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Posted Date: 17 May 2023

doi: 10.20944/preprints202305.1189.v1

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Article

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Javier Gallardo-Ignacio [1], Anislada Santibáñez [2], Octavio Oropeza-Mariano [3], Ricardo Salazar [4], Rosa Mariana Montiel-Ruiz [2], Sandra Cabrera-Hilerio [5], Manasés Gonzáles-Cortazar [2], Francisco Cruz-Sosa [1,*] and Pilar Nicasio-Torres [2,*]

[1] Departamento de Biotecnología, Universidad Autónoma Metropolitana-Iztapalapa, Av. Ferrocarril de San Rafael Atlixco No.186, Col. Leyes de Reforma 1ª Sección, Iztapalapa, 09310, CDMX, México.
[2] Centro de Investigación Biomédica del Sur, Instituto Mexicano del Seguro Social (CIBIS-IMSS), Argentina #1, Centro, Xochitepec 62790, Morelos, México.
[3] Cafeticultores MEPHAA de la Montaña, Paraje Montero, Malinaltepec, 41500, Guerrero, México.
[4] CONACYT, Universidad Autónoma de Guerrero, Av. Lázaro Cárdenas S/N, Chilpancingo de los Bravo 39086, Guerrero, México.
[5] Laboratorio de Bromatología, Facultad de Ciencias Químicas, Benemérita Universidad Autónoma de Puebla, Av. San Claudio S/N Ciudad Universitaria, 72000, Puebla, México.
* Correspondence: cuhp@xanum.uam.mx (F.C-S.); pisaliva@yahoo.com.mx (P.N-T.)

Abstract: Coffee is one of the most consumed beverages in the world; its production is based mainly on varieties of the *Coffea arabica* species. Mexico stands out for its specialty and organic coffee. In Guerrero the production is given by small indigenous community's cooperatives that market their product as raw material. Official Mexican Standards stipulate the necessary requirements for its commercialization within the national territory. In this work, the physical, chemical, and biological characterizations of green, medium, and dark roasted beans from *C. arabica* varieties were carried out. Analysis of HPLC showed higher chlorogenic acid (55 mg/g) and caffeine (1.8 mg/g) contents in the green beans of the Bourbon and Oro Azteca varieties. The caffeine (3.88 mg/g) and melanoidins (97 and 29 mg/g) contents increased according to the level of roasting; a dissimilar effect was found in the chlorogenic acid content (14.5 mg/g). The adequate nutritional content and the sensory evaluation allowed the classification of dark roasted as premium coffee (84.25 points) and the roasted medium as specialty coffee (86.25 points). The roasted coffees presented antioxidant activity without cytotoxic effect. The results obtained will serve as a basis for making decisions on the improvements of the coffees analyzed.

Keywords: antioxidant, chlorogenic acid, caffeine, cytotoxicity, melanoidins

1. Introduction

The coffee commercialization is mainly based on *Coffea arabica* with 70% of global production. The cultivation of *C. arabica* occurs mainly in Latin American countries such as Colombia, Honduras, Peru, and Mexico which have a production of 13.8, 5.6, 4.45, and 3.7 million bags of 60 kg, respectively [1]. Peru and Mexico are recognized for their organic and high-altitude coffee production.

Coffee presents chemical compounds such as phenolics where chlorogenic acid (CGA), caffeic, ferulic, catechin, epicatechin, and anthocyanins stand out; alkaloids compounds as caffeine and trigonelline, and those that form during roasting such as melanoidins and acrylamide (Figure 1) [2,3]. These compounds have important biological activities such as neurostimulators, antioxidants, anti-inflammatory, anticancer, among others [4–6]. The compounds content is related to the species, the conditions of cultivation, the processing of beans, and the procedure of coffee drink preparation [4].

In Mexico, there are more than 600 cooperatives dedicated to coffee cultivation whose producers have obtained certifications such as USDA Organic, Fair Trade, Shade Grown, Rainforest Alliance, and Small Producer, to achieve new markets and offer their products [7]. Coffee grown in 4 main regions: the Gulf of Mexico, the Soconusco, the north-central of Chiapas, and the Pacific Ocean slope, which includes Colima, Guerrero, Jalisco, Nayarit, and Oaxaca [8]. In Guerrero, *C. arabica* varieties

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are cultivated by people from the Mixtec and Tlapaneco ethnic groups, from La Montaña, Malinaltepec municipality [9].

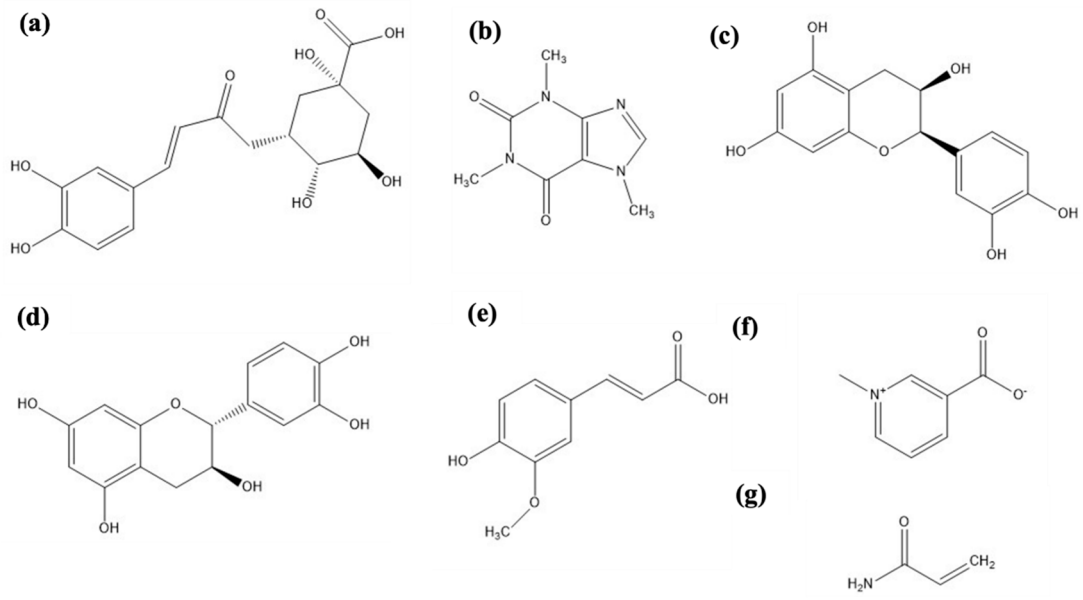

Figure 1. Chemical structures of main compounds identified in the beans of *Coffea arabica*, (a) 3-*O*-caffeoylquinic acid (CGA); (b) Caffeine; (c) Epicatechin; (d) Catechin; (e) Ferulic acid; (f) Trigonelline; (g) Acrylamide.

The coffee produced by these cooperatives is sold to intermediary companies such as Asociación Rural de Interés Colectivo de R.L. (ARIC), CAFECO Agroindustrial del Pacífico S.A. de C.V., and the Unión of Ejidos y Comunidades Luz de La Montaña, A.C. [10]. Some producers have organized themselves to give added value to their products by looking for methods that improve the quality of their products and marketing, one of them is the Cooperative Cafeticultores Mephaa de La Montaña. This Cooperative currently produces small batches of commercial and specialty coffee from mixtures of Typica, Bourbon, and Oro Azteca varieties of *C. arabica* which are marketed at regional and national levels.

In Mexico, there are Official Mexican Norms such as NMX-F-013-SCFI-2010 and PROY-NOM-255-SE-2021 that establish physical, chemical and nutriment specifications to market roasted and ground coffee within the national market. The Cooperative Cafeticultores Mephaa de la Montaña has implemented a physical analysis of green beans as a first step in improving their commercial coffees; therefore, it is important to know the cup quality of commercial and specialty coffee drinks obtained from medium and dark roasted beans that are currently marketed. To address this point, in the present study it was proposed to determinate the nutrimental and organoleptic properties and chemical content associated with the coffee quality and to its biological properties.

2. Results and Discussion

In the Mountain Region, coffee is produced by people of the Mixtec and Tlapaneca ethnic groups whose crops are mainly the Typica, Bourbon, Caturra, and Mundo Novo varieties of *C. arabica*, although hybrid varieties are being promoted such as Colombia, Costa Rica 95, Oro Azteca, and Sarchimor. The ripe cherries are cut and processed in artisanal way; cherries are dehydrated under the sun until to obtain dry fruits with a humidity of 11-12%, the shell is removed to obtain the green coffee beans (GC) [4].

The green beans are marketed as mixtures of different varieties to intermediaries or establishments dedicated to the preparation of coffee beverages. The coffee of the State of Guerrero is known for its natural dry process preserving the pulp, it gives a greater attributes in cup compared to the washed coffee; this type of coffee is processed by entire families characterized by preserving and the care of the environment. The coffee production is carry out as specialty coffee on a small scale

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and organically. For this reason is necessary to carry out studies that contribute to improve the yields and cost reduction, such as analysis of the quality of beans and beverages, as well as the search for new markets [11,12]. These advances will make it possible to offer new options of coffee to the consumers from *C. arabica* plants grown in indigenous areas of Mexico. In this work, the study of parameters associated with the quality of the beans and infusions of mixtures of Arabica varieties was carried out.

2.1. Nutritional composition

The bromatological analysis of green and processed beans coffee allows knowing the quality and nutritional composition of commercial coffees. The protein and carbohydrate content were similar between toasted beans and the mixture of Typica, Bourbon, and Oro Azteca (GCM) (Table 1); the fat content was higher in the dark roast coffee (DCR). The higher humidity content was found in GCM which decreases as the roasting temperature is increased, while the ashes generated are similar in GCM and DRC samples. The humidity (<6.0%) and ash (<6.5%) contents in the medium roasting coffee (MRC) and DCR commercial coffee beans are within the range established by the Official Mexican Norm for roasted and ground coffee NMX-F-013-SCFI-2010; instead, only the fat content of DCR beans as an etheric extract (18 – 8%) achieve the parameter stipulated by the norm. The humidity conditions of green beans should be 12 – 10% to prevent the growth of fungi and bacteria, preserve physical properties and nutritional content.

Table 1. Nutritional composition in the commercial coffee with *Coffea arabica* varieties in the mixture

	Content in percentage (%)				
Coffee	**Humidity**	**Ash**	**Fats**	**Proteins**	**Carbohydrates**
GCM	8.48 ± 0.13**	4.54 ± 0.06**	5.09 ± 0.89	12.34 ± 0.29	69.56 ± 1.06
MRC	4.23 ± 0.14*	3.84 ± 0.11	6.48 ± 0.34*	13.04 ± 0.28	72.41 ± 1.26
DRC	3.59 ± 0.12	4.44 ± 0.08**	8.15 ± 0.63**	13.01 ± 0.38	70.81 ± 1.11

Values are mean ± standard deviation (n = 3). According to the ANOVA and Tukey's test the means with * and ** were significantly different. Humidity F= 1251.75 $p \leq 0.0001$, $Tukey_{0.05}$= 0.33; Ash F= 62.89 $p \leq 0.0001$, $Tukey_{0.05}$= 0.20; Fats F= 16.26, $p \leq 0.0001$, $Tukey_{0.05}$= 1.65; Proteins F= 4.56 $p > 0.05$, $Tukey_{0.05}$= 0.80; Carbohydrates F= 7.55 $p \leq 0.0001$; $Tukey_{0.05}$= 2.25.

The main constituents of Arabica green coffee are carbohydrates (65 – 40%), soluble (sucrose, glucose, fructose, galactose, arabinose) and insoluble (hemicellulose and cellulose); proteins (15 – 11%) and free amino acids (1%); and lipids (18 – 15%) in the form of waxes that coat beans and triglycerides; the ash is at 5.4 – 3% and humidity 12 – 8%, which can be modified by the effect of roasting [13].

The oils released during roasting help to prevent the loss of volatile compounds and other compounds that are attributed to the flavor and aroma of the cup of coffee. The roasting temperature is related to the humidity content in the beans, as the temperature increases in roasting the beans take shades of darkening the inner oils are released giving a shiny aspect to the beans. In the *C. arabica* beans with light (176 °C), medium (204 °C), and dark (232 °C) roast the humidity content was reduced with the temperature increase, while protein (16%) and fat (16.2%) contents increased in the dark roast. Ashes and sugar contents were similar (2 °Brix) in the three roasts [14].

2.2. Chemical analysis

The main compounds related to the cup quality of the coffee drink, which give it astringency and flavor, are CGA, caffeine, caffeic acid, ferulic acid, vanillic acid, cinnamic acid, trigonelline, and volatile compounds such as furans, pyridines, pyrazines, and pyrroles [6,15]. In the High-Performance Liquid Chromatography (HPLC) analysis, compounds of greater predominance were identified in both infusions of greens and processed beans, CGA and caffeine with retention time of 8.51 min and 8.88 min (Figure 2).

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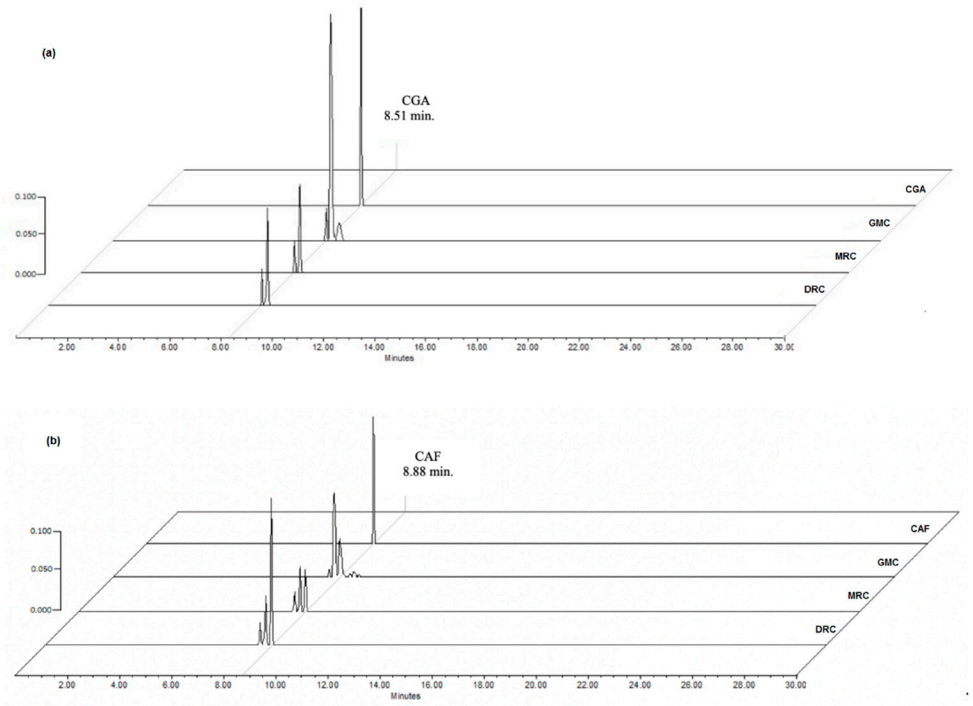

Figure 2. Chromatograms of HPLC at λ = 330 nm of **(a)** CGA standard (25 μg/mL), and infusions of GCM (125 μg/mL), MCR, and DCR (500 μg/mL); **(b)** at λ = 280 nm of caffeine (CAF) standard (10 μg/mL), and infusions of GCM (125 μg/mL), MCR and DCR (500 μg/mL).

The ^{1}H and ^{13}C nuclear magnetic resonance (NMR) analysis of the compound isolated from the MRC infusion validated that it corresponds to CGA; chemical displacements and coupling constants (Figure S1 and Table S1) correspond to those reported in the literature for this compound [16]. The Typica variety of green beans (Table 2) has the lowest contents of CGA (36.81 mg/g) and caffeine (1.16 mg/g); due to the GCM is composed in a greater proportion of the beans of the Typica species (Typica-Bourbon-Oro Azteca, 40-30-30%), the content of both compounds in the GCM is close to those detected in this variety. It has been reported that in Arabica green coffee the CGA content ranges between 52 – 76mg/g and is related to the variety of coffee, growing conditions, and degree of ripeness of the fruits [17]; the CGA content in the Bourbon and Oro Azteca varieties are within this range.

When green beans are subjected to the roasting process, the CGA content is reduced by the effect of temperature and time exposed, where its content can be reduced at least a 30% depending on the level of roasting; CGA is hydrolyzed into the molecules of simple phenols that compose it, caffeic acid, ferulic acid, and quinic acid [15]. The CGA contents were similar in GCM and MRC beans; while in the DRC beans roasted at 210 °C it was a 53% reduction in their content compared to unroasted beans (Table 2).

Studies carried out in different levels of roasting show that the content of CGA in the beans is correlated with the temperature and the time of roast, finding greater content in a light roast (186.5 °C, 7:15 min) of 11.24 mg/g of coffee, when the the roasting time is prolonging to a dark roast (186.5 °C; 14:02 min) the content was reduced by 70% [18]. In the coffee mixed, the Typica variety that predominates has a low content of caffeine (1.16 mg/g) compared to the other two varieties, so the content of caffeine in GCM presents low levels of 0.87 mg/g (Table 2). The values determined in this study for MRC and DRC commercial coffees are lower than those established for the commercialization of roasted coffee. According to the Mexican Norm (NMX-F-013-SCFI-2010) the caffeine content acceptable range is between 10 – 20 mg/g for roasted coffee. The caffeine content determined in green beans was increased in the roasted beans as the level of roasting increased (Table 2); however, this content is minor of the lowest level acceptable by the norm.

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Table 2. Contents of CGA, caffeine, and melanoidins in green and processed beans of varieties of *Coffea arabica*

Coffee beans	CGA	Caffeine	Melanoidins			
			Unclarified	Clarified		
	mg/g coffee				K_{mix} $^1cm^{-1}$	Lg^-
GCM	30.81 ± 2.22	0.87± 0.09	15.41±1.15	2.04 ±0.88	0.07	
Bourbon-GC	55.75 ± 2.31**	1.78± 0.12**	-	-	-	
Oro Azteca-GC	54.63 ± 2.43**	1.77± 0.15**	-	-	-	
Typica-GC	36.81 ± 0.10	1.16 ± 0.18	-	-	-	
MRC	30.26 ± 0.45**	2.52±0.17*	85.51±5.99*	18.95 ±1.9**	1.586	
DRC	14.52 ± 0.65	3.88±0.23**	96.79±3.44**	29.06 ± 7.7**	1.614	

Values are mean ± standard deviation (n = 9). According to the ANOVA and Tukey's test the means with ** were significantly different. CGA F= 328.13 $p \leq 0.0001$, $Tukey_{0.05}$= 2.66; caffeine F= 101.83 $p \leq 0.0001$, $Tukey_{0.05}$= 0.17 in mg/g of coffee in green beans; CGA F= 415.72 $p \leq 0.0001$, $Tukey_{0.05}$= 1.60; caffeine F= 678.07 $p \leq 0.0001$, $Tukey_{0.05}$= 0.20 in mg/g of coffee in processed beans. For melanoidins, values are mean ± standard deviation (n=3). According to the ANOVA and Tukey's test the means with * and ** were significantly different. Unclarified melanoidins F= 1423.09 $p \leq 0.0001$, $Tukey_{0.05}$= 4.055 in mg/g of coffee, and clarified melanoidins F= 26.45 $p \leq 0.0001$, $Tukey_{0.05}$= 11.516 in mg/g of coffee.

In a study conducted on coffees from Veracruz, Nayarit, Oaxaca, and Chiapas states of Mexico, it was found that the level of caffeine ranges between 2.9 – 7.0 mg/g of roasted coffee [19]. The caffeine content determined in the processed coffee from Guerrero state (Región de la Montaña) reported in this study is between these ranges. Some authors consider the use of magnesium oxide for the caffeine extraction and elimination of interferences for its quantification as is suggested in the NMX-F-182-SCFI-2011 and ISO 20481:2008 (reference methods for the determination of caffeine by HPLC), another factor to consider is the fertilization of crops using nitrogen [18].

Caffeine is a main component of coffee, drinks from dark roasts present higher caffeine content, when the beans are roasted, they lose moisture, increase in size and become porous, allowing a better extraction [20]. In Typica variety coffee beans from plants grown in two different places, the caffeine content in light roasting was 4.19 mg/g and 5.01 mg/g, when the level of roasting was increased to a dark level the content presented an increase (5.18 mg/g and 6.12 mg/g), a similar effect to that was reflected in this study [20]. Other studies in *C. arabica* have not reported variations in its content at different levels of roasting (11.9 – 13 mg /g) even using temperatures between 194 – 217 °C showing a thermostable behavior of caffeine [21].

Otherwise in other studies it has been reported that in Arabica beans from Brazil, the highest content of caffeine was presented in light (6.42 mg/g) and medium (5.77 mg/g) roast, compared to dark roast (2.63 mg/g). A similar effect was reported in the Typica and Bourbon varieties coffee beans, the highest content was presented in light roasting (14.59 mg/g), followed by medium and dark (5.57 mg/g). The caffeine content in green beans of Sidama (16.4 mg/g), Yirgacheffe (15.72 mg/g), and Harar (15.03 mg/g) varieties was reduced to 7.96, 8.87 and 4.52 mg/g, respectively, after the beans were subjected to a dark roasting process; the Sidama variety shown a more significant reduction of 60% [18,22].

Variations in the content of chemical compounds are related to temperature conditions for the beans processing, and beverage preparation [18]. In the infusion preparation, it is essential to consider the pressure, temperature, and contact time of the beans with water; within the populations the amount of coffee used can vary, for example for filtered coffees in Europe, the United States, and Canada 7 g per 100 mL of water is usually used, in Brazil 10 g, and in Italy 20 g are used [23]. In this study, infusions were prepared with 6.1 g of coffee (equivalent to one tablespoon) per 100 mL of water by extractions by French Press due to its practicality. In Mexico, it is common to coffee preparation use one or two tablespoons by coffee cup for Turkish coffee or drip coffee.

Several studies have shown that the content of chemical compounds is influenced by the processing method of beans and infusions preparation [24]. In a coffee cup (≈150 mL) the caffeine

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content varies between 40 to 180 mg. The moderate coffee drinker's consume per day between 200 to 400 mg of caffeine, and between 200 – 500 mg of CGA per cup. Infusions of MCR using 6.1 g of coffee in 150 mL of water would contain 15 mg of caffeine and 185 mg of CGA, while in DRC infusions the content would be 24 mg of caffeine and 89 mg of CGA per cup [4,25].

2.3. Melanoidins

During roasting practice, various processes can occur such as caramelization, like this, the phenolic compounds can be degraded by Maillard and Strecker reactions, or it can be followed by the formation of new compounds such as melanoidins, acrylamides, and hydroxymethylfurfural [26,27]. The molecular structure of melanoidins is not precisely known due to the diversity of couplings of their components, their presence is related to the beans roasting [28]. Melanoidins give to the beans a brown pigment, flavor, and color to the beverage and are associated with antioxidant activity that is enhanced by simple phenolic compounds such as caffeic, ferulic, and chlorogenic acids binding to their structure [15].

In this study, two procedures were performed to determine the content in the different infusions. In not clarified solutions, the melanoidins are present in the GCM and the content was more than doubled after beans are roasting in DRC (~2.87 fold) characterized by a dark brown coloration (Table 2). Carrez I and II solutions are used to precipitate proteins and remove turbidity and micelles reducing interference at the time of readings (λ = 420 nm). In the clarified samples the content was lower compared to those not clarified but with similar behavior in their content, it was related to the increase in the level of roasting. The samples subjected to the clarification process lost color and presented the formation of a precipitate which may be influencing the elimination of compounds of high molecular weight. The contents of melanoidins determined are lower than those reported in the literature 200 – 250 mg/g in coffee [29,30]. Other authors report that the highest melanoidin content was determined in soluble coffee obtained from Robusta (676 mg/g) and Arabica coffee (peaberry known as caracolillo in Spanish, 305 mg/g) roasted with sugar [30]. In the green coffee beans of the Arabica and Robusta species from Brazil, the presence of these compounds was low compared to the roasted beans, the dark beans of both species presented the highest contents but it was predominant in Robusta beans. There is a lack of adequate studies that relate the levels of roasting or color of the beans with the content of their compounds mainly caffeine, CGA, melanoidins, among others [20,21].

2.4. Sensory evaluation

The quality of coffee depends on the physical and organoleptic properties of the beverage, such as flavor, aroma, acidity, body, and balance. These properties are associated with the content of their chemical compounds (Figure 1), mainly CGA, caffeine, caffeic acid, ferulic acid, vanillic acid, cinnamic acid, trigonelline, and volatile compounds such as furans, pyridines, pyrazines, and pyrroles [15].

The GCM Infusions had a pH of 5.6, MRC infusions of pH = 4.74, and DRC infusions of pH = 6.15. The MRC infusions had higher acidity correlated with the CGA content (Table 2) as it was reported. Furthermore, the temperature used during the roasting process of coffee beans transforms carbohydrates into organic acids influencing the acidity of the drinks. It has also been reported that coffee infusions with higher caffeine content have bitter tastes, DRC infusions have the highest caffeine content, taste and color can be identified by sensory tests [14].

The green beans lack of taste and pleasant smell to the palate; when the beans are subjected to the roasting process a series of physical changes occur such as shape, water content, density and color. Among the chemical changes, occurs the degradation of sugars, CGA, caffeine, and trigonelline related to taste and aroma of coffee drink. The formation of volatile compounds also affects the acidity and aroma of the beverages [15,17,26,27].

Temperatures of 200 – 250 °C can be used for 5 to 20 min for the roasting of coffee beans, during this process the beans acquire colors, flavors, and aromas characteristic of coffee. The sensory attributes established by the Mexican Norm PROY-NOM-255-SE-2021 in fragrance/aroma, flavor, acidity, balance, and taster score must present a score ≥ 8, in residual flavor and body ≥ 7.5, and in

 doi:10.20944/preprints202305.1189.v1

uniformity, cup cleanliness and sweetness of 10. Natural coffees or honeyed coffees of specialty are those that present a total rating of 85 to 87.75 points according to the Mexican Norm.

The quality of coffee depends on the physical and organoleptic properties of the beverage, such as flavor, aroma, acidity, body, and balance. The sensory analysis of the infusions prepared from MRC, considered as natural specialty coffee, a score of 86.25 was obtained. The points obtained in each parameter are within the values considered by the Norm (Table 3). Natural coffee or dry process, it is common to obtain characteristic fruity aromas and flavors due to the preservation of the peel and pulp of the fruit [31], such as those determined in this study, which presents aromas of tropical fruits and flavors of white wine, grape, and honey.

Table 3. Sensory profile of commercial coffee blend of *Coffea arabica* varieties

Coffee beans	MRC	DRC
Aroma	8.00 ± 0.16*	7.75 ± 0.20
Taste	7.75 ± 0.29	7.75 ± 0.29
Aftertaste	8.00 ± 0.20	8.00 ± 0.20
Acidity	8.00 ± 0.61	8.00 ± 0.13
Body	8.25 ± 0.20*	8.00 ± 0.13
Balance	8.00 ± 0.29*	7.25 ± 0.29
Uniformity	10 ± 0	10 ± 0
Clean cup	10 ± 0	10 ± 0
Sweetness	10 ± 0	10 ± 0
Taster score	8.25 ± 0.29*	7.5 ± 0.41
Total Score	86.25	84.25

Values are mean ± standard deviation (n=4). According to the t-Student ($p \leq 0.05$) test the means with * were significantly different.

The score of DRC coffee (84.25) was lower than that required to be considered as natural specialty coffees but is within the natural coffees named premium (80 to 84.75 points) according to the Norm, attributes such as aroma, flavor, body, and balance presented the lowest score (Table 3). In this drink, a floral and fruity aroma with date, grape and red apple flavor was detected, with a tartaric citrus acidity, a juicy-silky body and medium-high sweetness.

The sensory analysis performed on the MRC beans is within the considered range of specialty coffees, a lower flavor was reported (7.75), a greater body (8.25); in the case of DRC beans showed a lower aroma (7.75), lower balance (7.25), as well as a smaller body than in MRC beans. In roasted coffee it is very common to find mixtures of *C. arabica* varieties identifying different organoleptic characteristics influenced by the method in which the cherries are subjected to obtain the green beans, other aspects that must be considered are the altitude at which the crops are located, and degree of maturity. The commercial coffees analyzed in this study are carefully collected by hand considering the uniformity of the beans of ripe cherries, eliminating green and dry fruits from *C. arabica* plants varieties grown from 1900 m.a.s.l. under the shade of other native trees of the place; besides, during drying, cherries are placed on beds to avoid contact with the soil and the growth of contaminating microorganisms. In addition, selected and sorted grains are stored in moisture-controlled spaces to preserve the quality of the green beans.

C. arabica L. beans from Brazil processed as natural coffee (87.8 points) presented better attributes in aroma, flavor, acidity, and body than honeyed coffee (83.8 points), possibly due to fermentation carried out by the microorganisms present in the fruit (Stanek et al., 2021). Likewise, natural coffee (dry, 80), wet (85), and semi-dry (86) with fermentation of *C. arabica* variety Colombia, presented similar attributes such as medium fruity body, medium fresh acidity and chocolate and caramel flavors. Sensory attributes are related to geographic conditions, climate, altitude, and crop field practices [32].

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2.5. Biological activities

2.5.1. Antioxidant effect

The antioxidant activity of the extracts of processed coffees was determined by the equivalents of Trolox and CGA related to the inhibitory effect of the radicals of DPPH, ABTS, and FRAP (Table 4). The biological activity of coffee is related to the content of the chemical compounds, MRC extract showed a greater capacity of radical scavenging in the tests carried out compared to the DRC extract. The CGA content in the infusion of MRC was related to the greater inhibitory activity, probably due to low degradation of phenolic compounds involved in antioxidant processes. Besides, DRC presented a higher content of melanoidin although its activity was lower than that presented by MRC. Finally, in the case of caffeine, it acts indirectly in the antioxidant processes increasing levels of glutathione [33]. Other molecules related to the main effect of beverages are ferulic acid, caffeic, vanillic, guaiacol, epicatechin, catechin, and anthocyanins which can unravel various mechanisms for the elimination or inhibition of free radicals [2,3,34].

Table 4. Antioxidant activity of commercial coffee with *Coffea arabica* varieties in mixture

Sample	DPPH		ABTS		FRAP		
	eq CGA	eq Trolox	eq CGA	eq Trolox	eq CGA	eq Trolox	eq FeSO4
MRC	1.60 ± 0.27*	52.74 ± 4.84*	16.09 ± 0.33*	14.39 ± 1.16	16.22 ± 1.04*	14.59 ± 2.35*	54.68 ± 1.46*
DRC	1.12 ± 0.37	42.52 ± 1.91	12.49 ± 0.46	12.15 ± 0.49	8.82 ± 0.94	6.38 ± 1.40	33.30 ± 0.63

Values are mean ± standard deviation (n=3). According to the t-Student ($p \leq 0.05$) test the means with * were significantly different.

The roasted beans of Colombia, Typica, and Bourbon varieties presented a similar effect like that reported in this study, the greatest antioxidant activity was found in the light and medium roasts through the tests carried out of DPPH and ABTS [18,32]. The high content of phenolic compounds provides greater radical inhibitory activity in beans with light roasting of the Cataui variety [35].

The concentration needed to inhibit 50% of DPPH and ABTS radicals depends on the content of phenolic compounds present like the CGA. According to the tests performed, the MRC extract (higher CGA content, Table 2) presents a greater antioxidant effect (Table 4), and a lower concentration is required to achieve IC_{50} compared to DRC (Table 5). In *C. arabica* the content of 5-CQA was higher in green beans than in roasted beans, so less content of the green extract was required to achieve CI_{50} using the DPPH test [36].

Table 5. Inhibitory concentration (IC_{50}) of commercial coffee with *Coffea arabica* mixture varieties

Assay	IC_{50}					
	MRC	DRC	MRC	DRC	CGA	Trolox
	mg/mL extract		µg/mL CGA content		µg/mL standard	
DPPH	2.22 ± 0.08	2.59 ± 0.05*	56.92 ± 1.90	66.20 ± 1.46*	28.18 ± 0.83	91.88 ± 3.75*
ABTS	0.38 ± 0.02	0.49 ± 0.02*	9.69 ± 0.35	12.67 ± 0.44*	6.51 ± 0.16*	6.29 ± 0.03

Values are mean ± standard deviation (n=3). According to the t-Student ($p \leq 0.05$), test the means with * were significantly different.

The main consuming countries are the United States and Europe; the highest consumption per person occurs in Nordic countries [37]. The beneficial effect of coffee is related to its consumption, the metabolism and sensitivity of people to the compounds present in the beverages; on average 2 to 4 cups of coffee are usually ingested per day. It is well known that coffee has stimulating, and antioxidant effects and its consumption is also related to its flavor and aroma. Other important effects reported are anti-inflammatory, help in the regulation of glucose and lipid metabolism, and as anticancer [3,38].

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2.5.2. Cytotoxic activity

Based on the IC_{50} determined for DRC (216.26 ± 27.7 µg/mL) and MRC (234.63 ± 29.6 µg/mL) infusions on the growth of the 3T3-L1 fibroblast cell line at 48 h of exposition, it was determined that the infusions not presented cytotoxic effects. A plant extract has been determined to be cytotoxic when they present IC_{50} values <100 µg/mL [39]. Studies on coffee extracts have reported a cytotoxic effect on prostate cancer cell lines DU145 and PC3, without showing toxic effects in macrophage cell lines (RAW 2647), hepatocytes (AML-12), and normal CCD-18Co fibroblast cells [35].

Studies show that CGA and other phenolic compounds have anti-inflammatory effects by activating and regulating proinflammatory cytokines, transcription factors such as tumor necrosis factor-alpha (TNF-α), and interleukins such as IL-8, and as anticancer in prevention by counteracting the actions of free radicals or activating chemoprotective mechanisms by the modulation of expression of enzymes involved in endogenous antioxidant defenses, DNA replication, cell differentiation and aging [4,6,23,38].

Coffee, being a food product in high demand in the world, must be free of harmful chemical compounds and biological agents, the beans when exposed to the environment and thermal processes that favor the formation of these toxic compounds, such as acrylamide and hydroxymethylfurfural considered carcinogenic or genotoxic [27,40]. The acrylamide content is regulated by each country, in the European Union the permissible limit is 400 µg/kg for roasted coffee, and 800 µg/kg for soluble coffee. The production of these compounds may be related to the size and humidity of the beans, free amino acids and sugar contents, and acidity. Studies reported variable amounts of acrylamide, but it has been shown that in dark coffees or with prolonged levels of roasting the acrylamide content may be lower than medium or light coffees [41,42]. The commercial samples analyzed showed biological effects of interest without presenting toxic effects, the content of harmful compounds in these coffees may be at low concentrations.

3. Materials and Methods

3.1. Biological material

The cherries of *Coffea arabica* varieties, Typica, Bourbon, and Oro Azteca, employed in the present study, were harvested in the 2020-2021 and 2021-2022 cycles in the region of "La Montaña", at the localities of La Soledad and Paraje Montero, municipality of Malinaltepec (longitude: 98.704167 and latitude: 17.164167), Guerrero, Mexico (Figure 2). Coffee cherries of each variety were dried on drying beds under the sun at room temperature for 15 days; after that time the husk was removed to obtain the GC.

Subsequently, the GC of the Typica, Bourbon, and Oro Azteca varieties were mixed (GCM) in a ratio of 40-30-30 %. The mixture of coffee beans was processed in a 100MEX® brand roaster to reach an Agtron Gourmet Bean roasting level of 45 (180 °C per 15 min) to obtain a medium roasting coffee (MRC) and with an Agtron Gourmet Bean roasting level of 35 (210 °C per 15 min) for a dark roast coffee (DRC).

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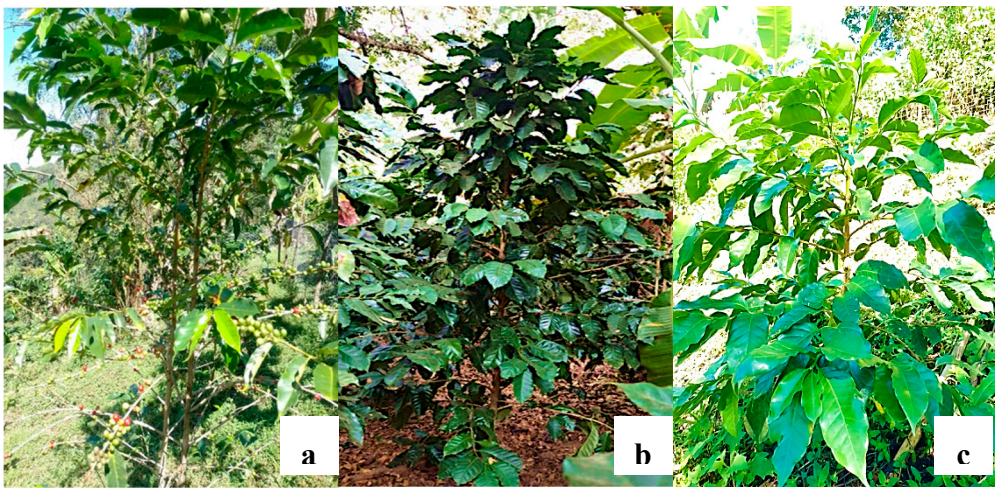

Figure 3. Photographs of the varieties *Coffea arabica* **(a)** Typica, **(b)** Bourbon, and **(c)** Oro Azteca grew in the Paraje Montero locality municipality of Malinaltepec at 1980 m.a.s.l.

3.2. Bromatological analysis

The analysis of the nutritional components of GCM, MRC, and DRC beans was performed based on the standard methods of the American Cereal Chemical Association (AACC International) [43]. The methods employed were moisture (44-15.02), protein (46-16.01), fat (30-25.01), and ash content values (08-01.01). Moisture (%) was determined by the weight difference of the grain before and after drying in a forced-air oven (VWR)® for 5 min. The ash content (%) was obtained by the difference in weight of the waste generated by incineration of the samples placed in a porcelain pot in a muffle at 550 °C for 4 h. The fats of the grains were extracted by Soxhlet with petroleum ether, the solvent was evaporated, and the extracted residue was kiln-dried at 100 °C, weighed, and expressed as % ethereal extract (crude fat). The protein content (%) was determined by the quantification of total free nitrogen by the modified Kjeldahl method. Carbohydrate content was calculated by subtracting the sum of the percentages of moisture, lipids, protein, and ash from 100%.

The values of humidity, ash, proteins, lipids, and carbohydrates obtained in the GCM, MRC, and DRC were expressed as an average of three replicates and their standard deviation (SD). Each variable was analyzed with a simple ANOVA and a Tukey post-test with a confidence level of 95% ($p< 0.05$) using the SAS System for Windows 9.1 software (Statistical software; SAS Institute, Inc.).

3.3. Chemical analyses

3.3.1 Infusion preparation

The beans of Typica-GC, Bourbon-GC, Oro Azteca-GC, GCM, MRC, and DRC were ground in an MCT-750 100MEX® grinder with a particle size of 1.0 mm. Infusions of each coffee were prepared in triplicate with 13.2 g in 200 mL of boiling water to 98 °C in a French press for 5 min. The infusions were filtered and the pH was measured with a potentiometer Oakton pH 510; subsequently the infusions were concentrated at reduced pressure in a rotatory evaporator (Heidolph Laborota 4000). Then the extracts were freeze-dried (Heto Drywinner DW3), and the powders were stored in amber glass containers at room temperature.

3.3.2. Chlorogenic acid and caffeine quantification

The concentrations of chlorogenic acid (CGA) and caffeine (CAF) in the Typica-GC, Bourbon-GC, Oro Azteca-GC, GCM, MRC, and DCR infusions were determined by High-Performance Liquid Chromatography (HPLC) in a Waters equipment consisting of a separation module (Waters 2695) and a photodiodes detector (Waters 2696). For this purpose, 10 µL of each infusion was injected at concentrations of 0.25, 0.5, and 1.0 mg/mL and eluted through an RP-18 column (250 x 4.6 mm, 5 µm, SUPELCO Discovery®, Merck) with a flow of 0.9 mL/min in a gradient system of 30 min based on

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water HPLC grade (VWR, Canada) with 0.5% trifluoroacetic acid (Sigma-Aldrich, MO, USA) (A) and acetonitrile-HPLC (Merck, Germany) (B). Gradient flow starts at A-100% with gradient changes to 95% in 2 min; to 70% in 2 min; to 50% in 17; to 20% in 3 min; 0% A and 100% B in 3 min; finally system return to initial conditions (A-100%) in 3 min. Data were processed with the Empower Pro 3.0 software (Waters, MA, USA) and the chromatograms were obtained at wavelengths (λ) of 330 nm for CGA and at λ = 280 nm for CAF. The identification of both compounds was determined by comparison of their retention time (RT) and absorption spectra. The CGA and caffeine concentrations were calculated according to external standards of CGA (3-(3,4-Dihydroxycinnamoyl) quinic acid; ≥95% purity Sigma-Aldrich) and caffeine (≥99% purity, Sigma-Aldrich). The calibration curves of CGA and caffeine were constructed using a lineal square model $y = mx + b$ with the Microsoft Office Excel 365 Software (Microsoft® Excel V.16.70) with correlation coefficients ≥0.9995; for CGA it was a regression equation of $y = 11702x + 19276$ with an $R^2 = 0.9982$ and for caffeine $y = 87483x + 38786$ with an $R^2 = 0.9993$.

The CGA and CAF contents were expressed in mg/g of coffee beans as the mean of nine analyses and their standard deviation (SD). The GC contents of the varieties, GCM, MRC and DRC infusions were compared with an ANOVA and a Tukey *post*-test with a 95 % confidence level ($p< 0.05$).

3.3.3. Chemical fractionation of MRC infusion

The MRC extract (2g) was fractionated by open column chromatography (2.7 cm diameter x 44 cm high) packed with 20 g of RP-18 silica gel (Supelco-Germany) and eluted with a gradient system of H_2O: CH_3CN. Aliquots of 10 mL were collected with an initial system of 100% H_2O (1-10) and polarity changes from CH_3CN at 5% (11-15) at 50% (16-17) and CH_3OH at 100% (18). The fractions were analyzed by reverse phase thin layer chromatography with an elution system of 90:10 H_2O:CH_3CN and were displayed in a UV lamp (UVP UVGL-58) at λ = 254 nm and λ = 365 nm. Aliquots with a similar chromatographic profile (13-15) were grouped and diluted in dimethyl sulfoxide to be analyzed by NMR of ^1H and ^{13}C spectra to 100 MHz, 2-dimensional (2-D) correlated spectroscopy (COSY), heteronuclear simple quantum coherence (HSQC), and heteronuclear multiple bond coherence (HMBC) at 400 MHz on a Varian INOVA-400 equipment.

3.4. Melanoidins

The melanoidin content was analyzed in infusions of GCM, MRC, and DRC by two different procedures:

1) Serial dilutions (2.0 - 0.0625 mg/mL) were prepared from a solution of 10 mg/mL of each infusion, and the absorbance of each concentration was measured at λ_{max} = 420 nm in a UV-VIS spectrophotometer (Genesys 20-Thermo Scientific). The melanoidin content in the infusions was determined by the Lambert-Beer formula: C = A/cb, C is concentration, A absorbance, b cell length (1cm), and c extinction coefficient (1.1289 L/g cm) [30,44].

2) Extracts from 2 g of each infusion were dissolved in 20 mL distilled water and they were filtrated through acrodiscs (0.45 µm) Pall®. The calibration curves of each coffee were built from dilutions with absorbances between 1.0 - 0.01. For melanoidin determination, 1 mL of the filtrate was diluted with water (1/5, v/v) and 1 mL of Carrez I and II solutions were added (Sigma-Aldrich), the solution was homogenized and completed to a volume of 10 mL. Then, each sample was centrifuged at 4000 rpm for 5 min, the clarified samples were filtered through acrodiscs (0.20 µm) Pall®. The corresponding readings for melanoidins were carried out to obtain the content and the specific extinction coefficient (K_{mix}) determined by Lambert-Beer's law.

The melanoidins content values in the GCM, MRC, and DRC were expressed as the mean of three analyses and their SD, compared with an ANOVA and a Tukey post-test with a 95 % confidence level ($p< 0.05$).

3.5. Sensory assessment

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Infusions in cups of MRC or DRC ground coffee (MCT-750 100MEX® grinder with a particle size of 1.0 mm) were prepared with 8.25 g in 150 mL of distilled water at 93 °C in a simple infusion. The sensory evaluation of the coffee in cup was carried out by fours tasters certified by the Mexican Association of Specialty Coffees and Cafeterias, AC (AMCCE, abbreviations in Spanish), following the cupping protocol by the Specialty Coffees of America Association (SCAA) [45]. The parameters of the SCAA are based on a reference of 100 points, a score of 0 to 10 points was given to each organoleptic characteristic of aroma/fragrance, flavor, residual flavor, sweetness, acidity, body, uniformity, balance, clean cup, and taster. The points total sum is the result of the sensory analysis, scores ≥ 80 points are considered very good coffees and are classified as specialty coffees.

3.6. Biological analyses

3.6.1. Antioxidant activity assays

Radical scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH)

In each well of microplates of 96 wells, 175 µL of DPPH (1 mg/ml of methanol) solution was added followed by 25 µL of CGA (100 – 3.0 µg/mL), Trolox (6-Methoxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, (100 – 3.0 µg/mL), MRC (5.0 – 0.1 mg/ml) or DRC (5.0 – 0.1 mg/ml) solutions. The absorbance was measured at the beginning of the reaction (A_0) and the plates were stored protected from light for 30 min; after this period the absorbance was measured (A_1) again with an ELISA lector (Perkin-Elmer Lambda 40 UV/Vis) to λ = 515 nm. The inhibition percentage was calculated with the equation:

$$\% \text{ inhibition} = \left[\frac{(A_0 - A_1)}{A_0}\right] \times 100$$

The CGA, Trolox, MRC, and DRC mean inhibitory concentration (IC_{50}) of scavenging DPPH radical was determined based on the curves generated by recording the inhibition percentages of the reaction against the concentration. In addition, the absorbance values of the samples were compared with the graphed curves of the inhibition percentage against the concentration of the standards and reported in GCA or Trolox equivalents (eq CGA and eq Trolox) [26].

The IC_{50} and GCA or Trolox equivalents were expressed as the mean of three replicates and their SD. Each variable of MRC and DRC was compared by a *t-Student* test with a $p < 0.05$ value.

ABTS (2,2′-Azinobis (3-ethylbenzothiazoline-6 sulfonic acid) radical

The 7 mM ABTS radical solution was prepared with persulfate of potassium 140 µM in the dark under agitation for 12 to 16 h; after, the solution was diluted with methanol until an absorbance value of 0.7 at λ = 734 nm. In each well, 230 µL of ABTS solution and 20 µL of CGA (13 – 0.3 µg/mL), Trolox (13 – 0.3 µg/mL), MRC (5.0 – 0.1 mg/ml) or DCR (5.0 – 0.1 mg/ml) was added. After a stirring of 30 sec, the absorbance was determined at λ = 734 nm. The antioxidant activity was expressed by inhibition percentage, IC_{50}, and CGA or Trolox equivalents as was previously described.

The IC_{50} and GCA or Trolox equivalents were expressed as the mean of three replicates and their SD. Each variable of MRC and DRC was compared by a *t-Student* test with a $p \leq 0.05$ value.

Ferric Reducing/Antioxidant Power (FRAP)

The FRAP solution was produced with 1 mL of 10 mM 2,4,6,-Tris(2-piridil)-s-triasine (TPTZ) solution and 1 mL 20 mM ferric chloride ($FeCl_3 \cdot 6H_2O$) solution in 10 mL of 300 mM sodium acetate buffer (CH_3COONa) to pH 3.6. The FRAP solution was prepared freshly and kept at 37 °C. To each well 175 µL of FRAP was added, 50 µL of CGA (13 – 0.3 µg/mL), Trolox (13 – 0.3 µg/mL), MRC (5.0 – 0.1 mg/ml) or DCR (5.0 – 0.1 mg/ml). The absorbance was measured at λ = 595 nm after 30 s of the reaction.

The IC_{50} and GCA, Trolox, or ferric sulfate equivalents were expressed as the mean of three replicates and their SD. Each variable of MRC and DRC was compared by a *t-Student* test with a $p \leq 0.05$ value.

 doi:10.20944/preprints202305.1189.v1

3.6.2. Cytotoxic evaluation

Cell line of 3T3-L1 fibroblasts committed to differentiation to adipocytes and derived from mouse embryonic cells (TCCA) was used for cytotoxic assays. The cells were cultivated in Dubelco's Modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum, L-glutamine (1 ml x100), antibiotic 3X (1 mix100), non-essential amino acids (1 mlx100), and sodium bicarbonate (3mLx100). Cellular culture was incubated at 37 °C and humidified atmosphere with 5% CO_2.

For cytotoxic assay, the 3T3-L1 cells were cultivated in plates of 96 wells with a cellular density of 3×10^4 cells per well. After 24 h of plate attachment, the non-adherent cells were eliminated and subsequently treated with DMSO 1% as the negative control, different concentrations of MRC and DRC infusions (15.6 –500 µg/mL) and paclitaxel as the positive control (0.6 –20 µg/mL). The plates were incubated for 24 and 48 h at the conditions above described.

After each incubation time, the medium was discarded and 80 µL of DMEM medium and 20 µL of (3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT, 5 mg/ml in PBS) were added to each well and incubated for 4 h at 37 °C to allow the formation of formazan crystals. The supernatant was removed and 100 µL of isopropanol was added and the plates were incubated at room temperature in stirring for 15 min until the formazan crystals were dissolved. The optical density of the solution was measured with a microplate spectrophotometer at λ = 490 nm.

The cell viability in percentage was determined by the following equation:

$$\text{Cell viability (\%)} = \left[\frac{(\text{negative control OD} - \text{tested sample OD})}{\text{negative contro OD}}\right] \times 100$$

The mean inhibitory concentration (IC_{50}) for each infusion was calculated with a dose-response curve regression analysis.

4. Conclusions

The artisanal coffees showed variable chemical contents related to the roasting process that beans were subjected. Their nutritional composition showed better content in the DRC beans according to NMX-F-013-SCFI-2010; while both infusions (presented variations in sensory attributes allowing the classification of the MRC coffees as a specialty while DRC as premium. The infusions of the coffees presented antioxidant activity as an important beneficial health source among their consumers without cytotoxic effects. The conditions of cultivation, the proportions of the varieties in the mixture, the processing of the beans, and the extraction of the infusions are variables that must be considered in the study of coffee. These parameters are related to the presence of major chemical compounds, CGA and caffeine, present in the beans and infusions. CGA was degraded by the effect of roasting, while caffeine and melanoidins were increased although below the suggested levels by the norm. The results obtained show the current conditions that will serve as a basis for improvements considering the suggestions established within the Standards for green and roasted coffee trying to preserve its biological potential.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org., Figure S1: Chemical structure of chlorogenic acid isolated from the infusion of beans *Coffea arabica* variety Oro Azteca; Table S1: ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) Spectroscopic Data of chlorogenic acid **(**MeOH-d_4, δ, ppm, J/Hz)

Author Contributions: Conceptualization, F.C-S. and P.N-T.; methodology, J.G-I., A.S., R.S., M.M-R., S.C-H., M.G-C. and O.O-M.; formal analysis, J.G-I., A.S., and P.N-T.; writing—original draft preparation, J.G-I., A.S., P.N-T., and F.C-S; writing—review and editing, J.G-I., A.S., R.S., M.M-R., S.C-H., M.G-C., O.O-M., F.C-S. and P.N-T.; supervision, F.C-S. and P.N-T; project administration, F.C-S. and P.N-T; All authors have read and agreed to the published version of the manuscript.

Funding: The first author (J.G-I) acknowledges that this work was supported Consejo Nacional de Ciencia y Tecnología of Mexico (CONACYT-Mexico), for the Basic Grant 58714 for his Doctoral studies at the Biotechnology Doctoral Program of Universidad Autónoma Metropolitana-Iztapalapa.

Data Availability Statement: All data generated during this study are included in this published article.

Acknowledgments: To the CIBIS-IMSS for the use of its facilities and materials to achieve the part of the work done.

Conflicts of Interest: The authors declare no conflict of interest.

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