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Influence of coffee roasting degree on antioxidant and metabolic parameters: Comprehensive *in vitro* and *in vivo* analysis

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ABSTRACT

This study aimed to assess the impact of roasting degree on antioxidant and metabolic parameters *in vitro* and *in vivo*. *In vitro*, we evaluated radical scavenging, lipid peroxidation, and the activity of digestive enzymes (α-glucosidase, α-amylase, and lipase). *In vivo*, we first examined coffee's effect on carbohydrate and lipid absorption in healthy rats, followed by a chronic evaluation of metabolic disorders and antioxidant markers using a diet-induced obesity model. *In vitro* results revealed that increased roasting degree reduced the antioxidant capacity of coffee brews. All brews showed lower inhibition of α-glucosidase and α-amylase, and lipase inhibition compared to the positive control (acarbose or orlistat). *In vivo*, all roasting degrees consistently reduced postprandial glucose levels by 20%. Notably, coffee with a high roasting degree (HRD) decreased serum triglycerides (TG) by ~44% after a lipid load, while other roasts did not. Chronic administration of unroasted (UN) or HRD coffee significantly reduced weight gain compared to the obese control (~15% and ~10%, respectively). Notably, all coffee samples improved lipid metabolism parameters. UN and HRD coffee significantly decreased adipocyte volume by 58% and 48%, respectively, compared to the obese control. Additionally, all groups exhibited less than 30% hepatic lipid droplets independent of roasting degree. HRD treatment notably increased liver catalase (CAT) activity and reduced lipid peroxidation in serum (~90%), liver (~59%), and adipose tissue (~37%) compared to the obese control group. These findings suggest that HRD in coffee may confer certain biological advantages.

1. Introduction

Coffee remains one of the world's most popular beverages, experiencing a steady increase in both production and demand. Global coffee output reached 174.5 million bags in the 2020/21 crop year (Setiyorini et al., 2023). The *Coffea arabica* is the main commercial variety of coffee, accounting for 70% of global production. In this regard, Latin American countries such as Colombia, Honduras, Peru, and México are market leaders (International Coffee Organization (ICO), 2022). México, the tenth-largest coffee producer worldwide, supplies a quarter of market

Abbreviations: UN, Unroasted; MRD, medium roasting degree; HRD, high roasting degree; MRP, Maillard reaction products; OSTT, Oral Starch Tolerance Tests; OGTT, Oral Glucose Tolerance Tests; AT, adipose tissue; OLTT, Oral Lipid Tolerance Tests; FFAs, free fatty acids; MDA, malondialdehyde; CAT, catalase; SOD, superoxide dismutase; TG, Triglycerides.

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demand, and is the leading producer of organic coffee (Gumecindo-Alejo et al., 2021). In recent years, several studies have reported bioactive effects associated with habitual coffee consumption. This has shifted the perception of coffee from a luxury stimulant to a health-promoting natural product, especially when consumed within usual levels of intake. Among the positive effects reported for coffee are anti-inflammatory and antioxidant effects, and improvement on glucose metabolism, obesity, metabolic syndrome, and type 2 diabetes (Santos and Lima, 2016). The biological activity of coffee is attributed to various bioactive components such as chlorogenic, ferulic, and caffeic acids, as well as epicatechin, catechin, anthocyanins, alkaloids like trigonelline and caffeine, and melanoidins. These constituents are responsible for the bioactive potential of coffee (Gallardo-Ignacio et al., 2023). Some of these bioactive compounds are generated during the coffee beans roasting process (Bastian et al., 2021), which involves the depletion of phenolic compounds and the formation of Maillard reaction products (MRP), altering the bioactivity of coffee (Alongi and Anese, 2018). There is ongoing debate about whether the roasting process affects positively, negatively, or negligibly the bioactive potential of coffee. For instance, Jung et al. (2017) reported that *in vitro* antioxidant and anti-inflammatory properties of coffee are negatively correlated with roasting levels. In contrast, Choi et al. (2018) found that light- and medium-roasted coffee increase the expression of antioxidant liver enzymes and decrease the expression of pro-inflammatory molecules. Moreover, we have previously observed a positive role of *Coffea canephora* in restraining obesity and related metabolic disorders, but the effect of roasting varied depending on the metabolic pathway and relevant markers (Gamboa-Gómez et al., 2023).

The variations in the impact of the roasting process on the bioactive potential of coffee can be attributed not only to the degree of roasting and the processing conditions. Most authors categorize roasting as light, medium, high, or very high based on the color changes and weight loss (Clarke et al., 1987) experienced by coffee during the process. However, the parameters leading to the same roasting degree, such as temperature and duration, can vary significantly among processes, depending on several factors, including the variety of coffee, the equipment available, and the scale of the experiment (Gallardo-Ignacio et al., 2023; Alongi and Anese, 2018; Choi et al., 2018). Depending on roasting parameters and coffee variety, the composition in bioactive compounds, such as phenolic compounds and MRP, of differently roasted coffees can vary considerably, as thus their potential bioactivity.

Despite these findings, controversy persists about the impact of roasting on coffee's bioactive properties, justifying further investigation. Therefore, this study aims to evaluate whether the roasting degree of Mexican *C. arabica* coffee influences antioxidant and metabolic parameters. To this end, we conducted *in vitro* assessments that included radical scavenging and lipid peroxidation for antioxidant capacity. Additionally, the activity of digestive enzymes such as α-glucosidase, α-amylase, and lipase was evaluated. For the *in vivo* assessment, obesity-associated metabolic disorders and antioxidant markers were examined.

2. Materials and methods

2.1. Coffee roasting process

Unroasted (UN) coffee beans (*C. arabica* L.) from the 2020 harvest in Veracruz, México, were generously donated by local farmers. Samples were identified by botanist Dr. Socorro Gonzalez of the Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional (CIIDIR) Durango herbarium and assigned voucher number 62782, in the collection of cultivated plants.

The roasting process was performed in an oven equipped with a needle valve to regulate gas pressure, which helps control the temperature (MT Maquinaria, Puebla, Mexico). Additionally, the oven has an air-based cooler that aids in maintaining the desired roasting conditions. For a medium roasting degree (MRD), hot air circulation at 240 °C was maintained for 4 min and 10 s. For a high roasting degree (HRD), the roasting was extended to 5 min and 10 s. Afterward, samples were quickly cooled and ground in a mill (Hamilton Beach 80335Rv, Richmond, Virginia, USA) to obtain a particle size of approximately 0.850 mm using a sieve adapted to the mill. Samples were stored at −20 °C in double-zipper Ziploc bags until analyses. Although sample particle size is a crucial parameter in the solid–liquid extraction of natural products for obtaining bioactive compounds, and smaller particle sizes have been reported to exhibit significantly stronger antioxidant activity (Prasedya et al., 2021), we used a particle size consistent with that used by the local farmers who donated the sample for this research. This particle size is also how they commercialize the coffee.

2.2. Moisture and color determination of coffee beans with different roosting degrees

The moisture content of roasted coffee beans was determined using the gravimetric method according to AOAC guidelines (AOAC®, 1995). Aliquots (1 g) of ground coffee were dried overnight at 75 °C under vacuum (Vuotomatic 50, Bicasa, Milano, Italy). The moisture content was calculated as the percentage ratio of the weight loss to the initial weight.

Color measurements were performed using a tristimulus colorimeter (Chromameter-2 Reflectance, Minolta, Osaka, Japan) equipped with a CR-300 measuring head. Ground coffee was compacted into a Petri dish to create a uniform layer with a thickness of 10 mm. The samples were positioned on white cardboard, and the colorimeter head was oriented perpendicular to the sample surface. Color values were expressed in CIE units, including L* (lightness/darkness), a* (redness/greenness), and b* (yellowness/blueness). Multiple measurements were taken at various points on each sample to ensure accuracy and reliability.

2.3. Coffee brews preparation with beans of different roosting degrees

Coffee brews were prepared according to the methodology reported by Gallardo-Ignacio et al. (2023). Briefly, extraction was performed using a French press with 6.6 g of coffee powder (equivalent to one tablespoon) per 100 mL of water.

The freeze-drying process releases the volatile compounds and preserves other bioactive compounds in coffee brews, maintaining bioactive properties such as enhanced antioxidant activity (Bettaieb et al., 2024). In this regard, the coffee brews were subsequently freeze-dried using the FreeZone 18 L Console Freeze Dry System (Kansas, USA), and the samples were stored in amber vessels until use.

2.3.1. Coffee brews yields

Coffee brew yield ($Y^{\%}$) was determined as follows:

$$Y^{\%} = [(\text{lyophilized coffee brew}_{(g)})/(\text{coffee powder}_{(g)})]*100 \quad (1)$$

Results are reported as means of two independent preparations.

2.4. Chemical characterization of coffee brews

2.4.1. Chlorogenic acid, caffeic acid and caffeine quantification

HPLC analysis was conducted using a Waters 2695 separation module (Waters, Milford, MA, USA) equipped with a Waters 2996 photodiode array detector (Waters, Milford, MA, USA). The separation was performed on an RP C-18 Superspher (Merck) column (250 × 4 mm; 5 μm) (Merck, Darmstadt, Germany). The gradient system was as follows: 0–1 min, 0% B; 2–3 min, 5% B, 4–20 min, 30% B; 21–23 min, 50% B; 24–25 min, 80% B; 26–27 min, 100% B. Finally, initial conditions of 0% B were returned during the 28–30 min. The flow rate was maintained at 0.9 mL/min, and the sample injection volume was 10 μL. Bioactive compounds like chlorogenic acid and caffeic acid were measured at 325 nm and caffeine was identified and quantified at 280 nm.

Five ascendant concentrations (12.5, 25, 50, 100, and 200 μg/mL) of a commercial standard of chlorogenic and caffeic acid, and (6.25, 12.5, 25,50, and 100 μg/mL) for caffeine (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) were injected by triplicate in the chromatographic method to build the calibration curves ($Y = 11702X + 19276$, $R^2 = 0.9982$; $Y = 54416X + 24248$, $R^2 = 0.9994$; $Y = 87483X + 38786$, $R^2 = 0.9993$) respectively.

2.4.2. Maillard reaction products determination

A non-specific marker of the Maillard reaction, involving the absorbance monitoring of various stages of coffee browning reaction was employed, as previously reported in the literature (Alongi et al., 2021) was employed. Early non-colored compounds were tracked at 280 nm, followed by the observation of more advanced compounds at 360 nm. Finally, high molecular weight compounds, specifically melanoidins, were assessed at 420 nm using a spectrophotometer (UV-2501PC, UV–VIS Recording Spectrophotometer, Shimadzu Corporation, Kyoto, Japan). The Maillard reaction indices (MRI) were estimated following the equation:

$$\mathrm{MRP} = ABS_{\lambda} \times F_D \quad (2)$$

Where ABS_{λ} represents the absorbance at the specified wavelength and F_D denotes the dilution factor.

2.5. In vitro assessment of coffee with different roasting degree

2.5.1. Antioxidant capacity

The antioxidant activity was assessed by DPPH assay, conducted using the methodology described by Xu & Chang (Xu and Chang, 2007). Additionally, the ABTS assay was performed as previously reported by Re et al. (1999). Lipid peroxidation was evaluated using the methodology previously described by Rocha-Guzmán et al. (2018). Results were expressed as Trolox equivalents. This water-soluble derivative of vitamin E, known for its antioxidant properties and simple structure, is widely used as a positive control in evaluating antioxidant activity. Additionally, antioxidant activity measured in Trolox equivalents can be accurately converted to other standard antioxidant compounds (Hwang and Lee, 2023).

2.5.2. Inhibition of digestive enzymes

The assessments of enzymatic inhibition for lipase, α-amylase, and α-glucosidase were carried out following the methodologies described by McDougall et al. (2009), Tamil et al. (2010), and Apostolidis et al. (2007), respectively. Dimethyl sulfoxide (DMSO) was used as a diluent. The positive control used for lipase assessment was Orlistat (Redustat®, Laboratorios Liomont, CDMX, Mex.) was selected as the positive control for lipase inhibition, while acarbose (Laboratorios Alpharma, CDMX, Mex.) was chosen for α-amylase and α-glucosidase inhibition. Orlistat, a lipophilic molecule, inhibits gastric and pancreatic lipases by covalently binding to the enzyme's active-site serine. Acarbose, on the other hand, is structurally similar to oligosaccharides and inhibits both α-amylase and α-glucosidase. However, both drugs cause significant gastrointestinal side effects, limiting their clinical use (Filippatos et al., 2008; Coniff and Krol, 1997). Consequently, plant-based lipase and glucosidase inhibitors with fewer side effects are emerging as promising alternatives for regulating lipid and carbohydrate absorption.

Results were expressed as the inhibition percentage (I%), which was estimated following the equation:

$$I\% = [ABS_0 - ABS_1)/ABS_0]*100 \quad (3)$$

where ABS_0 is the blank absorbance and ABS_1 is the absorbance for each coffee sample.

The median inhibitory concentration (IC_{50}) was determined by plotting the $I^{\%}$ against the logarithm (Log) of the sample concentration (Coruh et al., 2007).

2.6. In vivo assessment of coffee with different roasting degree

2.6.1. Experimental animals

Experiments were conformed to regulations established by the National Institutes of Health (NIH) (National Institutes of Health, 2002) and the Norma Oficial Mexicana (Norma Oficial Mexiana NOM-062-ZOO-1999). Furthermore, the study procedure was registered and authorized by the Research Committee of the Mexican Social Security Institute (R-2023-785-067).

The Bioterio of the "Centro Médico Nacional Siglo XXI" in Mexico City donated 32 female Wistar rats for acute assessment and 32 male Sprague Dawley rats for chronic assays.

In the acute test, since results can be obtained in just one day, using female rodents and this strain does not affect the outcomes because the overall energy balance is similar in both sexes and strains (Mauvais-Jarvis et al., 2017). However, for a chronic study on induced obesity, male rodents are generally preferred as they better exhibit metabolic diseases. Additionally, the Wistar strain is considered the most suitable model for studies on diet-induced obesity (Mauvais-Jarvis et al., 2017; Miranda et al., 2018).

All rats were 12 weeks old and housed in a controlled environment with a 12-h light-dark cycle at 25 ± 1 °C. A one-week acclimatization period was established before the experiments began. Throughout this period and during the experiments, the rats had unrestricted access to Rodent Lab Chow 5001 (Purina®, Québec, Canada).

2.6.2. In vivo acute assessment

2.6.2.1. Lipid and carbohydrates digestion and absorption. The effect of coffee roasting degree on the digestion and absorption of carbohydrates and lipids was evaluated through the oral starch tolerance text (OSTT) and the oral lipid tolerance test (OLTT). The negative control for OSTT was starch suspension (3 g/kg of body weight), while acarbose was the positive control (5 mg/kg of body weight, equivalent to the human dose of 50 mg consumed during a meal).

In the case of OLTT, the negative control was a lipid solution (corn oil/lard, 1:1) (at dose of 10 mL/kg of body weight), while orlistat (6 mg/kg of body weight, equivalent to the human dose of 60 mg consumed during a meal) was used as the positive control (Gamboa-Gómez et al., 2022).

The coffee dose to be administered to rats was based on an adult human consumption of 240 mL of coffee brew, i.e., approximately 1.6 cups, corresponding to 2.5 g lyophilized coffee brew, and considering an average adult human weight of 60 kg (Gallardo-Ignacio et al., 2023). The animal dose equivalent (mg/kg body weight) was thus calculated based on the equation reported by Reagan-Shaw et al. (2008):

$$\text{Animal dose equivalent} = [\text{Adult}_{\text{human dose}}]\,[\text{Km}_{\text{(adult human)}} / \text{Km}_{\text{(animal: rat)}}] \quad (4)$$

the adult human dose of lyophilized coffee brew was 41.67 mg/kg body weight; and Km represents the conversion values, which vary across different animal species and human age stages and correspond to 6 for rats and 37 for adult humans. The animal dose equivalent was 257 mg of lyophilized coffee brew per kg of body weight.

Under fasting conditions, groups of rats (n = 8) were administered either starch for the OSTT or the lipid solution for the OLTT. These treatments were combined with coffee samples, acarbose, or orlistat, depending on the corresponding group, via an intragastric tube. Blood samples were collected from the rats' lateral tail vein at baseline and at 30, 60, and 120 min for the OSTT or at 1 and 3 h for the OLTT after the administration of treatments (Gamboa-Gómez et al., 2022). Glucose concentrations were determined using a glucometer (Stat Strip® Glucose, Nova Biomedical, Waltham, MA, USA). Serum for TG measurements was obtained from blood samples, and TG levels were assessed using a commercial enzymatic kit (Biosystem Laboratories,

Barcelona, Spain), following the manufacturer's instructions.

The onset time, peak levels, and area-under-the-curve (AUC) of glucose and TG variation were analyzed to determine the relative rate of carbohydrate or lipid digestion and absorption.

2.6.3. *In vivo chronic assessment*

To evaluate the chronic bioactive potential of coffee with varying degrees of roasting, we utilized an induced obesity model. The obesity-promoting diet comprised 13.9% protein, 15.1% lipids, with 27% of saturated fat derived from lard, and 71% carbohydrates, including 46% from corn crystalline fructose. The diet's caloric content was approximately 511.1 kcal/100 g.

All experimental groups received the same obesogenic diet. The rats were divided into the following groups (n = 8), receiving: (1) an inert vehicle (drinking water) as the obese control, (2) brew from unroasted coffee (UN), (3) brew from medium roasted degree (MRD), and (4) brew from high roasted degree (HRD). The same coffee dose as in the acute assessment was used. The coffee brew treatments were administered via an orogastric tube in the early morning, specifically during the final hour of the dark phase. This timing was selected to coincide with the anticipated peak concentration of caffeine at the end of the dark phase when the animals are still awake. The rationale behind this choice was to minimize any additional wakefulness, considering the known half-life of caffeine, which is approximately 1 h (Olini et al., 2013). Our objective was to mitigate the presence of caffeine in the circulatory system once the animals entered their primary sleep period. This approach is critical as circadian misalignment, such as activity or feeding during the usual sleep phase, is known to disrupt metabolic rhythms and can lead to metabolic abnormalities like obesity and type 2 diabetes mellitus (Jha et al., 2015). By administering the treatment during the dark phase when rodents are naturally active, we aimed to align with their innate circadian rhythm, potentially reducing unwanted metabolic disturbances that could confound the results. However, this timing may also enhance the metabolic alterations in our animal model, reflecting real-world scenarios where irregular feeding times contribute to metabolic dysregulation (Jha et al., 2015). Thus, while the study design aims to mitigate additional wakefulness, it simultaneously considers the impact of circadian timing on metabolic outcomes, providing a nuanced understanding of how coffee consumption might affect metabolic and antioxidant parameters under disrupted circadian conditions.

Throughout the 36-week trial period, the rats had free access to water and food. Weekly measurements of body weight gain and daily documentation of food and water consumption were recorded.

Before euthanasia (1 week later), an oral glucose tolerance test (OGTT) was conducted following the methodology outlined by Udia et al. (2013) with some modifications. After an 8-h fasting period, blood samples were collected from the rat's lateral tail vein at 30, 60, and 120 min following a glucose load of 2 g/kg body weight. Glucose concentration was measured using a glucometer (Stat Strip® Glucose, Nova Biomedical, Waltham, MA, USA). The onset time, peak levels, and AUC of glucose variation were analyzed to assess the relative rate of glucose metabolism in obese rats.

To euthanize the rats at the end of the experimental period, under fasting conditions (8 h), sodium phenobarbital was injected at doses of 50 mg/kg of body weight for anesthesia, followed by thoracotomy. Subsequently, the left ventricle was exposed, and blood was drawn with a syringe, then to obtain serum, samples were centrifuged 15 min at 3000×*g*. The liver and adipose tissue (AT) were promptly extracted and washed with a cold commercial sterile saline solution (~2 °C, 0.9% NaCl, pH 7) (Pisa®, Jalisco, Mexico) to maintain adequate pH levels. Subsequently, the organs were dried, weighed, portioned, and frozen in liquid nitrogen for future analysis. Additionally, a segment of both the liver and AT was immersed in a 10% formalin solution for further histological examination.

Serum measurements included fasting glucose, triglycerides (TG), high-density lipoprotein (HDL-c), total cholesterol (TC), and very-low-density lipoprotein cholesterol (VLDL-c), aspartate aminotransferase (AST) and alanine aminotransferase (ALT), which were assessed using a commercial assays kit and an automated A15 spectrophotometer (Biosystem Laboratories, Barcelona, Spain).

Additionally, the serum concentration of free fatty acids (FFAs) was determined following the methodology reported by Falholt et al. (1973), and the results were expressed in μg of palmitic acid equivalents/mL. Palmitic acid, which constitutes 20–30% of total FFAs, is well incorporated into adipose tissue, making it a suitable standard (Murru et al., 2022).

Furthermore, changes in insulin sensitivity were estimated through the product of TG and glucose (TyG) index calculated as follows (Guerrero-Romero et al., 2010):

$$\text{Ln}\ [\text{fasting TG}_{(\text{mg/dL})} \times \text{fasting glucose}_{(\text{mg/dL})}/2] \quad (5)$$

We chose the TyG index due to their high sensitivity and specificity is comparable to the Euglycemic-Hyperinsulinemic, Clamp for assessing insulin resistance (Guerrero-Romero et al., 2010). In addition, one of the main advantages is that fasting state of glucose and triglycerides levels for TyG index assessment not insulin levels are required that make it less costly and accessible.

2.6.3.1. Lipid content and adipose hypertrophy assessment. Adipose tissue homogenate was obtained by pulverizing it with liquid nitrogen and subsequently homogenizing it in a phosphate buffer (50 mM, pH 7) containing EDTA (0.5 mM). The protein concentration was then determined using the Bradford method (Bradford, 1976).

The adipose TG and FFAs contents were determined using the methodology described by Folch et al. (1957) and Falholt et al. (1973), respectively. To adjust variations in tissue amount and ensure that the subsequent measurements of TG and FFA are accurate and reflective of the tissue's actual content, rather than being influenced by differences in sample size or tissue density results were expressed as μg/mg of protein.

The AT hypertrophy was assessed by evaluating cell size following the methodology described by Booth et al. (2008). Briefly, 10% formalin preserved samples were embedded in paraffin, and 4–5 μm-thick tissue sections were prepared for histological analysis. These sections were stained with hematoxylin and eosin and observed under a 40x magnification microscope. For each animal, five images were captured, and ten fields were evaluated in each section. A pathologist (J.L.G.) conducted the histological evaluation. Adipocytes were examined via systematic random sampling, and the radius (*r*) of each cell was manually measured using a micrometer. Subsequently, the cell volume (*V*, picolitres) was calculated using Equation (6).

$$V = 1/4(3\pi r^3) \quad (6)$$

2.6.3.2. Liver lipid content and steatosis. The TG and FFAs concentration were determined as previously described in section 2.6.3.1.

For steatosis evaluation, liver samples preserved in formalin were embedded in paraffin, and 4–5 μm-thick tissue sections were prepared and stained with hematoxylin and eosin. Five images were captured from each section, and ten fields were assessed per image. J.L.G. conducted a histological evaluation of the liver tissue. The severity of liver steatosis and inflammatory cell infiltration was assessed using a semi-quantitative scoring system reported by Harb et al. (2019). Macro vesicular liver steatosis was quantified as the proportion of hepatocytes containing fat droplets that were the same size as or larger than the nucleus and often displaced the nucleus. The number of focal inflammatory cell infiltrates was also determined.

2.6.3.3. Oxidative stress markers. An evaluation of oxidative stress markers was performed in serum, and adipose and liver tissues. Lipid peroxidation was assessed following the methodology of Rocha-Guzmán

et al. (2018). Results were expressed as μmol of malondialdehyde (MDA) equivalents per milligram of protein, using a 1,1,3,3-Tetraethoxypropane (TEP) calibration curve prepared by diluting a standard MDA solution [(hydrolysis of 1,3,3,3-TEP 99% (20.5 μL) in 39% HCl (200 μL)].

The activity of antioxidant enzymes, including catalase (CAT) and superoxide dismutase (SOD), was evaluated using methods described by Sinha (1972) for CAT and Ukeda et al. (1998) for SOD, respectively.

Regarding SOD, one unit (U) of enzyme activity was defined as the enzyme amount required to inhibit 50% of the initial rate of blue tetrazolium chloride (NBT) reduction. Then, the enzyme activity was expressed as U/mg of protein and was calculated based on the percentage of inhibition (equation (1)) and the following Equation (7):

$$\text{SOD activity} = [(I^{\%})/(50\%)(\text{sample volume})][(1/(\text{Protein content of sample})] \quad (7)$$

To evaluate CAT activity, a calibration curve was constructed using H_2O_2. One unit (U) of activity was defined as the consumption of one μmol of H_2O_2 consumed during 1 min.

The results were expressed as U/mg of protein using the following equation:

$$\text{CAT activity} = (\Delta\mu\text{mol of } H_2O_2/(\text{incubation time})(\text{sample volume}))^* (\text{ABS of } H_2O_2 \text{ at } 0.2\text{M/ABS blank})^*(1/\text{Protein content of sample}) \quad (8)$$

Where:

$\Delta\mu$mol of H_2O_2 = μmol of H_2O_2 (Blank) - μmol of H_2O_2 (Reaction)

Incubation time (min) = 3.

(ABS of H_2O_2 at 0.2M/ABS Blank) = Factor to allow for any deviation in H_2O_2 substrate concentration.

2.7. Statistical analysis

The statistical analysis performed aimed to assess the data normality was the Shapiro-Wilk test.

Parametric data were analyzed using Tukey's post-hoc test.

The results were presented as mean ± standard error (SE). A significance level of $p < 0.05$ was used to determine statistical significance.

The IBM SPSS Statistics for Windows, version 20 (IBM Corp., Armonk, New York, USA) were employed for the statistical analyses.

3. Results

3.1. Moisture, color, and chemical characterization

Results moisture and color of coffee beans are shown in Table 1. As expected, moisture significantly decreased with increasing roasting intensity (0.5-fold for MRD and 0.3-fold for HRD compared with UN). Compared with UN, color decreased in lightness (L*) and increased in redness (a*) (7-fold increase for MRD and 5- fold increase for HRD). Regarding b* values, both roasted coffees showed a tendency towards colors with more yellow tones.

Regarding the coffee brew yields, UN, MRD, and HRD were approximately 19%, which is equivalent to ~12 mg of lyophilized sample per mL of coffee brew.

Regarding the MRI results, Table 2 illustrates the presence of early non-colored compounds (monitored at 280 nm), more advanced Maillard reaction products (monitored at 360 nm), and advanced high molecular weight compounds (monitored at 420 nm) in coffee brews with varying degrees of roasting. Some significant differences in MRI were found between MRD and HRD, such as for example MRI 420 of 7.4 in MRD and 6.4 in HRD. However, these differences were negligible, (less than 10%), especially when comparing the MRI profile of MRD and HRD with that of UN, which in all cases presented considerably lower values. These results indicate primarily the presence of differing amounts of early and high molecular weight compounds in MRD and HRD, particularly melanoidins, differently from UN.

Chemical characterization identified and quantified bioactive compounds such as chlorogenic acid, caffeic acid, and caffeine (Fig. 1). Both, chlorogenic (~85% for MRD and ~92% for HRD) and caffeic acid (~33% for MRD and ~96% for HRD) levels progressively decreased with an increase in the degree of roast, while caffeine levels were not affected by the roasting process.

Table 1
Moisture and L*, a* and b* values of unroasted, dark, and very dark roasted coffee powders.

Roasting degree	Roasting time (min)	Moisture (%)	Color		
			L*	a*	b*
UN	0	8.72 ± 0.02^{a}	58.19 ± 0.40^{a}	0.75 ± 0.06^{c}	13.33 ± 0.49^{a}
MRD	4.3	4.59 ± 0.00^{b}	23.35 ± 0.33^{b}	5.01 ± 0.09^{a}	2.51 ± 0.17^{b}
HRD	5.1	3.22 ± 0.01^{c}	22.17 ± 0.16^{c}	4.04 ± 0.13^{b}	1.12 ± 0.10^{c}

Data presented as Mean ± Standard Error. Between rows, different letters (a-b) indicate a significant difference between study groups determined by Tukey's test ($p < 0.05$). Unroasted (UN), Medium Roasted Degree (MRD), and High Roasted Degree (HRD) coffee brews samples.

3.2. In vitro assessment

3.2.1. Antioxidant activity

The results of antioxidant activity *in vitro* are shown in Fig. 2. It can be observed that UN had the highest effect in the three assessments: ABTS (~0.8-fold), DPPH (~0.7-fold), and lipid peroxidation (~0.5-fold), compared with the roasted counterparts.

3.2.2. Inhibition of digestive enzymes

Regarding digestive enzymes, all coffee brews showed lower inhibition percentages of α-glucosidase and α-amylase than the positive control (acarbose) (Fig. 2).

In concordance, the IC_{50} results showed that all coffee brews had higher values than acarbose (~4-fold for α-glucosidase and 6-fold for α-amylase) (Table 3).

In relation to lipase enzyme inhibition, all coffee brews showed lower inhibition percentages than the positive control (orlistat) (~22%) (Fig. 2). In concordance, IC_{50} results showed higher values than orlistat for all coffee brews (~3.5-fold).

3.3. In vivo acute assessment

3.3.1. Carbohydrate and lipid absorption in normal weight healthy rats

Results of OSTT and OLTT are shown in Fig. 3. After 120 min of starch administration, rats administered UN, MRD, or HRD exhibited a decrease in serum glucose concentration compared with the negative group (~20%). However, this decrease in serum glucose was lower to that with the positive control (acarbose). These results were consistent with the AUC values.

Regarding OLTT results and their AUC, only rats that received HRD

Table 2
Maillard reaction indices (MRI) of compounds monitored at 280, 360 and 420 nm.

Sample	MRI_{280}	MRI_{360}	MRI_{420}
UN	68.45 ± 1.06^{b}	55.40 ± 0.57^{a}	2.19 ± 0.10^{c}
MRD	152.00 ± 7.07^{a}	52.15 ± 0.07^{a}	7.42 ± 0.16^{a}
HRD	113.00 ± 15.56^{a}	38.40 ± 3.25^{b}	6.40 ± 0.18^{b}

Data presented as Mean ± standard error. Between rows, different letters (a-b) indicate a significant difference between study groups determined by Tukey's test ($p < 0.05$). Unroasted (UN), Medium Roasted Degree (MRD), and High Roasted Degree (HRD) coffee brews samples.

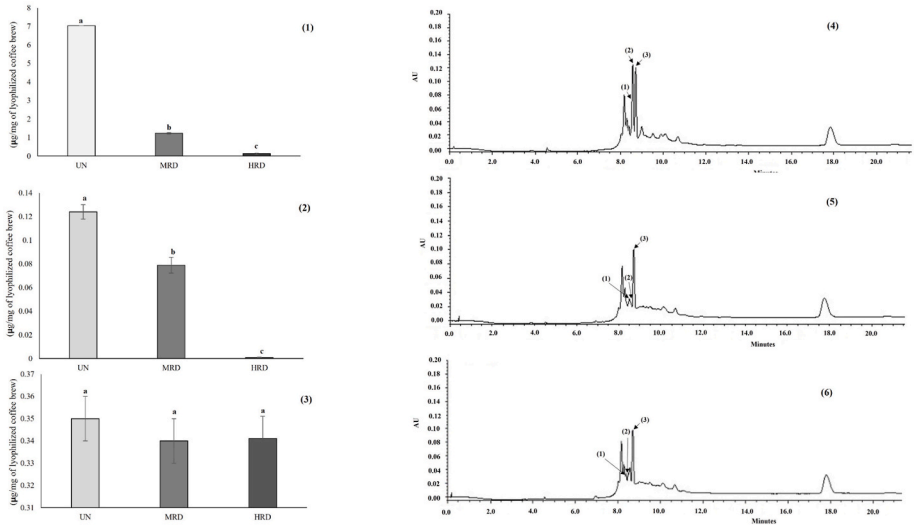

Fig. 1. Chemical characterization: (1) Chlorogenic acid, (2) caffeic acid, (3) caffeine quantification, and representative chromatogram: (4) Unroasted (UN), (5) Medium Roasted Degree (MRD), and (6) High Roasted Degree (HRD) coffee brews samples. The numbers displayed above each peak of each chromatogram represent the corresponding compounds: 1- chlorogenic acid, 2- caffeic acid, and 3-caffeine.

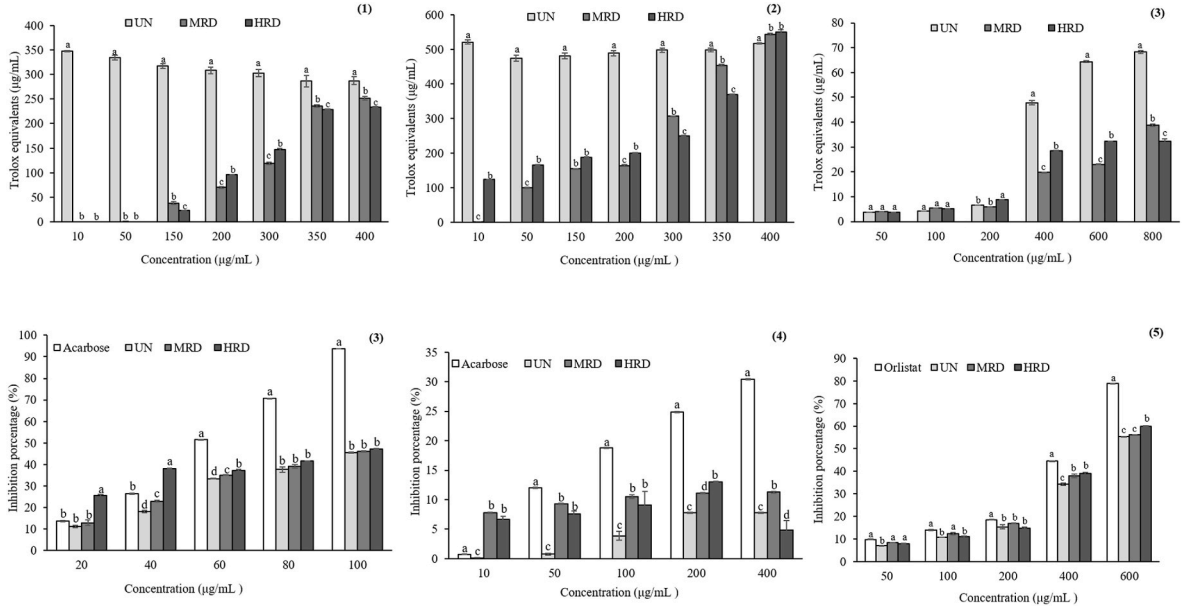

Fig. 2. *In vitro* antioxidant activity (1) ABTS, (2) DPPH, and (3) lipid peroxidation assessment, along α-glucosidase (4), α-amylase (5), and lipase (6) enzyme inhibition evaluation of Unroasted (UN), Medium Roasted Degree (MRD), and High Roasted Degree (HRD) coffee brews samples. Acarbose and orlistat were used as positive controls for enzyme inhibition assessment. Values are expressed as mean ± standard error. Different letters (a–c) assigned to the columns in each figure indicate a significant difference ($p < 0.05$) between the study groups. Significance was determined using analysis of variance (ANOVA) followed by Tukey's test.

Table 3
Inhibitory median concentration (IC_{50}, in μg/mL) of digestive enzymes.

Enzyme	Orlistat	Acarbose	UN	MRD	HRD
Lipase	4.0 ± 0.01^{c}	–	6.24 ± 1.0^{b}	15.3 ± 1.0^{a}	5.6 ± 1.2^{b}
α-amylase	–	562.3 ± 3.6^{c}	3194.9 ± 37.^{a}	3421.6 ± 42.6^{a}	5342.0 ± 42.0^{b}
α-glucosidase	–	48.9 ± 1.1^{c}	143.6 ± 14.8^{b}	162.5 ± 11.2^{b}	213.8 ± 4.9^{a}

Mean ± standard error. Between rows, different letters (a-c) indicate a significant difference between study groups determined by Tukey's test ($p < 0.05$). Unroasted (UN), Medium Roasted Degree (MRD), and High Roasted Degree (HRD) coffee brews samples.

treatment showed a decreased in serum TG after 3 h of lipid load compared with the negative control (~44%). However, this decrease was lower to that with the positive control (orlistat). On other hand, the AUC for MRD and HRD was statistically similar between, but lower than the positive control.

3.4. *In vivo chronic assessment*

3.4.1. *Weight gain, glucose metabolism and serum lipid profile*

After 36 weeks on an obesogenic diet, rats administered with UN or HRD showed a significant reduction in weight gain compared to the obese control group (~15% and ~10%, respectively) (Fig. 4).

In terms of glucose metabolism, none of the treatments exhibited a significant difference in serum glucose concentrations after 120 min of

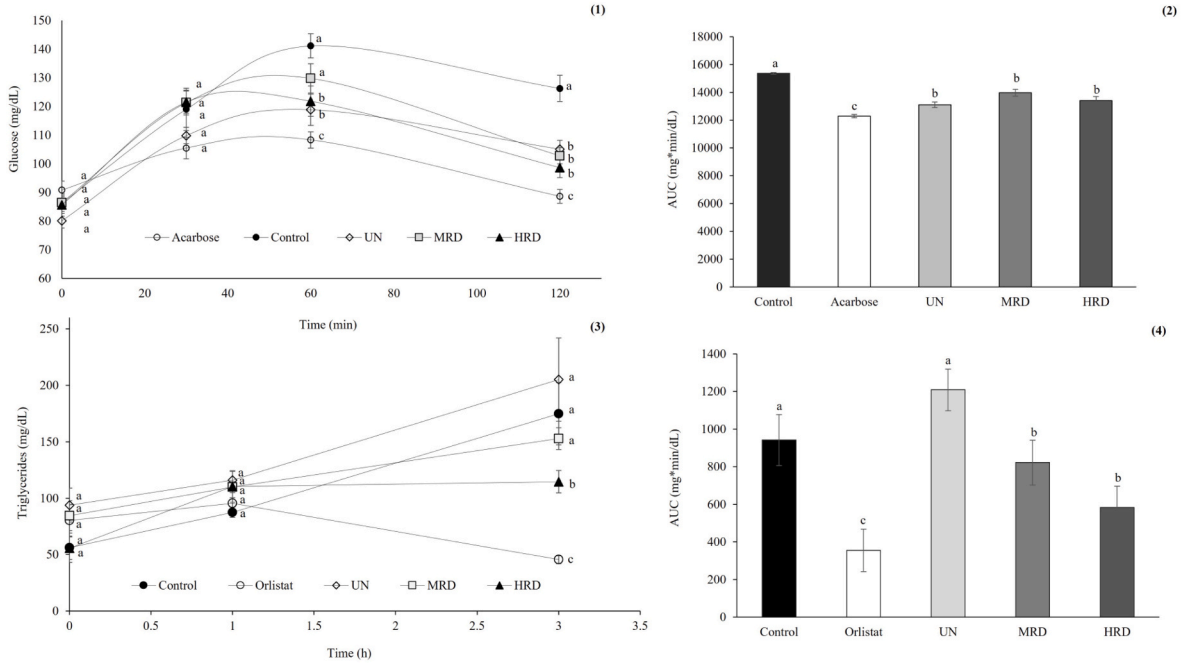

Fig. 3. OSTT (1), and Area-under-time-curve for OSTT (2), OLTT (3), and Area-under-time-curve for OLTT (4) of healthy rats that were administered with Unroasted (UN), Medium Roasted Degree (MRD), and High Roasted Degree (HRD) coffee brews samples. Acarbose and Orlistat were used as positive controls. Values are expressed as Mean ± Standard Error. Different letters (a–c) in each figure indicate a significant difference ($p < 0.05$) between the study groups. Significance was determined using analysis of variance (ANOVA) followed by Tukey's test.

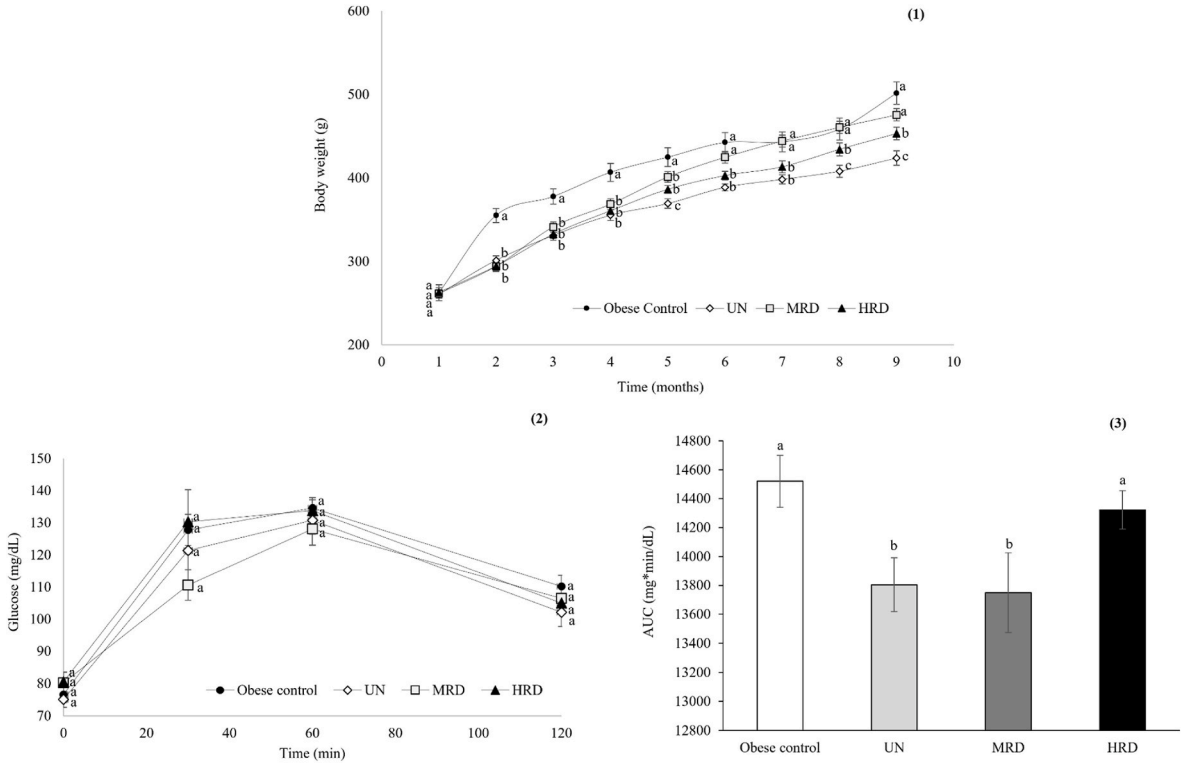

Fig. 4. OSTT (1), and Area-under-time-curve for OSTT (2), OLTT (3), and Area-under-time-curve for OLTT (4) of healthy rats that were administered with Unroasted (UN), Medium Roasted Degree (MRD), and High Roasted Degree (HRD) coffee brews samples. Acarbose and orlistat were used as positive controls. Values are expressed as mean ± standard error. Different letters (a–c) in each figure indicate a significant difference ($p < 0.05$) between the study groups. Significance was determined using analysis of variance (ANOVA) followed by Tukey's test.

Table 4
Serum metabolic parameters in obese rats administered with Unroasted (UN), Medium Roasted Degree (MRD), and High Roasted Degree (HRD) coffee brews samples.

	Obese control	UN	MRD	HRD
Glucose (mg/dL)	168.0 ± 14.9[a]	169.1 ± 8.6[a]	166.5 ± 10.3[a]	158.5 ± 18.2[a]
Triglycerides (mg/dL)	153.4 ± 5.1[a]	92.3 ± 4.1[b]	116.7 ± 4.1[b]	98.2 ± 8.1[b]
Free fatty acids (μg eq. of palmitic acid/mg of protein)	0.11 ± 0.0[a]	0.05 ± 0.0[b]	0.03 ± 0.0[b]	0.02 ± 0.0[b]
Total cholesterol (mg/dL)	62.1 ± 2.5[a]	52.8 ± 2.6[b]	66.5 ± 2.5[a]	55.0 ± 1.8[b]
High-density lipoprotein (mg/dL)	26.7 ± 2.7[b]	36.1 ± 1.2[a]	36.1 ± 2.7[a]	36.7 ± 1.3[a]
Very-low density lipoprotein (mg/dL)	29.9 ± 1.0[a]	19.3 ± 1.0[b]	22.1 ± 1.3[b]	18.5 ± 1.8[b]
Aspartate aminotransferase (U/L)	87.9 ± 8.9[a]	100.2 ± 8.2[a]	80.7 ± 4.6[a]	96.9 ± 8.9[a]
Alanine aminotransferase (U/L)	26.3 ± 1.5[a]	28.9 ± 2.1[a]	31.1 ± 2.9[a]	27.1 ± 1.5[a]
TyG index	9.5 ± 0.1[a]	9.0 ± 0.1[a]	9.2 ± 0.2[a]	9.0 ± 0.1[a]

Values are means of duplicated determinations ± standard error. Different letters (a-c) between rows indicate a significant difference between study groups ($p < 0.05$) by Tukey's test.

glucose loading compared to the obese control (Fig. 4). This trend was similarly reflected in fasting glucose results and consequently in the assessment of insulin resistance, as indicated by the TyG index (Table 4).

Regarding lipid profile, all rats that were administered any of the coffee brews showed decreased concentrations of TG (~40% for UN, ~24% for MRD, and ~36% for HRD, respectively) and FFA (~54% for UN, ~72% for MRD, and ~81% for HRD, respectively) compared with the obese control. Rats administered UN and HRD coffee brews also showed decreased concentrations of total cholesterol (~15% and ~11%, respectively). Furthermore, all treatments resulted in increased HDL values (~37%) and decreased VLDL concentrations (~26%) compared with the obese control (Table 4).

3.4.2. *Lipid content and adipose tissue hypertrophy assessment*

The results of lipids (FFA and TG) in adipose tissue are depicted in Table 5. All treated groups exhibited a decreased concentration of FFAs, with an approximate reduction of ~49%. Significant reductions in TG levels were observed only in rats administered UN and HRD coffee brews, showing decreases of 44% and 35%, respectively, compared to the obese control.

Consistent with the lipid content in adipose tissue, the results of adipose hypertrophy showed that only rats treated with UN and HRD coffee brews exhibited a significant decrease in adipocyte volume compared to the obese control (58% and 48% reduction, respectively).

Table 5
Lipid content: free fatty acids (FFAs) and triglycerides (TG) concentration in liver and adipose tissue of obese rats who received Unroasted (UN), Medium Roasted Degree (MRD), and High Roasted Degree (HRD) coffee brews.

Sample	Liver		Adipose tissue	
	FFAs (μg/mg of protein)	TG (μg/mg of protein)	FFAs (μg/mg of protein)	TG (μg/mg of protein)
Obese control	1.0 ± 0.1[a]	57.7 ± 1.7[a]	7.8 ± 0.1.5[a]	207.1 ± 15.5[a]
UN	1.0 ± 0.1[a]	56.1 ± 4.1[a]	3.4 ± 0.6[b]	116.2 ± 7.0[b]
MRD	1.1 ± 0.1[a]	47.1 ± 2.0[a]	4.0 ± 0.6[b]	186.2 ± 12.1[a]
HRD	1.5 ± 0.1[a]	55.3 ± 2.6[a]	2.9 ± 0.4[b]	134.9 ± 5.3[c]

Values are means of duplicated determinations ± standard error. Different letters (a-c) between columns indicate a significant difference between study groups ($p < 0.05$) by Tukey's test.

3.4.3. *Steatosis degree, lipid content, and liver damage markers*

The results of liver steatosis are presented in Fig. 5. All groups exhibited less than 30% of droplets in the hepatic area, indicative of grade 1 steatosis. This suggests that fatty hepatocytes occupied less than 30% of the hepatic parenchyma. Regarding the lipid concentration in the liver (TG and FFAs) and enzyme liver damage markers (AST, and ALT) levels, no significant differences were observed compared with the obese control (Table 5).

3.4.4. *Oxidative stress markers*

The results of the effect of coffee brews on lipid peroxidation expressed as equivalents of MDA concentrations in serum, liver, and AT are shown in Fig. 6. Rats administered with UN coffee brew, exhibited a significant decrease in serum (~90%) and liver (~45%) of MDA levels compared to the control group, while rats administered with MRD coffee brew, showed a significant decrease in liver MDA (~45%) and AT (~30%). Finally, rats administered with HRD coffee brew, demonstrated a significant decrease in serum (~90%), liver (~59%), and AT (~37%) of MDA levels compared to the obese control group.

On the other hand, evaluations of SOD and CAT revealed no significant differences between the obese control and the treated groups, except for the HRD group in the liver, where CAT activity exhibited a significant increase of 27% compared with the obese control (Fig. 6).

4. Discussion

Despite a growing body of scientific research supporting the potential health benefits of coffee, there is some debate regarding whether the roasting degree of beans influences these effects. In this context, we focused our investigation on evaluating whether the roasting intensity of Mexican *C. arabica* coffee beans influences its bioactive potential.

Although previous studies have shown that preparation methods like espresso yield greater extraction of bioactive compounds and antioxidant activity (Kim and Ahn, 2023), we selected the French press in this study because it is an infusion method preferred by the Mexican population.

Our *in vitro* evaluation revealed that the degree of roasting exerted a variable influence on the antioxidant activity of the coffee brews, dependent on the roasting degree and its underlying mechanism of antioxidant action. The relative contributions of different antioxidant groups to the overall activity varied across the roasting levels, with the most significant changes observed in roasted coffees compared to unroasted ones. We attribute this activity to a combined mechanism involving phenolic and melanoidin antioxidants, with the latter potentially playing a dominant role. This hypothesis is supported by previous reports suggesting that the hydrogen atom transfer mechanism associated with melanoidin antioxidant activity offers certain advantages (Laukaleja and Kruma, 2019). However, the existing literature on the relationship between coffee roasting degree and antioxidant activity is not entirely clear. Previous studies reported both increases and decreases in antioxidant activity with higher degrees of roasting (Laukaleja and Kruma, 2019).

The reported differences in antioxidant activity could be attributed to the initial changes during roasting. Early in the process, there may be a release of lower molecular weight phenolic compounds, which can contribute to antioxidant activity. However, at more advanced stages of roasting, degradation and/or structural changes of these bioactive compounds can occur, leading to a change in antioxidant activity (Herawati et al., 2019).

Coffee contains several potential nutraceuticals, including those with anti-hyperglycemic and anti-hyperlipidemic activities. These activities are mediated by the inhibition of carbohydrate and lipid absorption through the inhibition of digestive enzymes such as amylase, glucosidase, and lipase (Alongi and Anese, 2018; Duangjai et al., 2021). However, it has been reported that these effects could be influenced by the degree of coffee roasting. For instance, Duangjai et al. (2021) and

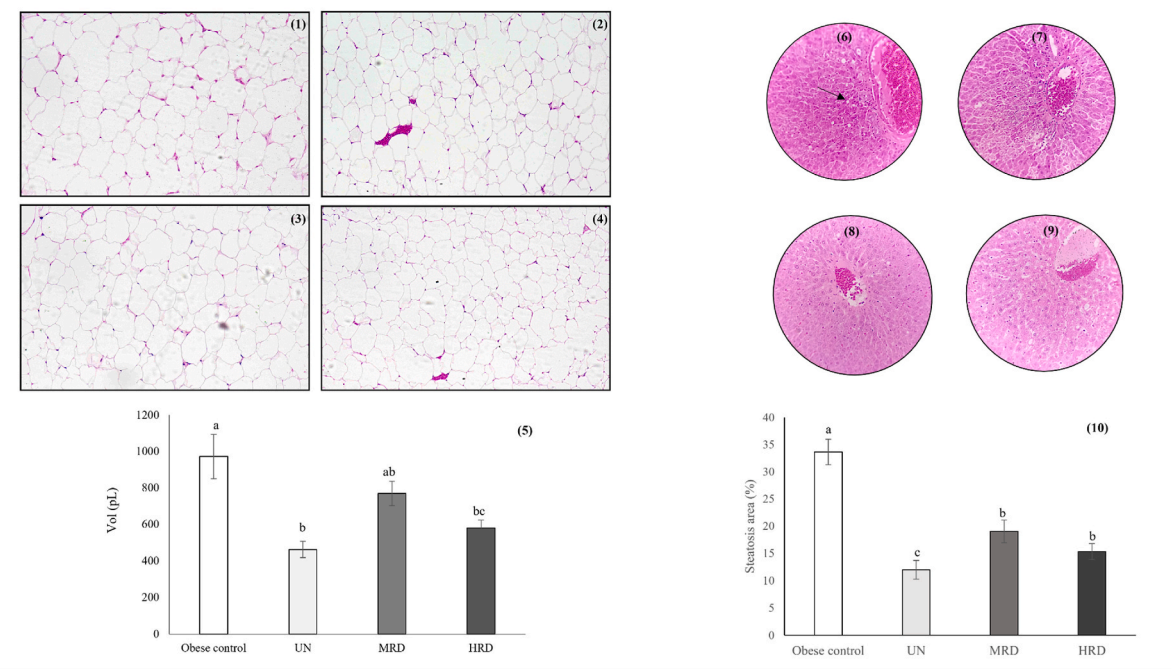

Fig. 5. Representative photographs of Hematoxylin-eosin–stained of adipose and liver tissues (40X): of obese control (1) and (6), Unroasted (UN) (2) and (7), Medium Roasted Degree (MRD) (3) and (8), High Roasted Degree (HRD) (4) and (9), adipocytes volume values (5) and steatosis area percentage (10) of obese rats who received coffee brews with different roasting degree. Values are means ± standard error. Different letters (a–c) between each point on the curve or by columns per figure indicate a significant difference between study groups ($p < 0.05$) by Tukey's test.

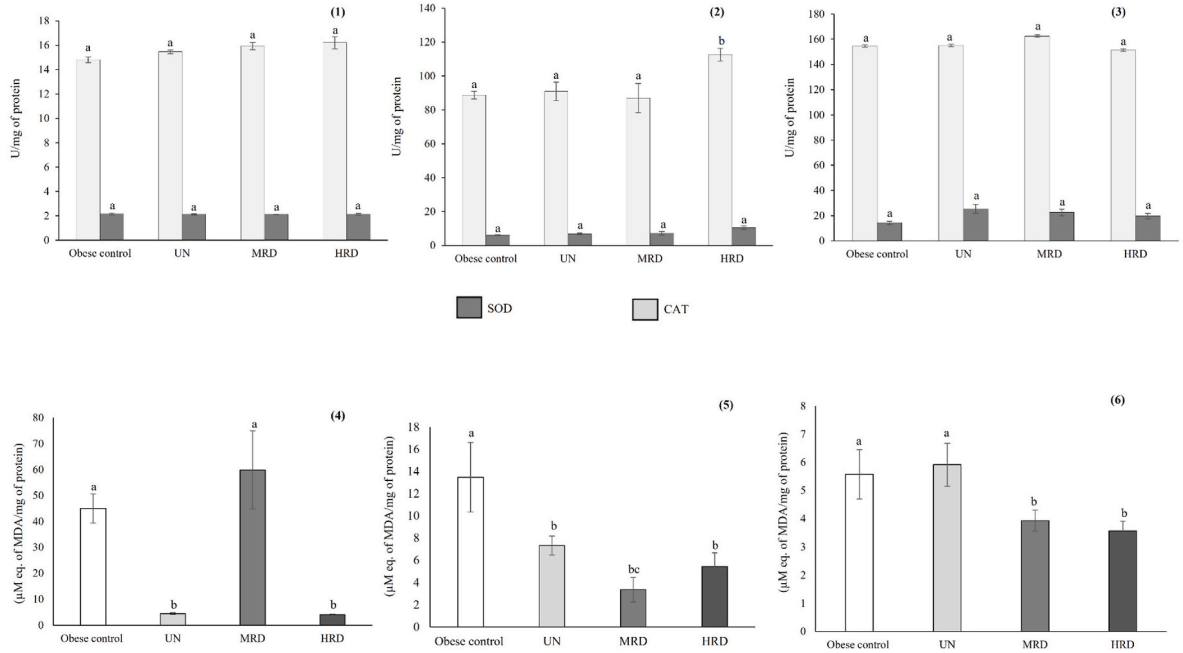

Fig. 6. Oxidative stress markers evaluation: (1) SOD and CAT activity in serum, (2) SOD and CAT activity in liver, (3) SOD and CAT activity in adipose tissue, (4) lipid peroxidation in serum, (5) lipid peroxidation in liver (2), and lipid peroxidation in adipose tissue of obese rats that were administered with Unroasted (UN), Medium Roasted Degree (MRD), and High Roasted Degree (HRD) coffee brews samples. Values are expressed as mean ± standard error. Different letters (a–c) assigned in each figure indicate a significant difference ($p < 0.05$) between the study groups. Significance was estimated using analysis of variance (ANOVA) followed by Tukey's test.

Alongi and Anese (2018) reported higher α-glucosidase inhibition with greater roasting. Contrary to these findings, we did not observe statistically significant differences in glucosidase activity attributed to roasting, nor did we observe a relevant inhibitory effect on amylase. This could be due to various factors, including sample variety and roasting process variables.

To elucidate the effect of coffee roasting degree on lipid and carbohydrate absorption, an *in vivo* evaluation was conducted. The results indicated that all groups receiving coffee brew treatment exhibited reduced glucose levels, independent of the roasting degree. Notably,

although the postprandial glucose reduction following coffee brew administration was lower than the positive control acarbose, the treated groups showed an approximately 20% decrease. In this context, previous studies suggested that a similar decrease in postprandial glucose levels, which was achieved by administering supplements or functional foods, could be a complementary strategy for managing hyperglycemia in diabetes (Petsiou et al., 2014).

Previous studies demonstrated that coffee has the potential of decrease the intestinal absorption of both cholesterol and TG (Pires et al., 2022). However, the influence of roasting degree on this effect remains unclear. Our *in vivo* evaluation demonstrated that the roasting degree of coffee impacted lipid absorption. Specifically, only groups receiving roasted coffee exhibited a reduction in postprandial TG, with a stronger effect observed for higher roasts. It is important to note that this lipid-lowering effect was lower than the positive control (orlistat). This are in concordance with the observed *in vitro* results.

To evaluate the effect of chronic intervention with coffee of different roasting degrees, an obesity animal model was utilized. Obesity was induced in rats with a high-fructose and saturated fat diet for 36 weeks. Our findings revealed that both UN and HRD coffee brews restrained body weight gain in obese rats (~15% and 10%, respectively) compared with the obese control. This effect was independent of food or water intake, as these remained comparable among all groups throughout the intervention period (data not shown). Consistent with our results, previous studies have reported that both UN and roasted coffee can decrease body weight gain in rats induced to be obese (Gamboa-Gómez et al., 2023). Coffee may prevent weight gain through several mechanisms, including elevated resting metabolic rate, increased lipolytic activity (fat breakdown), norepinephrine release, cellular thermogenesis, and accelerated energy expenditure (Baspinar et al., 2017). Polyphenols such as chlorogenic and caffeic acid, as well as melanoidins, are considered the main bioactive compounds responsible for coffee's anti-obesity effect (Tarigan et al., 2022). Consistent with previous findings, our results showed that the levels of identified compounds like caffeine remained relatively unchanged during roasting. In contrast, chlorogenic and caffeic acid contents decreased with increasing roast degree, while low-molecular-weight Maillard reaction products increased and eventually polymerized into melanoidins (Tarigan et al., 2022).

Considering the above mentioned, we attributed the decreased body weight in the group that received HRD mainly to melanoidins, as previous studies have reported that melanoidins reduce daily energy intake and modulate postprandial glycemia (Nikpayam et al., 2019).

Unlike in previous studies (Gamboa-Gómez et al., 2023; Reis et al., 2019) our work did not exhibit significant differences in glucose metabolism parameters or insulin resistance between obese control rats and those administered either treatment. This can be attributed to different factors ranging from harvest, variety, storage, processing, among others (McQuaid et al., 2011).

In obesity, alterations in serum TG, FFAs, HDL, VLDL, ALT, and AST are clinically significant. Obesity often leads to elevated TG and VLDL levels, which are markers of dyslipidemia and increased cardiovascular risk. Higher FFAs levels in obesity contribute to insulin resistance and fat accumulation in the liver, exacerbating conditions like non-alcoholic fatty liver disease. Low HDL levels, common in obesity, further increase cardiovascular risk. Elevated ALT and AST are indicators of liver injury, reflecting the impact of obesity on liver function and the potential progression to more severe liver diseases (Zhang et al., 2014).

Conversely, all coffee treatments, regardless of roast degree, improved lipid metabolism parameters such as serum TG, FFA, HDL, and VLDL. This finding suggests that the coffee's effects on lipid metabolism may be attributed to compounds that are relatively unaffected by the roasting process. However, further studies are necessary to elucidate this hypothesis.

Obesity is characterized by an increase in lipids stored within AT, leading to both hypertrophy (increased cell size) and hyperplasia (increased cell number). An expansion of AT mass, in particular abdominal obesity, is associated with an increased risk of type 2 diabetes and insulin resistance (Perrone et al., 2012). In this study, we evaluated the effects of coffee intervention on these alterations in AT and investigated whether the roasting process influences these effects. Our results showed that UN and HRD coffee treatments significantly decreased lipid content in adipocytes, thereby improving hypertrophy. This finding aligns with the observed decrease in body weight.

In the case of UN coffee, we attribute this effect primarily to the presence of chlorogenic acid, the most abundant and active compound in unroasted coffee. Previous studies have reported its ability to reduce lipid accumulation in adipocytes (Pimpley et al., 2020). For HRD coffee, the effect is likely due to compounds formed during the advanced stages of roasting (Perrone et al., 2012).

Regarding liver steatosis, all treated groups displayed a reduction in steatosis area (~30%) compared to the obese control group, independent of the roasting degree. This observation is consistent with our previous findings in *Coffea canephora* var. under different roasting conditions, suggesting that the effect is independent not only of roast degree but also of the roasting process and coffee variety (Rocha-Guzmán et al., 2018). The main bioactive compound attributed to this effect is caffeine, which is unaffected by roasting degree. Caffeine has been shown to improve hepatic steatosis through its lipolytic activity and its ability to activate the cAMP/CREB/SIRT3/AMPK/ACC pathway (Zhang et al., 2015).

In summary, our results of histological analyses of adipose hypertrophy and hepatic steatosis suggest that coffee consumption may directly influence fat accumulation and adipose tissue function, thereby impacting liver health by either preventing or exacerbating fat buildup. However, further studies are needed to precisely determine the underlying mechanisms.

One of the most studied biological effects of coffee, particularly influenced by its roasting degree, is its antioxidant activity. In this regard, we selected serum, AT, and liver to evaluate oxidative stress markers due to their relevance in obesity-related metabolic dysfunction. Serum serves as a systemic indicator of oxidative balance, reflecting the overall impact of oxidative stress across organ systems, and is the first point of contact for absorbed coffee bioactive compounds. The liver, a central organ for detoxification and metabolism including the processing of coffee bioactive compounds is a critical site for assessing oxidative damage and antioxidant response. The AT, as a primary site of fat storage, plays a significant role in metabolic regulation and inflammation, both closely linked to oxidative stress. Studying the effects of bioactive compounds in AT can provide insights into their antioxidant effects, which may be prolonged due to potential reabsorption and extended presence within the body (Čolak and Pap, 2021; Del Rio et al., 2010; Manach et al., 2004). Therefore, we evaluated the effect of coffee roasting degree on oxidative stress markers in obese rats, focusing on the activity of antioxidant enzymes (CAT and SOD) and lipid peroxidation levels in serum, liver, and adipose tissue. CAT and SOD are crucial antioxidant enzymes, particularly in obesity, where oxidative stress is elevated. SOD converts superoxide radicals ($O_2^{\bullet-}$) into hydrogen peroxide (H_2O_2) and oxygen, reducing oxidative damage linked to inflammation and insulin resistance. CAT then breaks down hydrogen peroxide into water and oxygen, preventing further oxidative harm (Aliko et al., 2018). Our results showed that only rats administered highly roasted coffee exhibited a significant increase in CAT activity in the liver. Given that catalase depletion is associated with non-alcoholic fatty liver disease (Shin et al., 2019), this increase in hepatic CAT activity is biologically significant. It suggests a protective mechanism against oxidative stress, supporting liver function and potentially reducing the risk of obesity-related metabolic disorders.

Additionally, compared to the obese control group, all coffee treatments decreased MDA levels in the liver, with the highest reduction observed in the HRD group (approximately 50%). Consistent with our findings, previous studies have also reported that roasted coffee,

particularly intense roasts, can decrease liver MDA levels compared to unroasted coffee [79]. Like the liver, the HRD group exhibited the most significant decrease in MDA levels within AT, whereas UN coffee did not significantly affect MDA levels in AT.

Our findings on antioxidant stress markers align with previous observations. Budryn et al. (2017) reported that unroasted and roasted coffee extracts in reducing MDA levels in organs affected by oxidative stress, with activity dependent on the target organ. These observations suggest that the variability between organs and treatments may arise from two factors: alterations in coffee composition induced by roasting, and differences in the absorption and bioavailability of coffee brew compounds. In this regard, melanoidins formed during the HRD might explain the strongest effect observed in this group. Somoza (2005) reported that melanoidins can exhibit bioavailability, with absorption rates of up to 30%. This characteristic could, at least partially, account for the observed antioxidant effect in the HRD group.

Our results demonstrated that the primary antioxidant effect of coffee treatments was observed in the liver. This may be attributed to the liver's role in 'first pass' metabolism, where bioactive compounds are absorbed and metabolized before entering general circulation (Neilson et al., 2017). More studies in the field are required.

5. Limitations of the study

This study has some limitations that should be acknowledged. Fist, we did not investigate the potential side effects of coffee brew consumption. However, no unusual symptoms or abnormalities were recorded during the study, and no significant changes were observed in any of the experimental groups. Although several studies have examined the potential toxic effects of coffee, few have focused on the influence of coffee bean quality and roasting degree on toxicity using brewed coffee, still a controversial issue, given that in some cases a greater harmful effect is mentioned in medium roasts than in high roasts but attributed to other factors such as grain quality (da Silva et al., 2021). Further research in this area is needed.

Secondly, we didn't make measurements of parameters such as body composition or energy expenditure, insulin tolerance testing or insulin level measurement. However, for assessing insulin sensitivity we evaluate the product of TG and glucose (TyG) index, which has been shown to be comparable with the gold standard method (Euglycemic-Hyperinsulinemic, Clamp) for assessing insulin resistance (Guerrero-Romero et al., 2010).

6. Conclusion

Our study explored the effects of coffee roasting intensity on its bioactive potential. Our results showed that roasting influenced coffee's antioxidant potential and its effects on lipid metabolism. Specifically, higher roast levels were associated with decreased postprandial triglycerides and limited body weight gain in obese animal models. Moreover, all coffee treatments improved lipid metabolism parameters, suggesting benefits beyond roasting intensity.

These findings suggest that higher roasting degrees in coffee may offer potential benefits in weight management and lipid health in obese individuals. Further research is needed to elucidate the underlying mechanisms and determine the optimal roasting degree for specific health outcomes.

Financial interests

The authors declare they have no financial interests.

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Ethical approval

All procedures were performed in accordance with the guidelines established by the National Institutes of Health (NIH) for the care and use of laboratory animals (Guide for the Care and Use of Laboratory Animals, 8th Edition).

CRediT authorship contribution statement

Claudia I. Gamboa-Gómez: Investigation, Project administration, and, Funding acquisition, experimental design, result analysis and discussion, Writing – original draft. **Juliana Morales-Castro:** lab experimentation, data collection. **Jazel Barragan-Zuñiga:** result analysis, discussion, and, Writing – original draft. **Mayra Denise Herrera:** data collection and result analysis. **Alejandro Zamilpa-Álvarez:** lab experimentation, data collection, final revision. **José Luis Gónzalez:** data collection and result analysis. **Gerardo Martínez-Aguilar:** result analysis and discussion. **Elisa P. Morales-Castro:** lab experimentation, data collection. **Monica Anese:** Writing – review & editing. **Marilisa Alongi:** lab experimentation, data collection, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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