storage, roasting, and commercialization of coffee (Aguilar-Alvarez *et al*., 2021, Maman *et al*., 2021). For this reason, the OTA concentration during storage was determined in this study. The results showed no significant difference in the concentration of OTA in green coffee stored in modified atmospheres at two conditions (environmental and 18 ºC) for 12 months, whose initial concentration was 0.942 μg/kg, and the final concentration after 12 months of storage was 1.11 μg/kg. OTA in coffee beans can be due to environmental conditions and process conditions

(wet, mechanical, or dry processes) (Vieira *et al*., 2015). Several authors (Pitt *et al*., 2013; Barcelo and Barcelo, 2018) studied the formation of OTA in the dry process, demonstrating that a higher concentration of OTA is generated during this processing compared to the wet processing, due of the antagonism of other microorganisms such as lactic acid bacteria and yeasts during fermentation. OTA occurrence was observed before storage, indicating the possibility that harvesting and post-harvest handling of coffee cherries are the critical steps leading to contamination. Accordingly, Suarez-Quiroz *et al*. (2004) studied the evolution of mycobiota during coffee processing, where it was observed that there was a high level of mold infection in all processes after drying (parchment and dried cherries). It was found that 80 % of the wet process beans, 72 % of the mechanical process, and 92 % of the dry process were contaminated with fungi, *Penicillium*, *Mucor*, *Cladosporium*, and *Aspergillus* spp., including well-known potential OTA producing fungi (*A. ochraceus* and *A. niger*). Broissin-Vargas *et al*. (2018) determined the concentration of OTA in green coffee stored in jute bags for a year, where it was observed at six months of storage there was OTA production (1.10 ± 01.1 μg/kg). Finally, at twelve months, an OTA concentration (5.09 ± 0.2 μg/ kg) observed that the high temperature and relative humidity in the warehouse and chemical changes in GC favored the population dynamics of the fungal community. In another study, Akbar *et al*. 2020 reported that *A. westerdijkiae* strain was able to produce Ochratoxin A during coffee storage; when the storage temperature was increased to 35° C, the concentration of CO_2 was higher than 1000 ppm, and drought stress was imposed ($a_w < 0.90$).

It is essential to mention that the OTA concentrations found in this study GC stored in modified atmosphere packaging are not considered a hazard in the OTA standard risk management: Guidelines for the purchase of green coffee (International Coffee Organization, 2005). Therefore, these OTA concentrations do not represent any risk to the sanitary quality of the beans.

Conclusions

The results obtained in this work showed that the storage conditions analyzed are a viable alternative for the conserving of green coffee beans for one year, avoiding the loss of quality markers associated with the storage of green coffee beans in jute bags. The use of modified atmospheres (vacuum and nitrogen) and two storage conditions (environmental and 18 ºC) allowed the conservation of green coffee beans' main physicochemical and biological parameters during one year of storage. Storage of GC in modified atmospheres preserved water activity, which is an important quality indicator of this product, as it is closely related to color change, loss of reducing sugars, fungal growth, and OTA production. However, more studies are still needed on the effect of modified atmosphere packaging during storage on the aromatic and sensory parameters of green and roasted coffee, which affect the quality of the beverage produced with these beans.

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