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"El saber de mis hijos hará mi grandeza"

Physicochemical, aromatic, sensory properties and antioxidant activity of roasted coffee (*Coffea arabica* L.) treated with cold plasma technology

Propiedades fisicoquímicas, aromáticas, sensoriales y actividad antioxidante del café tostado (*Coffea arabica* L.) tratado con tecnología de plasma frío

Paloma Patricia Casas-Junco[1], Juan Arturo Ragazzo-Sánchez[1], Josué Raymundo Solís-Pacheco[2], Blanca Rosa Aguilar-Uscanga[2], Sonia Guadalupe Sáyago-Ayerdi[1], Montserrat Calderón-Santoyo[1]*

[1] Laboratorio Integral de Investigación en Alimentos. Tecnológico Nacional de México/Instituto Tecnológico de Tepic. Av. Tecnológico #2595, Col. Lagos del Country, C.P. 63175. Tepic, Nayarit, México.

[2] Laboratorio de Microbiología Industrial. Centro Universitario de Ciencias Exactas e Ingeniería. Universidad de Guadalajara, Jalisco, México. Boulevard Marcelino García Barragán #1421, Col. Olímpica, C.P. 44430. Guadalajara, Jalisco, México.

ABSTRACT

The cold plasma technology has gained popularity in the food industry, since it causes minimal alterations in nutritional content, does not leave chemical residues, and in some cases, does not affect the sensory quality of food. The aim of this research was to evaluate the physicochemical, aromatic, and sensory properties, as well as antioxidant activity of roasted coffee treated with cold plasma technology. Samples were treated with Dielectric Barrier Discharge (DBD) to produce plasma at an input power of 30 W and an output voltage of 850 V, using helium gas (1.5 L min^{-1}). The cold plasma was applied at different times (0, 1, 4, 8, 12, 16, 20, 24 and 30 min). Cold plasma treatment showed no significant difference in the color parameter. Moreover, we detected no differences in the aromatic and sensory profile submitted to plasma treatment for 30 min compared with the untreated samples. On the other hand, a 12 % reduction of the total content of soluble polyphenols and an increase of 14 % in the antioxidant capacity were observed in samples treated with cold plasma. This is a novel technology with the potential of ensuring the safety and maintain the sensory characteristics of roasted coffee.

Keywords: Dielectric Barrier Discharge (DBD) plasma; reactive species; antioxidant capacity; minimally processed food.

RESUMEN

La tecnología de plasma frío ha cobrado auge en la industria alimentaria, ya que provoca mínimas alteraciones en el contenido nutricional, no deja residuos químicos y en algunos casos no afecta la calidad sensorial de los alimentos. El objetivo de esta investigación fue evaluar las propiedades fisicoquímicas, análisis sensorial, aromática y actividad antioxidante del café tostado tratado con plasma frío. Las muestras fueron tratadas con descarga de barrera dieléctrica (DBD) con potencia de entrada de 30 W y voltaje de salida de 850 V, usando gas helio (1.5 L min^{-1}). El plasma frío fue aplicado en diferentes tiempos (0, 1, 4, 8, 12, 16, 20, 24 y 30 min). El tratamiento con plasma frío no mostró diferencias significativas en el parámetro de color. Además, no se observaron diferencias en el perfil aromático y sensorial sometido a tratamiento con plasma durante 30 min en comparación con las muestras no tratadas. Por otro lado, se observó una reducción del 12 % del contenido total de polifenoles solubles y un aumento del 14 % en la capacidad antioxidante en muestras tratadas con plasma frío. Esta es una tecnología novedosa con potencial para asegurar la inocuidad y mantener las características sensoriales del café tostado.

Palabra claves: Plasma de barrera dieléctrica (DBD); especies reactivas; capacidad antioxidante, alimento mínimamente procesado.

INTRODUCTION

Coffea arabica L. is one of the most popular coffee varieties with 70 % of the global market (Caballero, 2003). Mexico ranked 11th among the largest coffee producers worldwide, with 2.4 % of global production (SIAP, 2018). Unfortunately, the coffee quality is affected by environmental, genetic, pre-harvest, and postharvest operations (Haile and Kang, 2019). Casas *et al*. (2017) mention that the fungi *A. niger, A. versicolor*, and *Byssochlamys spectabilis* naturally produce Ochratoxin A (OTA) in roasted coffee from Nayarit, Mexico. This toxin represents a health risk for humans and animals since OTA has been reported as carcinogenic (Abdullah Al-Abdalall and Abdullah Al-Talib, 2014).

A promising physical approach for shelf life is the application of non-thermal plasma. Dielectric barrier discharge (DBD) is a plasma generation class characterized by the mode of operation and system configuration which generate ionization of the process gas molecules and the formation of reactive chemical species, such as ions and radicals, heat (gas temperature less than 500 K), and UV light (Keener, 2008).

Various authors have assessed quality attributes like taste, flavor, color, texture, enzymatic activity (peroxide and pectin methylesterase) of fruit and vegetal treated with this technology, showing its potential as an innovative treatment for enzymes inactivation and quality preservation in food products (Lee *et al*., 2018; Tappi *et al.,* 2016).

Thus, the aim of this work was to evaluate the effect of cold plasma on the phenolic compounds content and antioxidant capacity (AOX), as well as physicochemical, aromatic, and sensorial parameters of roasted coffee (*C. arabica* L.).

*Autor para correspondencia: Montserrat Calderón Santoyo
Correo electrónico: mcalderon@tepic.tecnm.mx
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MATERIALS AND METHODS

Raw materials

Roasted coffee (*C. arabica* L.) grains produced in Nayarit, Mexico, were obtained from local markets for this study. The coffee had a roasted appearance, an attractive aroma, and a desirable visible coffee quality. Coffee samples were kept in storage in zip-lock bags while awaiting analysis to avoid the loss of volatile compounds.

Plasma generator equipment

The plasma generating equipment's design and construction correspond to the Plasma Physics Laboratory of the National Institute for Nuclear Research in Toluca, Mexico. The system was made up of the 13.56 MHz radio frequency generator (RF) designed to directly supply a pair of stainless-steel plates in a glass DBD reactor (Peña-Eguiluz *et al*., 2010).

Roasted coffee powder treated with cold plasma

Roasted coffee (*C. arabica* L.) grains were pulverized, and 0.5 g of sample was placed on plate Petri on thin layers. They had approximately 1 mm of thickness and were immediately treated with cold plasma energy at atmospheric pressure. Plasma was generated with commercial helium gas (Praxair, Mexico) at a flow of 1.5 L/min, and the energy was applied at different times (0, 1, 4, 8, 12, 16, 20, 24, and 30 min) and 30 W input power and an output voltage of 850 V. Before and after each plasma treatment, we determined antioxidant activity and total polyphenol content. In the case of physicochemical and sensory properties, treatment times were 0 and 30 min.

Physicochemical quality parameters in roasted coffee

Surface color

The color assessment was evaluated with a Hunter Lab MINOLTA Chroma Meter CR300 colorimeter (Hunter Laboratory, México) in the reflectance mode for roasted coffee. The color was expressed as L (brightness), a (redness), and b (yellowness) values. Results were expressed as the average of four measurements. Besides, the total color difference (ΔE) was calculated as below (Kovačević *et al*., 2016) (Eq. 1):

$$\Delta E = \sqrt{(L_0 - L)^2 + (a_0 - a)^2 + (b_0 - b)^2} \quad (1)$$

Where L_0, a_0, and b_0 were the untreated control values, and L*, a*, and b* were the values for treated roasted coffee.

Aroma compounds evaluation

Samples subjected to a 30 min cold plasma treatment were analyzed with a gas chromatograph (GC-7890) coupled to mass spectrometry (NIST) system with ion trap mass spectrometer from Agilent Technologies Instruments operated in the split mode. A CP- Wax (60 m X 0.25 mm i.d.) column with a 0.25 μm film thickness was employed for chromatographic separation of aromatic compounds. Ion trap mass spectrometer was operated in the electron ionization (EI) mode. The NIST library was used to detect possible intermediates, and also our mass library was created with this program. The free volatile compounds were identified by GC/MS by means of retention time and mass spectrum. Helium at 1.6 mL/min was used as the carrier gas. Divinylbenzene / carboxen / polydimethylsiloxane (DVB/CAR/PDMS) fiber with coating thicknesses of 50 and 30 μm was used (Akiyama *et al*., 2008).

The oven temperature program was as follows 50 °C for 2 min (rate 2 °C min^{-1}) to 220 °C for 85 min. Hydrogen was used as the carrier gas at 100 kPa; the injector temperature and the splitless flow were set at 260 °C and 30 mL min^{-1}, respectively, after a splitless time of 1 min. Solid-phase microextraction (SPME) was carried out according to Solis-Solis *et al*. (2007), with some modifications. Samples of 0.5 g of non-treated and treated roasted coffee were mixed with 1 g NaCl and 1.6 mL deionized water and subsequently sealed with PTFE-silicone septa. Sample vial equilibration was incubated at 40 °C for 30 min; the DVB/CAR/PDMS fiber was then exposed to the headspace above the sample for 30 min followed by 10 min of thermal desorption of the adsorbed substances in the injector port.

Polyphenols extraction

0.5 g of roasted coffee were mixed with 20 mL of acidified methanol solution (0.8 M HCl, 50:50, v/v) and extracted by shaking for 1 h in a wrist-action (Burrel, USA) at maximum speed (room temperature). The extracts were then centrifuged (Hermle, Z32HK, Labortechnik GmbH, Wehingen, Germany) at 6000 x *g* for 15 min at 4 °C. The supernatants were recovered, and the residues were re-extracted using 20 mL of an acetone solution (70:30, v/v, 60 min), supernatants were collected in a flask with the combination of acidified methanol solution and acetone solution (50:50, v/v). The supernatants were considered as total soluble polyphenols (TSP) and were used to evaluate the AOX. Samples were stored at 4 °C in the dark (Pérez-Jiménez *et al*., 2008).

Total Soluble Polyphenols (TSP) content

The soluble polyphenols content was determined by the method of Alvarez-Parilla *et al*. (2010) with some modifications. An 250 μL aliquot of extracts from roasted coffee were mixed with 1000 μL Na_2CO_3 (sodium carbonate, 7.5 w/v). After 3 min, 1250 μL of Folin–Ciocalteu's reagent were added, and the mixture heated in a water bath for 15 min at 50ºC, which resulted in a final volume of extract sample of 270 μL. A microplate reader (Bio-Tek®, Synergy HT, Winooski, VT, USA) in a multi-mode spectrophotometric detection with 96-well plates and the Gen5 Program was used. Gallic acid was used as a standard (0.0125 – 0.2 mg/mL $R^2 \geq 0.9997$), and results were expressed as mg of gallic acid equivalents (mg GAE/g DW), the absorbance was read at 750 nm against a blank.

AOX activity determination

This assay was based on the ability of different substances to scavenge 2,2´-Azinobis-3-ethyl benzothiazo-

line-6-sulfonic acid (ABTS) radical cation. The method proposed by Re *et al*. (1999) with some modifications was used. The radical cation was prepared by mixing a 7 mM stock solution with 2.42 mM potassium persulfate and kept in the dark at room temperature for 14 h. The ABTS solution was adjusted with phosphate buffer at an absorbance of 0.70 (±0.02). Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) was used as a standard and methanol as a blank. Extract samples of 20 μL were read on a microplate reader (Biotek, Synergy HT, Winooski, VT, USA) with 300 μL capacity, and 280 μL of the ABTS radical was added. Then, it was incubated at 37 °C in the dark and read for 6 min at 734 nm; a calibration curve was prepared using an aqueous solution of Trolox as a standard. We reported the results as mmol TE per 100 g sample DW.

Sensory evaluation

The roasted coffee (untreated control and 30 min cold plasma treated) was prepared by adding the coffee powder (1:4 w/v) and sugar 5 % (w/v) in boiling water. Prepared coffee was evaluated for sensory attributes by 40 untrained judges. The experiment was divided into two phases: (1) triangular test was performed to compare between three samples simultaneously, two untreated samples and one treated with 30 min cold plasma, without having to specify the sensory characteristic(s) that differ; (2) hedonic test was based on the evaluation of color, odor, taste and overall acceptability parameters. A 9-point hedonic scale (1: dislike extremely to 9: like extremely) was used to analyze the sensory score corresponding to each tasted sample (Kumar *et al*., 2012).

The sensory results were analyzed by a two-factor analysis (panelist and product) with an ANOVA test and differences were established by the LSD test.

Statistical Analysis

A unifactorial design where the independent factor was the treatment time, was employed. Statistical analysis was performed using SAS for Windows V9. Analysis of variance (ANOVA) and multiple comparison procedures (Least Significant Difference–LSD) test were conducted to determine whether there were significant differences ($p<0.05$) among treatments for physicochemical properties, sensory analysis, and antioxidant compounds.

RESULTS AND DISCUSSION

Color evaluation in cold plasma treated roasted coffee

Hunter color parameters for roasted coffee, both treated with cold plasma and untreated, are shown in Table 1. No differences in the surface measured parameters were observed ($p > 0.05$), and the value of ΔE of 0.78 was obtained. In conditions of ideal vision, values of $\Delta E \leq 1$ represent a minimum difference of color, hardly perceptible for the human eye (Vervoort *et al*., 2012).

The impact of cold plasma on color depends on critical factors associated with the product such as type (cut, solid, liquid), plasma treatment conditions (input voltage, time, power, working gas), and storage conditions (Pankaj *et al*., 2018).

Tabla 1. Valores de color superficial en café tostado sin tratar y muestras tratadas con plasma frío.
Table 1. Surface colour values of untreated roasted coffee and cold plasma treated samples.

Treatments	L*	a*	b*
Rosted coffee untreated (0 min)	22.85 ± 0.02[a]	11.36 ± 0.03[a]	17.16 ±0.05[a]
Roasted coffee with cold plasma treatment (30 min)	22.89 ±0.05[a]	10.90 ± 0.03[a]	17.02 ± 0.01[a]

a=Similar letters uppercase indicates no significant difference ($p < 0.05$) between treatments.

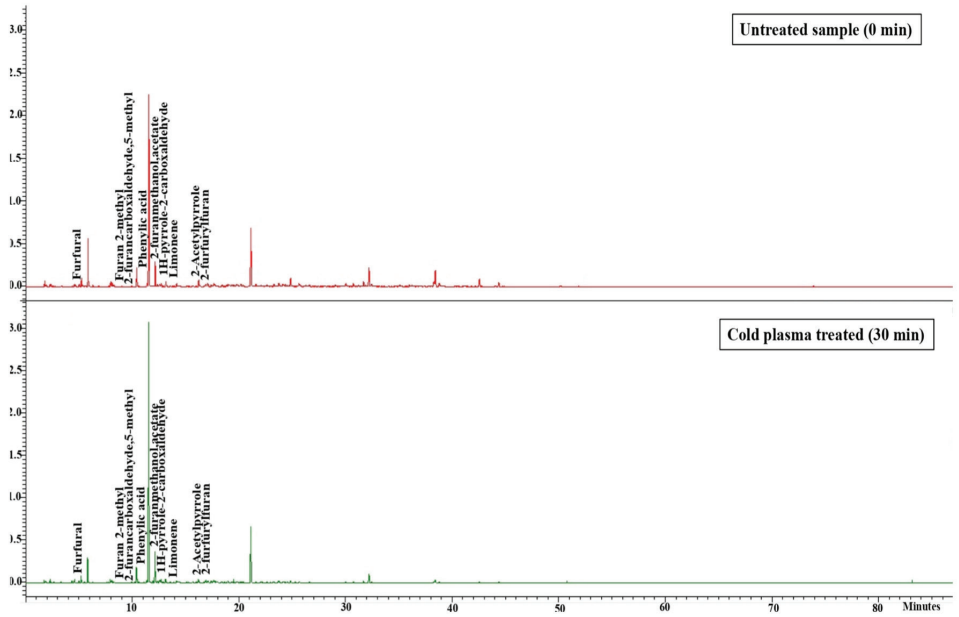
Aroma compounds determination

The aroma compounds detected in the untreated roasted coffee and cold plasma treated samples were not statically significant ($p > 0.05$). A total of 9 volatile compounds were identified in both samples (Figure 1).

This suggests that cold plasma treatment did not significantly affect the covalent bonds responsible for aroma, compounds' integrity and flavor characteristics (Bressanello *et al*., 2017). However, the chemistry of cold plasma collision reactions generates N_xO_y, O_3 (ozone), peroxy-radicals in a discharge produced O atom and attacks molecular O_2 by a three-body reaction to yield ozone induced oxidation in foods (Gavahian *et al*., 2018). Also, oxygen species (ROS) can be formed in nature under UV-light influence generating photo-oxidation of lipids (Vandamme *et al*., 2015). Some studies have demonstrated that, especially fatty foods, can interact with ROS (OH and $^1O_2$) and be linked by double bonds (Van Durme and Vandamme, 2016). Therefore, the primary oxidation products (non-volatile components) and secondary oxidation products (volatile components) contribute to off-flavors, for example, vinyl ketones, *trans*, *cis*-alkadienals, alkanals, hydrocarbons present oils and fats (Kerrihard *et al*., 2015).

Vandamme *et al*. (2015), performed a qualitative and semi-quantitative identification of volatile oxidation compounds from by-products in vegetable oil samples treated with DBD plasma jet (Ar / 0.6 % O_2), generating a wide range of highly reactive oxidative species as atomic oxygen, hydroxyl radicals and singlet oxygen, while maintaining ambient temperatures. Alves *et al.* (2017), showed processed by ozone and plasma (oxygen gas) for a long period (1 to 3 min), and obtained oxidation of limonene, y-terpinene and linalool, causing off-flavor and volatile composition on orange juice.

The main parameters involved in by-products formation are food matrix, voltage, treatment time, oxygen concentration, plasma configuration, and also water and carrier gas concentration. From those parameters, high lipid content in food matrices is critical for off-flavor formation during cold plasma treatment. The coffee beans have a low lipid content, and thus, no formation of off-flavor molecules was observed.



Compounds	Retention time
Furfural	5.24
Furan 2-methyl	9.97
2-furancarboxaldehyde, 5 – methyl	10.48
Phenylic acid	11.39
2-furanmethanol, acetate	12.15
1H-pyrrole-2-carboxaldehyde	12.71
Limonene	14.18
2-Acetylpyrrole	16.24
2-furfurylfuran	17.41

Figura 1. Cromatogramas GC-MS de compuestos aromáticos detectados en café tostado sin tratar y tratado con plasma frío durante 30 min.
Figure 1. GC-MS chromatograms of aromatic compounds detected in untreated roasted coffee and cold plasma samples treated for 30 min.

Total Soluble Phenols (TSP) content and AOX in roasted coffee treated with cold plasma

TSP content in roasted coffee showed significant ($p < 0.05$) variations during the cold plasma treatment, decreasing from 67.74 ± 2.31 GAE/g to 59.12 ± 3.92 GAE/g after 30 min of treatment (Table 2). A decrease of 12 % in total phenolic concentration (TPS) after cold plasma treatment, compared with control.

The plasma technology generates reactive oxygen species (ROS) such as hydroxyl radicals, atomic oxygen, and singlet oxygen and ozone (Brandenburg *et al*., 2007). The TSP can capture radicals and are susceptible to ozone attack (Stalter et al., 2011), explaining the reduction in TSP through the time of plasma application. According to these authors, a phenolic compounds reduction was observed, probably due to oxidation caused by active particles and radicals, depending on the plasma exposure time (Sarangapani *et al*., 2017). Moreover, molecular ozone acts on the aromatic compounds favoring the formation of hydroxylated and quinone compounds, because the formation of aliphatic compounds is originated from the rupture of the aromatic ring (Perez *et al*., 2002).

Almeida *et al*. (2015), showed that the total phenolic content was affected ($p<0.05$) only after 60 s under indirect plasma exposure on orange juice. Solís *et al*. (2013), observed a reduction of 38 % in total polyphenol content in chamomile samples with 10 min of plasma energy at 750 volts, and of 33 % in cinnamon samples treated at 650 volts.

Regarding the AOX in roasted coffee, this increased 14 % after 30 min of treatment with cold plasma ($p < 0.05$) (Table 2). The influence of plasma species and phenolic derivatives

Tabla 2. Fenoles solubles totales (FST) y capacidad antioxidante (ABTS) en café tostado tratado con plasma frío (30 W potencia de entrada y voltaje de salida de 850 V)
Table 2. Total soluble polyphenols (TSP) and antioxidant capacity (ABTS) in roasted coffee treated with cold plasma (30 W input power and at output voltage of 850 V).

Treatment time (min)	TSP (mg GAE/g DW)	ABTS value (mmol TE/g DW)
Control	67.74±2.31^{b}	528.96±8.65^{f}
1	65.06±0.31^{a,b}	438.94±19.82^{a,b}
4	63.27±4.52^{a,b}	418.27±1.24^{b}
8	58.12±5.76^{a}	306.36±17.35^{d}
12	59.42±5.24^{a}	361.92±21.87^{e}
16	60.06±0.47^{a,b}	480.65±13.94^{a}
20	61.36±0.17^{a,b}	593.59±29.51^{c}
24	60.78±1.02^{a,b}	464.55±13.95^{a}
30	59.12±3.92^{a}	604.06±18.83^{c}

a=Similar letters uppercase indicates no significant difference ($p < 0.05$) between treatments.

(hydroxyl radicals, peroxyl radicals), atomic or singlet oxygen caused reaction, both direct and indirectly (Brandenburg *et al*., 2007). Therefore, depending on the chemical structure of each of the polyphenols, the antioxidant properties might vary. These chemical conditions influence the ability of polyphenols to scavenge free radicals, changing their biological activity (Vajragupta *et al*., 2000). The UV-B radiation during cold plasma, plays a crucial role in the individual compounds of the polar fraction due to penetration or favored biosynthesis (Ramazzina *et al*., 2015; Grzegorzewski *et al*., 2010), which facilitate the increment of antioxidants (others than polyphenols) in roasted coffee.

Solís *et al*. (2013) reported an increase of antioxidant activity in cinnamon samples at 750 V (21.4 %), 850 V (12.2 %), and 650 V (14.4 %), during 10 min of treatment. Also, Trouillas *et al*. (2006) observed an increase in the antioxidant properties of plasma-treated basmati rice.

The variable effect of cold plasma on functional food components may be due to differences in the food matrices, plasma equipment configuration, and processing parameters, particularly the gas used. Accordingly, further work is needed to clarify the reaction chemistry between plasma RS and antioxidants in food products. For example the elucidate the molecule fragments resulted from the erosion and UV radiations produced during cold plasma treatments to gain further insight into the relationship between antioxidant activity and polyphenols content in treated coffee samples.

Sensory evaluation

Sensory quality to evaluate triangle test was determined after taking into consideration sensory scores given by untrained judges. The results showed no significant difference between treated and untreated samples ($p < 0.05$). Thus, cold plasma treated samples were found to be acceptable in terms of sensory attributes.

Figure 2 shows the results of a hedonic test of sample C201 (treatment cold plasma) and sample C685 (untreated). The judges evaluated "like moderately" for both samples.

Study by Basaran *et al*. (2008) report no significant difference on the sensory analysis, of nut samples treated with cold plasma LPCP using air gases or sulfur hexafluoride (SF_6) (appearance, color, odor, texture).

Another study of cold plasma showed that high lipid such as alkanes, alkenes, aldehydes, alcohols, ketones, and acids produce unpleasant flavors, which are sensory described as fishy, metallic, rancid, and oxidized (Kim *et al*., 2013; Jayasena *et al*., 2015). The factor of relative humidity on fresh fruits and vegetables generates "sour" or "chemical" odor associated with reactive nitrogen species (nitrogen monoxide, nitrogen dioxide and oxygen) and peroxynitrite (Schnabel *et al*., 2015; Von-Woedtke *et al*., 2012). Furthermore, it should be mentioned that coffee bean has a low content of a_w (10 %) and lipids (16 %) (Alvarado and Puerta, 2002). Hence, the probability of generating new compounds with sensory impact was reduced, and no differences in sensory attributes were observed.

CONCLUSION

DBD Plasma treatment, a novel technology in value-added coffee processing, results from the research. Good preservation of color, sensory and aromatic properties were obtained after a 30 min treatment at 30 W input power and 850 volts with Helium gas 1.5 L/min. An increase of 14 % in AOX and a decrease of 12 % with respect to TSP during plasma exposure for 30 min were observed. Therefore, it is important to continue the research, especially in the interaction of individual reactive species and /or unravel oxidation reaction pathways in food to improve the quality attributes.

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AUTHOR CONTRIBUTIONS

Paloma P. Casas-Junco, acquisition of data and writing of the manuscript; Josué R. Solís-Pacheco, supervision of cold plasma treatments; Juan A. Ragazzo-Sánchez, aroma compounds analysis, and article critical revising; Blanca R. Aguilar-Uscanga, supervision of physicochemical and senso-

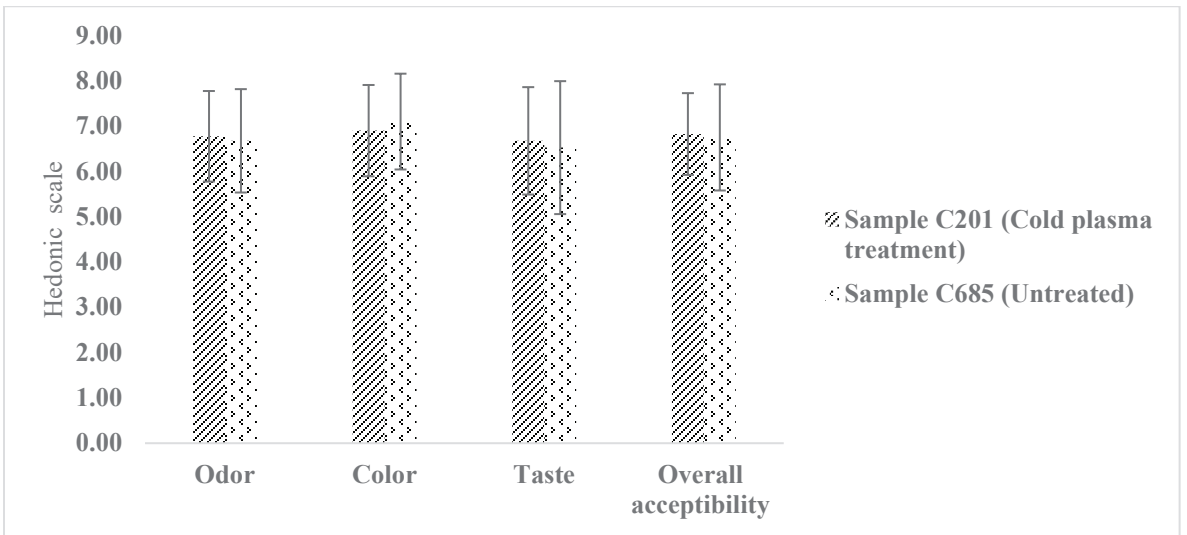

Figura 2. Evaluación sensorial de café tostado (tratado con plasma frío y sin tratar) con una escala hedónica de 9 puntos.
Figure 2. Sensory evaluation of roasted coffee (cold plasma treated and untreated) on a 9-point hedonic scale.

rial analysis; Sonia G. Sáyago-Ayerdi, supervision of TSP and AOX analysis; Montserrat Calderón- Santoyo, conception, and design of the study, analysis and interpretation of data and article critical revising.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest and none to declare concerning the results in this manuscript.

DATA AVAILABILITY

Data are available under request.

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