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Evaluation of the Antioxidant Capacity of Deep-frozen Green Coffee and Roasted Coffee

Evaluación de la capacidad antioxidante de café verde ultracongelado y café tostado

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Abstract: There is little information on the antioxidant composition of green or roasted coffee. This work evaluated the antioxidant capacity (AC) and the content of total phenolic compounds (TPC) in the ethanolic extracts of deep-frozen green coffee (DFGC), green coffee without deep-freezing (GCWDF), and roasted coffee (RC). The AC was evaluated by the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) and DPPH (2,2-diphenyl-1-picrylhydrazyl) methods, while the content of phenolic compounds was determined by the Folin-Ciocalteau method. The average values of the AC (mmol Trolox/g coffee) were 1.35 for DFGC at a temperature interval between -40ºC and -10 ºC, 0.22 for GCWDF, and 0.15 for RC between100 ºC and 250 ºC by the ABTS method. These values correspond to a % inhibition of this radical of 90.5, 81.5, and 62.3, respectively. By the DPPH method, the AC (mg caffeic acid/g coffee) was 21.31 for DFGC, 20.91 for GCWDF, and 7.72 for RC. These values correspond to a % inhibition of this radical of 94.4, 94.4, and 87.6, respectively. The TPC content (mg gallic acid/g coffee) was, on average, 158.4 for DFGC, 132.6 for GCWDF, and 99.3 for RC. To know the content of antioxidant compounds considering the rate and speed at which they can be extracted, the effective diffusion coefficient (D_{eff}) in m^2/s, and the first-order kinetic constant (k) in s^{-1} of the TPC extraction process were also determined. The results of D_{eff} were 6.8×10^{-11}, 4.7×10^{-11}, and 5.83×10^{-11}, and those of k were 2.15×10^{-3}, 1.57×10^{-3}, and 1.58×10^{-3} on average for DFGC, GCWDF and RC, respectively.

Keywords: frozen coffee, antioxidant test, total phenolic compounds, effective diffusion coefficient.

Resumen: Existe poca información sobre la composición de los antioxidantes del café verde o tostado. En este trabajo se evaluó la capacidad antioxidante (CA) y el contenido de compuestos fenólicos totales (CFT) en los extractos etanólicos de café verde ultracongelado (CVUC), café verde sin ultracongelación (CVSUC) y café tostado (CT). La CA se evaluó mediante los métodos ABTS y DPPH, mientras que el contenido de CFT se determinó por el método de Folin-Ciocalteau. Los valores promedio de la CA (mmol Trolox/g de café) fueron: 1.35 para CVUC en el intervalo de temperatura de -40 ºC a -10 ºC, 0.22 para CVSUC y 0.15 para CT en el intervalo de 100 ºC a 250 ºC por el método ABTS. Estos valores corresponden a un % de inhibición de este radical de 90.5, 81.5 y 62.3, respectivamente. Por el método DPPH, la CA (mg de ácido cafeico/g de café) fue de 21.31 para CVUC, 20.91 para CVSUC y 7.72 para CT. Estos valores corresponden a un % de inhibición de este radical de 94.4, 94.4 y 87.6, respectivamente. El contenido de CFT (mg de ácido gálico/g de café) fue en promedio de 158.4 para CVUC, 132,6 para CVSUC y 99.3 para CT. Para conocer el contenido de compuestos antioxidantes considerando la tasa y velocidad con la que se pueden extraer, se determinó el coeficiente de difusión efectiva (D_{eff}) en m^2/s y la constante cinética de primer orden (k) en seg^{-1}, del proceso de extracción de CFT. Los resultados de D_{eff} fueron de 6.8×10^{-11}, 4.7×10^{-11} y 5.83×10^{-11} y los de k fueron de 2.15×10^{-3}, 1.57×10^{-3} y 1.58×10^{-3} en promedio, para el CVUC, CVSUC y CT, respectivamente.

Palabras clave: café congelado, prueba de antioxidantes, compuestos fenólicos totales, coeficiente de difusividad efectiva.

Introduction

Coffee is one of the most consumed products worldwide due to its organoleptic and stimulating properties. Still, the beans of *Coffea arabica* L. are also a natural source of thermosensitive antioxidant compounds, so it is imperative to explore new technologies to preserve its antioxidant capacity (AC; Kungsuwan et al., 2023).

Deep freezing is widely used in the food industry to preserve food. It consists of cooling the food until it reaches a temperature below the water freezing point (0 °C at 1 atm). In this operation, which is carried out using deep freezers, temperatures around - 40 °C are reached. Deep freezing can be performed slowly, at freezing speeds of less than 5 cm/h or 4 °C/min and for periods of more than 4 hours, or quickly for short periods, at a speed of 5 to 20 cm/h (Ishevskiy & Davydov, 2017). In the food industry, quick freezing is preferred since the number of microorganisms that survive inside the food is less than that in slow freezing. Under these quick-freezing conditions, the needle-shaped water crystals formed inside the food are smaller than those in slow freezing, guaranteeing the food's quality after thawing. In deep-frozen food, the biological and biochemical aging processes are delayed, and microbial activity is reduced. This is explained by the dependence of the speed of chemical reactions on temperature, and because there is a decrease in the permeability of the microbial cytoplasmic membrane, these minimally processed foods retain their organoleptic (color, flavor, and texture) and biochemical (nutrients and vitamins) properties as if they were fresh (Biglia et al., 2016).

Traditionally, coffee drinks are preferably obtained from roasted coffee. However, there is scientific evidence that green coffee could be more beneficial for health since there is greater availability of phenolic compounds that give green coffee a higher AC than roasted coffee (Hall et al., 2022). Deep freezing can improve or maintain the quality of natural products with or without processing, especially those that contain biocompounds that degrade due to increased temperature. Such is the case of green coffee, whose phenolic compounds are thermolabile and degrade or transform during the roasting process.

"Green coffee" are the beans of *Coffea arabica* or *Coffea canephora* that have not been treated and keep all their layers. A thorough review of articles where clinical trials were conducted reveals that the benefits of green coffee polyphenolic compounds on human health range from improving skin smoothness, cognitive functions, blood pressure, plasma lipids, and body weight to reducing the risk of suffering from metabolic syndrome (Bosso et al., 2021). Green coffee is a natural source of chlorogenic acids (CGA; Marín & Puerta, 2008). They fulfill numerous biological processes such as antioxidants, protection of plants against microorganisms, ultraviolet light, damage by herbivores, and physical damage, and pest and disease resistance (Leitão et al., 2011; Marín & Puerta, 2008).

Depending on the variety and the region where it is grown, the concentration of these compounds in coffee beans ranges from 4.1 to 11.3 g per 100 g of green coffee on a dry basis (Rojas-González et al., 2022). CGA are esters obtained from the reactions of quinic acid with trans-cinnamic acids such as caffeic, ferulic, and coumaric. The main CGA in green coffee are caffeoylquinic, feruloylquinic, and dicaffeoylquinic acids, all phenolic compounds responsible for their AC (Farah & Donangelo, 2006). CGA is proven to be effective against some parasites

(Smith et al., 2016), bacteria (Runti et al., 2015), and viruses (Kaihatsu et al., 2014). In addition, they have shown strong biological activity with anti-inflammatory (Artusa et al., 2022), neuroprotective (Tian et al., 2015), anticancer (Ismail et al., 2021), antidiabetic (Febrina et al., 2021), and anticarcinogenic effects (Reichal & Rajeshkumar, 2022).

Very few consumers resist the aroma of good coffee. However, this delicate and captivating aroma, flavor, and fragrance only appears in roasted coffee, obtained after roasting the coffee beans at temperatures of 100 to 250 °C. The main volatile compounds found in roasted coffee are furans, pyrazines, and pyridines (Somporn et al., 2011), which are products of caramelization, pyrolysis, and Maillard reactions (Pérez et al., 2021; Tarigan et al., 2022) and of the degradation of the CGA isomers themselves (Dawidowicz & Typek, 2017). A study has reported that the reduction of CGA is related to the roasting temperature. It is mentioned that when green coffee beans were roasted at 230 °C for 12 min, the total content of CGA was almost halved, while at 250 °C for 21 min, the reduction was even more significant (Moon et al., 2009). In general, the roasting process of green coffee beans considerably reduces the amount of CGA isomers (Eröz Poyraz et al., 2016). Thus, the AC of roasted coffee can decrease because of the unfortunate degradation and reduction of CGA unless the set of new compounds that are formed during the roasting process (quinic acid, CGA isomers, and CGA in the form of lactones) compensate for this decrease in its AC.

On the other hand, to take advantage of the AC of the different antioxidant compounds present in green or roasted coffee, the coffee beans must be subjected to an adequate extraction process that guarantees their availability. There are many traditional and new extraction methods, such as maceration, soxhlet extraction, turbo-extraction, ultrasonic and microwave-assisted extraction, and supercritical fluid extraction, among others (Gligor et al., 2023; Menzio et al., 2020; Vandeponseele et al., 2022). In the solid-liquid or solid-fluid extraction, it has been verified that the phytochemical profile, AC, and quality of the extracts depend on the extraction method, in which the transport properties are involved, for instance, the effective diffusion coefficient (D_{eff}) and the kinetics of the extraction process (Akemi Toda et al., 2021; Gligor et al., 2023).

Therefore, one way to keep the amount of CGA in green coffee unaltered would be to subject it to a physical treatment, such as deep-freezing, so that the AC of the coffee could be preserved. As far as is known, there are no data on the impact of deep freezing on the AC of green coffee or on the effect it could have on the extraction kinetics and the diffusion coefficients in the extraction process ofantioxidant compounds. In addition, since chemical reactions are not favored by lowering the temperature, much less at temperatures below -10°C, the formation of new compounds that could modify their AC is impossible.

It is likely that through the deep-freezing process, the AC of green coffee can be preserved or enhanced and that this phenomenon is related to the diffusion coefficients of the phenolic compounds extracted from particles with different porosities, which would be caused by thawing water crystals of various sizes that formed at different deep-freezing temperatures. Therefore, in order to test this hypothesis, the main objective of this work was to evaluate the AC in deep-freezing green coffee and roasted coffee, Because there is enough scientific data to suggest that frozen coffee has more available phenolic compounds than roasted coffee, temperature likely affects the oxidation of antioxidant compounds.

Materials and Methods

Reagents and Standards

Folin-Ciocâlteu reagent, $ABTS^{+}$ (diammonium 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate)), DPPH (2,2-Diphenyl-1-(2,4,6- trinitrophenyl)hydrazyl) and the standards Trolox reagents, gallic acid and caffeic acid were acquired from Sigma-Aldrich (Naucalpan, State of Mexico, Mexico). Ethanol, methanol, sodium bicarbonate, potassium persulfate, and other chemical reagents used in this work are analytical grade and were acquired from J. T. Baker (Ecatepec, State of Mexico). The hulling green coffee and the ground green coffee (*Coffea arabica* L.) were provided by Joel Romero Gómez, owner of the Rancho Escondido, located at 1,100 meters above sea level in the town of San Marcos de León, in the municipality of Xico, in the state of Veracruz, Mexico.

Conditioning of Green and Roasted Coffee Samples

The green coffee with a moisture content of 9.5 % by weight was grounded in a coffee grinder (MrCoffee BVMC-BMH23/26). It was received in thermal and hermetic bags with a content of 0.5 kg, which was characterized as a poly-dispersed system. Through a sieve analysis, the different fractions were obtained, and the most abundant (>50 %) was chosen with a particle size distribution in the 0.833 to 1.168 mm interval according to Tyler's standard mesh opening.

Deep Freezing Process

Fifty grams of ground green coffee were placed inside sealed freezer bags. The samples were kept in a domestic refrigerator at 4 °C for 30 min. Subsequently, they were placed inside deep-freezing equipment (Tecnomac model BK5-AG. Citalsa, Italy) for 30 min at temperatures of -10, -20, -30 and -40 °C. The thawing time was 30 min at 4 °C. Subsequently, the solid-liquid extraction process was carried out.

Roasting Process

Fifty grams of husked green coffee were placed in metal containers. The samples were subjected to the roasting process using a controlled heating grill (Thermo Scientific, model Sp88857100). Roasting temperatures were 100, 150, 200, and 250 °C, according to the methodology presented by Osorio-Pérez et al. (2020). The excessive temperature of 250 °C was selected to observe how the treatment could affect the bioactive compounds. The roasting time was as follows: Each sample was put under the indicated temperature, and the sample was kept for 20 minutes once reached. The roasted coffee was ground in a coffee grinder (MrCoffee BVMC-BMH23/26) and subjected to the same sieving process described above. The roasted coffee was kept at 4 °C in sealed thermal bags until the solid-liquid extraction process began.

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Solid-liquid Extraction Process

For each extraction system, 50 mL of ethanol was added to 5 g of ground coffee in a Falcon flask. The mixtures were shaken at 200 rpm for 20 min in an orbital shaker (Lab-Line Incubator-Shaker, USA) at 20 °C. After extraction, the samples were centrifuged and filtered using a 0.45 µm membrane (syringe filter Corning, Germany). Coffee extracts were obtained and placed in amber glass containers until TPC and AC were determined.

Determination of Antioxidant Capacity by the ABTS Radical Scavenging Assay

The AC by the ABTS radical scavenging assay was determined through a procedure adapted and used in other previously published works (Guadarrama-Lezama et al., 2012). This procedure starts with preparing the ABTS radical solution, for which 6.6 mg of potassium persulfate and 0.0384 g of the ABTS reagent were weighed and dissolved in 10 mL of deionized water. The prepared solution was placed in an amber glass road and allowed to stand for 24 hours in cooling (5 ± 1 °C). Subsequently, 0.5 mL were taken from the previous solution and were to a volume of 25 mL with anhydrous ethanol (mother solution). The absorbance of the mother solution was adjusted until a constant value (0.700 ± 0.02) was obtained for 7 minutes at a wavelength of 734 nm. From the mother solution, 1.98 mL were taken and added to 0.2 mL of coffee extract. The mixture was placed in a quartz cell, and the absorbance was measured to a wavelength of 734 nm in a UV-Vis spectrophotometer (Scientific Vela Quin, VW 5600UV, AIII2001, Mexico) at intervals of 1 to 7 min. The data obtained were adjusted to a Trolox calibration curve obtained using the methodology described by Masek et al., 2017. The determinations were made in triplicate. The inhibition percentage was obtained using the method used in other works (Equation 1; Guadarrama-Lezama et al., 2012; Ormaza-Zapata et al., 2022):

$$\%\text{Inhibition} = \frac{\text{As}_{t0} - \text{As}_{tf}}{\left(\text{As}_{t0} - \left[\frac{\text{Ad}_{t0} - \text{Ad}_{tf}}{\text{Ad}_{t0}}\right]\right)} \times 100 \qquad (1)$$

Where As_{t0} and As_{tf} are the sample's absorbances at initial and final times, Ad_{t0} and Ad_{tf} are the dissolvent's absorbances at initial and final times.

Determination of Antioxidant Capacity by the DPPH Method

For this assay, the DPPH radical solution was first prepared, for which 0.0025 mg of the DPPH reagent were weighed and methanol was added until reaching a volume of 100 mL (DPPH solution). At a volume of 3.9 mL of the DPPH solution, 0.1 mL of the coffee extract was added. The absorbance of the mixture was determined at the initial time and after 30 minutes of incubation at a wavelength of 515 nm. According to the methods described by other authors, the effect of the extraction solvent in the samples was considered (Ibarra-Cantún et al., 2022; Mishra et al., 2012; Villaño et al., 2007). Caffeic acid was selected as a standard, and the results were expressed as mg of caffeic acid per g of coffee on a dry basis. The determinations were made in triplicate.

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Determination of Total Phenolic Compounds by the Folin-Ciocalteau Method

The content of TPC was determined using the Folin-Ciocalteau reagent and following the methodology described in previous works (Guadarrama-Lezama et al., 2012). To prepare the Folin-Ciocalteu reagent solution, 0.5 mL of Folin's reagent was mixed with 4.5 mL of deionized water, resulting in a diluted solution. The coffee extract was diluted in ethanol in a 1:5 (v/v) ratio in each determination. From this mixture, 0.1 mL was taken and mixed with 0.75 mL of the Folin's reagent diluted solution. The solution was allowed to be incubated for 5 min, and 0.75 mL of a sodium bicarbonate solution (60 g/L) was added, stirred (200 rpm), and allowed to stand for 90 min. Then, the mixture was leaked using a 0.45 µm membrane (Syringe Filter Corning, Germany). The absorbance of the solution at 750 nm was determined. The data were adjusted to a gallic acid (GA) calibration curve obtained with the methodology described by Huang et al. (2005) and Singleton et al. (1999). The TPC content was expressed in mg of GA per gram of coffee on a dry basis. The determinations were made in triplicate.

Kinetics of Total Phenolic Compounds Extraction

The solid-liquid extraction process described previously was repeated with each of the DFGC, GCWDF, and RC samples to quantify the concentration of TPC in the ethanolic extracts as a function of time. The concentration (mg of GA/g of coffee) was determined when the contact time between the phases was 5, 10, 15, 20, 25, 30, and 35 min. The TPC content was determined according to the procedure described in the previous section. Before the extraction process, the % moisture content of the coffee samples was determined (Cortés-Camargo et al., 2023).
The results were adjusted to the first-order kinetic model, represented by Equation 2.

$$LnC_t = LnC_0 - kt \qquad (2)$$

Where C_0 and C_t (mg GA/g coffee) are the initial concentration at any time, respectively, *t* is the time in seconds, and *k* is the kinetic coefficient of the extraction process in s^{-1}.

Determination of the Effective Diffusion Coefficient (D_{eff})

To determine the D_{eff} of the extraction process of TPC of the coffee samples, the analytical solution of the second Fick law in spherical coordinates was used, as described by Equation 3 (Huamaní-Meléndez & Darros-Barbosa, 2018):

$$\frac{C_t}{C_0} = \frac{6}{p^2}\sum_{n=1}^{\infty}\frac{1}{n^2}exp\left(-\frac{D_{eff}n^2p^2t}{r^2}\right) \qquad (3)$$

Where C_0 and C_t are the initial concentration and at any time, respectively, in mg of GA/g of coffee; D_{eff} is the effective diffusion coefficient, in m^2/s; r is the radius of the particles, in m; and *t* is the extraction time, in seconds. To solve Equation 3, a program written in Matlab was utilized.

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Statistical Analysis

Data were analyzed using a one-way analysis of variance (ANOVA) and a Tukey's test for a level of significance $p \leq 0.05$ using the Minitab 17 software (Minitab Inc., State College, PA, USA). The results are given as averages with standard deviations (±). All experiments were completed in triplicate.

Results and Discussion

Antioxidant capacity through the ABTS radical

Table 1 shows the results of the AC obtained by the ABTS radical scavenging assay for the different coffee samples. The AC in mmol Trolox/g of coffee ranges from 1.33 to 1.36 for DFGC, 0.22 for GCWDF, and 0.13 to 0.17 for RC. These results show a significant difference in deep-freezing temperature on the AC of green coffee in the range from -40 to -10 °C. On the other hand, in the RC samples, the temperature from 100 to 250 ºC produces a zig-zag behavior in the AC value. The explanation for this phenomenon has been widely reported (Dawidowicz & Typek, 2017). Most authors agree that it is due to the degradation of phenolic compounds, particularly CGA, and the formation of new compounds during the roasting process, which could compensate for the AC in coffee drinks (Moon et al., 2009; Perrone et al., 2012).

What is evident in the results of this work is the fact that DFGC and GCWDF have a higher AC than RC. On average, the AC of the DFGC, the GCWDF, and RC was 1.35, 0.22, and 0.15 mmol Trolox/g, respectively. A comparison of these three samples indicates that the AC of the DFGC is six times larger than that of GCWDF and nine times greater than that of RC. Regarding the comparison of the AC of green coffee and roasted coffee, there are contradictory results; some researchers report that green coffee has a greater AC (Bobková et al., 2020), while others report the opposite (Muñoz et al., 2020). The controversy over these differences focuses on the degradation and transformation reactions of the phenolic compounds that occur in coffee beans due to temperature (Yahayu et al., 2020). For 100 % Arabica green coffee without any thermal treatment, the AC ranging from 0.47 to 1.1 mmol Trolox/g has been reported (Masek et al., 2020). For roasted coffee, values of 3 to 6 mmol Trolox/g have been published (Díaz et al., 2018).

Figure 1 shows the results of the inhibition of the ABTS radical in the DFGC, GCWDF, and RC samples. On average, the inhibition percentages of the DFGC, the GCWDF, and the RC were 90.50, 81.55 and 62.27 %, respectively. Similar values of the percentage inhibition of the ABTS radical (90 %) have been reported in ethanolic extracts of green coffee (Masek et al., 2020). On the other hand, for RC, the percentage of inhibition was between 16 and 42 %, depending on the time and the roasting temperature (Yahayu et al., 2020). In this work, a comparison of the three samples indicates that the % inhibition of the ABTS radical of DFGC is 11 % higher than that of GCWDF and 45 % higher than that of RC.

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Table 1. Antioxidant capacity obtained by the ABTS radical scavenging assay

Ground coffee sample	Temperature (°C)	AC (mmol Trolox/g coffee)
DFCG	-40	1.36 ± 0.02[a]
	-30	1.35 ± 0.01[b]
	-20	1.34 ± 0.01[b]
	-10	1.33 ±0.02[c]
GCWDF	25	0.22 ± 0.04[b]
RC	100	0.14 ± 0.02[c]
	150	0.16 ± 0.01[a]
	200	0.13 ± 0.01[b]
	250	0.17 ± 0.05[c]

Note. *The results are expressed as the average value ± standard deviation of three repetitions. Values in columns with different letters indicate significant differences ($p \leq 0.05$).
Source: Prepared by the authors.

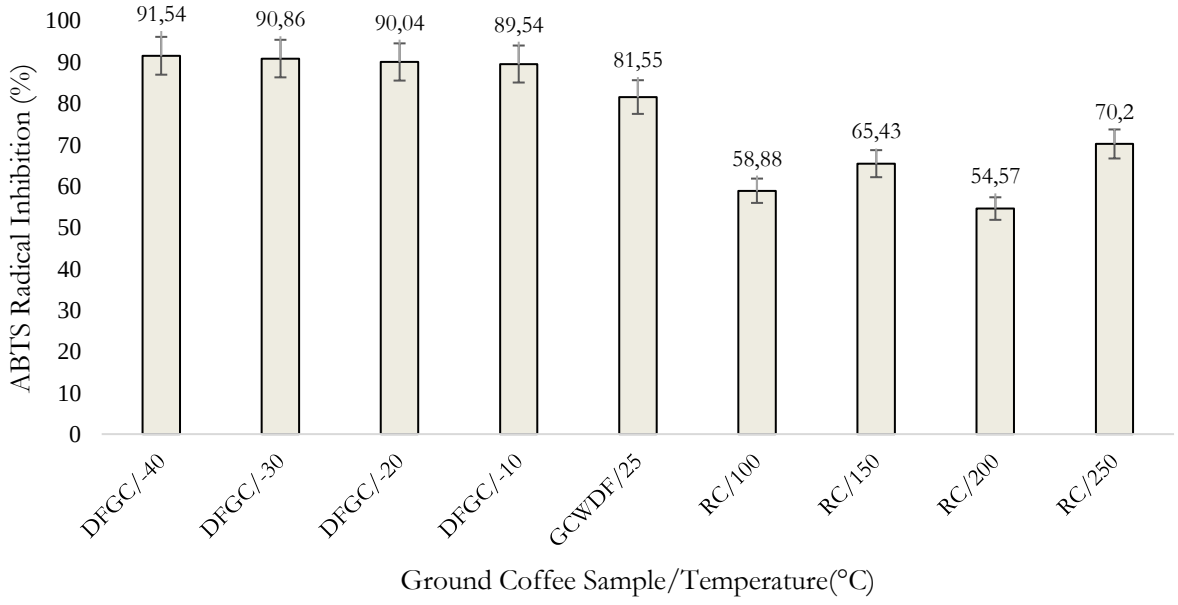

Figure 1. Percentage of ABTS radical inhibition. *The bars above the columns represent the standard deviation percentage of three repetitions.
Source: Prepared by the authors.

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Antioxidant Capacity Obtained by the DPPH Radical Scavenging Method

Table 2 shows the AC results obtained by the DPPH radical scavenging method for the different coffee samples. In the DFGC samples, for every 10 °C increase in temperature from -40 to -10 °C, AC decreased by 8.5 % on average. On the other hand, in the RC samples, the effect of the temperature from 100 to 250ºC is reflected in the AC content; that is, it decreases deeply.
A comparison of the AC of these three samples indicates that the AC of DFGC is practically equal to that of GCWDF, but it is 2.76 times higher than that of RC on average. This behavior is similar to that obtained with the ABTS method.

Table 2. Antioxidant capacity obtained by the DPPH radical scavenging method

Ground coffee sample	Temperature (°C)	AC (mg caffeic acid/g coffee)
DFGC	-40	22.92 ± 0.15[a]
	-30	22.37 ± 0.14[a]
	-20	20.01 ± 0.15[a]
	-10	19.95 ± 0.31[b]
GCWDF	25	20.91 ± 2.38[c]
RC	100	13.15 ± 3.07[b]
	150	8.86 ± 2.37[b]
	200	2.83 ± 0.47[a]
	250	6.03 ± 0.63[b]

Note. *The results are expressed as the average value ± standard deviation of three repetitions. Values in columns with different letters indicate significant differences ($p \leq 0.05$).
Source: Prepared by the authors.

Figure 2 shows the results of the % of DPPH antiradical activity of DFGC, GCWDF, and RC samples. The increase in this parameter because of temperature is only 2.7 % when the temperature decreases from -10 to -40 ºC. On average, the % of DPPH antiradical activity of the extracts of DFGC, GCWDF, and RC was 94.34, 94.38 and 87.63 %, respectively. A comparison of these three samples indicates that the % DPPH antiradical activity of DFGC is almost equal to that of GCWDF and 8 % higher than that of RC. For green coffee, percentages of inhibition of this radical have been reported from 64 to 83 % (Masek et al., 2020; Patriche et al., 2015), and for roasted coffee at 215 °C from 46 to 99 % (Yahayu et al., 2020).

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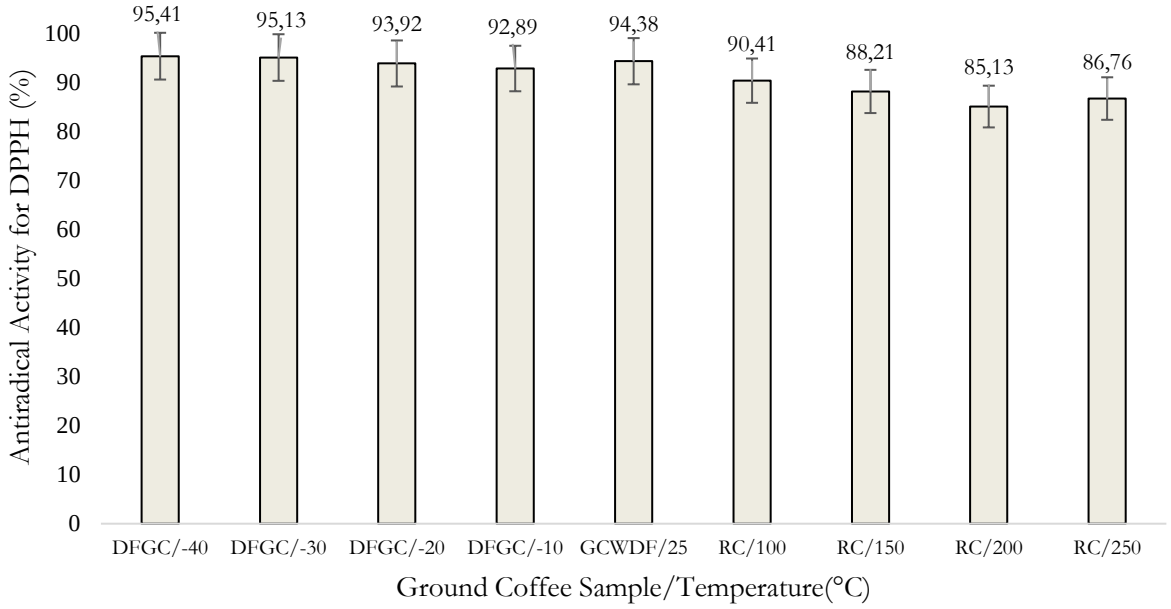

Figure 2. Percentage of antiradical activity for DPPH. *The bars above the columns represent the standard deviation percentage of three repetitions.
Source: Prepared by the authors.

Total Phenolic Compounds by Folin-Ciocalteu Method

Figure 3 shows the results of the content of TPC expressed in mg of gallic acid per gram of coffee on a dry basis obtained by the Folin-Ciocalteau method for the different coffee samples. The highest value of PC content was found in the samples of DFGC, regardless of temperature. At higher temperatures, the TPC content was always lower. The effect of the increase in temperature on TPC content is negative. The same phenomenon is repeated in the roasted coffee samples; the effect of temperature from 100 to 250 ºC is also negative since the TPC content decreased by 20 % on average for each 50 ºC increase in roasting temperature. On average, the TPC content of DFGC, GCWDF, and RC was 158.4, 132.6, and 99.4 mg GA/g coffee, respectively. A comparison of the AC of these three samples indicates that the TPC content of DFGC was 19.4 % higher than that of GCWDF and 59 % higher than RC's.

Values of TPC content in green coffee in the range of 62.1 to 71.2 mg GA/g in coffee samples from different geographic regions have been reported. For this same coffee, but roasted, the TPC content was 41.8 to 38.8 mg GA/g (Bobková et al., 2020). In most cases, the effect of temperature is negative; that is, the higher the temperature, the lower the TPC content.

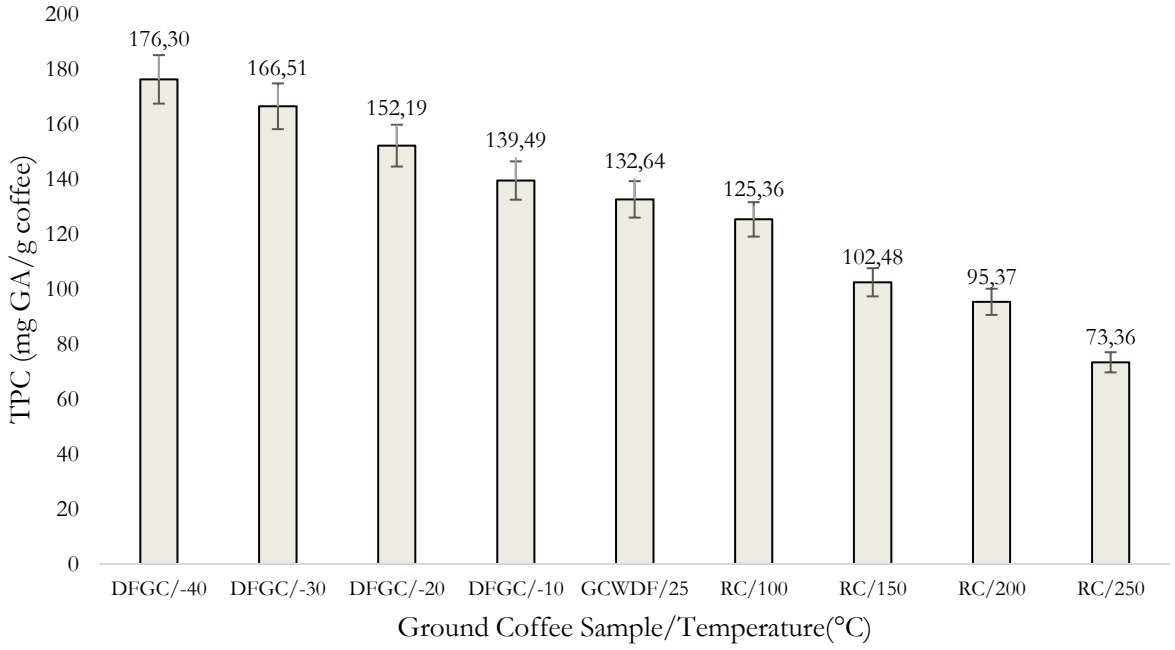

Figure 3. Total phenolic compounds for the Folin-Ciocalteu method. *The bars above the columns represent the standard deviation percentage of three repetitions.
Source: Prepared by the authors.

The Extraction Process of TPC and its Relationship with Moisture Content, Kinetics, and D_{eff}

The average moisture content of DFGC is 8.43 %; this value is 11.5 % higher than that of GCWDF (7.56 %), and it is also 2.2 times greater than that of the RC (3.87 % on average), as shown in Table 3. These results were expected since these are coffee samples with different heat treatments. Moisture content plays a vital role in the extraction process of polar compounds. In this case, the extracts with the highest TPC also have the highest moisture content; this behavior is closely related to temperature; that is, the lower the temperature, the higher the TPC content, as can be seen in Figure 3. This behavior has also been reported in the coffee oil extraction process, which was explained by the fact that the effect of temperature on the increase in equilibrium concentration in the extraction process is greater than the effect on the increased diffusivity (Dibert & Cros, 1989)

Table 3. Physical properties of coffee samples at different temperatures

Ground coffee sample	Temperature (°C)	Moisture content %	$D_{eff} * 10^{11}$ ($m^2 \cdot s^{-1}$)	$k * 10^3$ (s^{-1})
DFGC	-40	8.65 ± 0.15[b]	7.73 ± 0.11[a]	2.53 ± 0.14[b]
	-30	8.56 ± 0.13[b]	7.04 ± 0.16[b]	2.25 ± 0.22[c]
	-20	8.32 ± 0.12[a]	6.48 ± 0.23[c]	2.02 ± 0.25[d]
	-10	8.18 ± 0.19[c]	5.95 ± 0.12[a]	1.82 ± 0.31[a]
GCWDF	25	7.86 ± 0.18[c]	4.70 ± 0.31[d]	1.57 ± 0.12[b]
RC	100	5.16 ± 0.15[b]	5.34 ± 0.14[a]	1.43 ± 0.11[a]
	150	4.52 ± 0.14[d]	5.67 ± 0.25[b]	1.53 ± 0.24[c]
	200	3.15 ± 0.13[b]	6.09 ± 0.18[b]	1.67 ± 0.21[c]
	250	2.65 ± 0.14[d]	6.28 ± 0.33[c]	1.72 ± 0.23[c]

Note. *The results are expressed as the average value ± standard deviation of three repetitions. Values in columns with different letters indicate significant differences ($p \leq 0.05$).
Source: Prepared by the authors.

The kinetics of the process in the extraction were satisfactorily adjusted ($r^2 > 0.99$) to the first-order equation in all cases. The first-order rate constant ranged from 1.43×10^{-3} to 2.53×10^{-3} s^{-1}. These values are characteristic of rapid extraction processes and agree with those reported by other authors who have studied the ethanolic extraction of different solid-liquid systems, including green and roasted coffee (Akemi Toda et al., 2021). Figure 4 clearly shows that the TPC extraction speed in DFGC is greater than that of the others, but in general, it is a fast extraction process because 50 % is reached in less than 10 min. On average, the kinetic coefficient of DFGC is 37 and 36 % higher than that of GCWDF and RC, respectively.

This work found that in the ethanolic extraction process of total phenolic compounds, the D_{eff} is between 4.70×10^{-11} and 7.73×10^{-11} m^2/s, regardless of the conditions of the coffee samples. These values are in the same interval as those reported in the extraction of coffee oil with ethanol in conventional and pressurized processes (Akemi Toda et al., 2021) and also correspond to those reported for the diffusion of caffeine in the decaffeination process (Huamaní-Meléndez & Darros-Barbosa, 2018; Spiro & Chong, 1997), but are superior in two orders of magnitude to those reported for the diffusion of CO_2 in fresh roasted and ground coffee (Anderson et al., 2003).

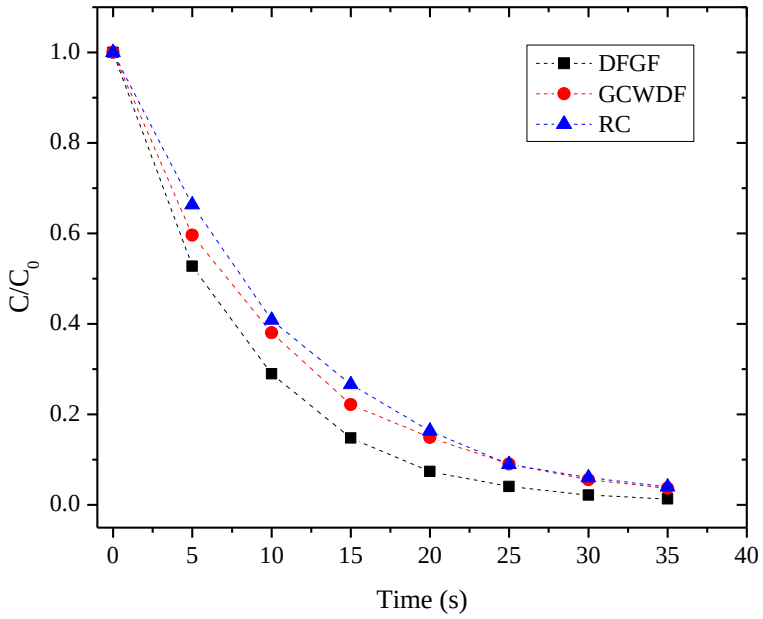

Figure 4. Kinetics of extraction of TPC in the different coffee samples.
Source: Prepared by the authors.

The moisture content in the coffee samples modifies the ethanol-water relationship in the extraction process, which could explain the diffusion results and the process's kinetics.

As seen in Table 3, the values of the practical and kinetic diffusion coefficients in the green coffee samples are positively related to the moisture content; that is, the higher the moisture content in the coffee sample, the higher the values of D_{eff} and k. This is contrary to what happens in roasted coffee samples, where the behavior is reversed. This fact may be related to the convective diffusion process in the extraction process.

On the one hand, the chemical affinity of the polar compounds that coffee naturally contains, with the ethanol-water mixture that is formed, favors a rapid and effective diffusion of these during the extraction process. On the other hand, in roasted coffee samples where the percentage of humidity is lower, the diffusion of the formed compounds is lower. However, their chemical affinity is preferentially to ethanol. This set of compounds which are of a different chemical nature than those of green coffee; although they are indeed responsible for the pleasant aroma and fragrance of roasted coffee, these diffuse more slowly during extraction, and the extracts obtained provide a lower concentration of total phenolic compounds, and consequently offer lower antioxidant activity, as shown in Figure 3.

Analyzing the results in detail, it is worth noting that, on average, the value of D_{eff} in DFGC is 45 % and 16 % higher than that of GCWDF and RC, respectively. These results seem to be

related to the moisture content of the coffee samples; that is, the diffusion of phenolic compounds is favored in samples with higher moisture content. In general, regardless of the extraction method and the source, the extraction of polyphenolic compounds is more efficient using solvents of higher polarity (Pérez et al., 2021). The challenge of this study lies in the evaluation of compounds that are synthesized during the freezing or roasting process. It is possible that new molecules are being formed that could cause an increase or decrease in antioxidant activity. In future studies, quantifying the antioxidant compounds formed by chromatographic analysis could be relevant. Likewise, from this study, it is expected that the consumption of frozen coffee will increase more than traditional roasted coffee, which could result in more health benefits from the associated intake of antioxidants.

Conclusions

The ethanolic extract obtained from DFGC has a higher AC than that obtained from GCWDF and RC. Expressed in mmol Trolox per gram of coffee, the AC of DFGC is six and nine times higher than that of GCWDF and RC, respectively. This gives the DFGC extract a percentage of inhibition of the ABTS radical of 90.5 %, which is 11 and 45 % higher than that obtained from GCWDF and RC extracts. In grams of caffeic acid per gram of coffee, the AC of green coffee is barely 2 % greater than that of GCWDF, but it is almost three times greater than that of RC. In this case, DFGC shows a percentage inhibition of the DPPH radical of 94.4 %, which is practically equal to that of GCWDF and almost 8 % higher than that of RC.
Similarly, the content of TPC in DFGC is, on average, 19 and 59 % higher than that of GCWDF and RC. The polyphenolic compound extraction process satisfactorily fits the first-order kinetic model, and both the kinetic coefficient and the effective diffusion coefficient are related to the moisture content of the coffee sample. The diffusion and extraction speed of the compounds that give the AC to coffee, whether frozen or roasted, is related to the ethanol-water ratio of the solvent. Because of the effect of the polarity of the solvent, the extraction of phenolic compounds in frozen coffee is favored in coffee samples with higher moisture content. Therefore, the deep-freezing process is highly recommended to preserve the AC of green coffee. Moreover, there remains the challenge of designing new products from deep-frozen green coffee for the technology of coffee drinks.

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Authors' Contributions

All authors made written contributions and corrections to the manuscript. The contributions made by the authors were the following: Mónica Monserrat Aguayo-Muñoz: Data collection and analysis and writing of the manuscript. Andrea Yazmín Guadarrama-Lezama: Development and

supervision of the methodology and analytical tests. César Pérez-Alonso: Preparation of tables and figures, use of software and revision of the manuscript in the English language. Julian Cruz-Olivares: Administration, design and development of the project. Writing and editing the final manuscript.

Ethical implications

This article has no ethical implications for its development.

Conflict of interest

The authors declare that there are no conflicts of interest in this study.

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References

Akemi Toda, T., Franco Visioli, P. de C., Lopes de Oliveira, A., & da Costa Rodrigues, C. E. (2021). Conventional and pressurized ethanolic oil extraction from spent coffee grounds: Kinetics study and evaluation of lipid and defatted solid fractions. *The Journal of Supercritical Fluids*, *177*(105232). https://doi.org/10.1016/j.supflu.2021.105332

Anderson, B. A., Shimoni, E., Liardon, R., & Labuza, T.P. (2003). The diffusion kinetics of carbon dioxide in fresh roasted and ground coffee. *Journal of Food Engineering*, *59*, 71-78. https://doi.org/10.1016/S0260-8774(02)00432-6

Artusa, V., Ciaramelli, C., D'Aloia, A., Facchini, F. A., Gotri, N., Bruno, A., Costa, B., Palmioli, A., Airoldi, C., & Peri, F. (2022). Green and roasted coffee extracts inhibit interferon-β release in LPS-stimulated human macrophages. *Frontiers in Pharmacology*, *13*(1), 1-12. https://doi.org/10.3389/fphar.2022.806010

Biglia, A., Comba, L., Fabrizio, E., Gaya, P., & Aimonino, D. R. (2016). Case studies in food freezing at very low temperature. *Energy Procedia*, *101*(1), 305-512. https://doi.org/10.1016/j.egypro.2016.11.039

Bobková, A., Hudácek, M., Jakabová, S., Belej, L., Capcarová, M., Curlej, J., Bobko, M., Arvay, J., Jakab, I., Jozef Capla, J., & Demianová, A. (2020). The effect of roasting on the total polyphenols and antioxidant activity of coffee. *Journal of Environmental Science and Health Part B*, *54*(12), 1-6. https://doi.org/10.1080/03601234.2020.1724660

Bosso, H., Barnalho, S. M., de Alvares Goulart, R., & Machado Bueno Otoboni, A. M. (2021). Green coffee: economic relevance and a systematic review of the effects on human health. *Critical Reviews in Food Science and Nutrition*, *1*, 1-8. https://doi.org/10.1080/10408398.2021.1948817

Cortés-Camargo, S., Román-Guerrero, A., Alvarez-Ramirez, J., Alpizar-Reyes, E., Velázquez-Gutiérrez, S. K., & Pérez-Alonso, C. (2023). Microstructural influence on physical properties and release profiles of sesame oil encapsulated into sodium alginate-tamarind mucilage hydrogel beads. *Carbohydrate Polymer Technology and Applications*, *5*(100302), 1-9. https://doi.org/10.1016/j.carpta.2023.100302

Dawidowicz, A. L., & Typek, R. (2017). Transformation of chlorogenic acids during the coffee beans roasting process. *European Food Research and Technology*, *243*, 379-390. https://doi.org/10.1007/s00217-016-2751-8

Díaz, F. O., Ormaza, A. M., & Rojano, B. A. (2018). Effect of coffee roasting (*Coffea arabica* L. var. Castillo) on cu profile, antioxidant compound content and antioxidant activity. *Información Tecnológica*, *29*(4), 31-42. http://dx.doi.org/10.4067/S0718-07642018000400031

Dibert, K., & Cros, E. (1989). Solvent extraction of oil and chlorogenic acid from green coffee. Part II: Kinetic data. *Journal of Food Engineering*, *10*, 199-214. https://doi.org/10.1016/0260-8774(89)90026-5

Eröz Poyraz, I., Öztürk, N., Kiyan, H. T., & Demirci, B. (2016). Volatile compounds of *Coffea arabica* L. Green and roasted beans. *Anadolu University Journal of Science and Technology. C-Life Sciences and Biotechnology*, *5*(1), 31-35. https://doi.org/10.18036/btdc.13390

Farah, A., & Donangelo, C. M. (2006). Phenolic compounds in coffee. *Brazilian Journal of Plant Physiology*, *18*(1), 23-36. https://doi.org/10.1590/S1677-04202006000100003

Febrina L., Happyana, N., & Syah, Y. M. (2021). Metabolite profiles and antidiabetic activity of the green beans of Luwak (civet) coffees. *Food Chemistry*, *355*(1), 1-9. https://doi.org/10.1016/j.foodchem.2021.129496

Gligor, O., Clichici, S., Moldovan, R., Muntean, D., Vlase, A. M., Nadas, G. C., Matei, I. A., Filip, G. A., Vlase, L., & Crisan, G. (2023). The effect of extraction methods on phytochemicals and biological activities of green coffee beans extracts. *Plants*, *12*, 712. https://doi.org/10.3390/plants12040712

Guadarrama-Lezama, A. Y., Dorantes-Alvarez, L., Jaramillo-Flores, M. E., Pérez-Alonso, C., Niranjan, K., Gutiérrez-López, G. F., & Alamilla-Beltrán, L. (2012). Preparation and characterization of non-aqueous extracts from chilli (*Capsicum annuum* L.) and their microencapsulates obtained by spray-drying. *Journal of Food Engineering*, *112*, 29-37. http://dx.doi.org/10.1016/j.jfoodeng.2012.03.032

Hall, R. D., Trevisan, F., & de Vos, R. C. H. (2022). Coffee berry and green bean chemistry – Opportunities for improving cup quality and crop circularity. *Food Research International*, *151*, 1-20. https://doi.org/10.1016/j.foodres.2021.110825

Huamaní-Meléndez, V., & Darros-Barbosa, R. (2018). Diffusion of water and caffeine beans using the hemispherical geometry approach. *Journal of Food Process Engineering*, e12809. https://doi.org/10.1111/jfpe.12809

Huang, D., Boxin Ou, B., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry*, *53*, 1841-1856. https://doi.org/10.1021/jf030723c

Ibarra-Cantún, D., Ramos-Cassellis, M. E., Sánchez-Arzubide, M. G., Castelán-Vega, R. C., & Marin-Castro, M. A. (2022). Compuestos fenólicos totales y actividad antioxidante de la fermentación en estado sólido de bagazo de manzana (*Malus domestica* Borkh., var. Panochera). *Ciencia y Tecnología Agropecuaria*, *23*(2), e2103. https://doi.org/10.21930/rcta.vol23_num2_art:2103

Ishevskiy, A. L., & Davydov I. A. (2017). Freezing as a method of food preservation. *Theory and Practice of Meat Processing*, *1*(2), 43-59. https://doi.org/10.21323/2414-438X-2017-2-2-43-59

Ismail, T., Donati-Zeppa, S., Akhtar, S., Turrini, E., Layla, A., Sestili, P., & Fimognari, C. (2021). Coffee in cancer chemoprevention: an updated review. *Expert Opinion on Drug Metabolism & Toxicology*, *17*(1), 69-85. https://doi.org/10.1080/17425255.2021.1839412

Kaihatsu, K., Kawakami, C., & Kato, N. (2014). Potential anti-influenza virus agents based on coffee ingredients and natural flavonols. *Natural Products Chemistry & Research*, *2*(2), 1-7. https://doi.org/10.4172/2329-6836.1000129

Kungsuwan, K., Sawangrat, C., Ounjaijean, S., Chaipoot, S., Phongphisutthinant, R., & Wiriyacharee, P. (2023). Enhancing Bioactivity and Conjugation in Green Coffee Bean (Coffea arabica) Extract through Cold Plasma Treatment: Insights into Antioxidant Activity and Phenolic–Protein Conjugates. *Molecules*, *28*(20), 7066. https://doi.org/10.3390/molecules28207066

Leitão, S., Guerra-Guimarães, L., Bronze, M. R., Vilas-Boas, L., Sá, M., Almeida, M. H., & Silva, M. C. (2011). Chlorogenic acid content in coffee leaves: possible role in coffee leaf rust resistance. In *Proceedings of the 24th International Conference on Coffee Science (ASIC2010)(Bali)*.

Marín, C., & Puerta, G. I. (2008). Contenido de ácidos clorogénicos en granos de coffea arabica l y c canephora según el desarrollo del fruto. *Cenicafé*, *59*(1), 7-28. https://biblioteca.cenicafe.org/handle/10778/60

Masek, A., Chrzescijanska, E., Latos, M., Zaborski, M., & Podsedek, A. (2017). Antioxidant and antiradical properties of green tea extract compounds. *International Journal of Electrochemical Science*, *12*, 6600-6610. https://doi.org/10.20964/2017.07.06

Masek, A., Latos-Brozio, M., Kałuzna-Czaplinska, J., Rosiak, A., & Chrzescijanska, E. (2020). Antioxidant properties of green coffee extract. *Forests*, *11*(557), 1-13. https://doi.org/10.3390/f11050557

Menzio, J., Binello, A., Barge, A., & Cravotto, G. (2020). Highly-efficient caffeine recovery from green coffee beans under ultrasound-assisted SC–CO_2 extraction. *Processes*, *8*, 1062. https://doi.org/10.3390/pr8091062

Mishra K., Ojha H., & Chaudhury, N. K. (2012). Estimation of antiradical properties of antioxidants using DPPH assay: A critical review and results. *Food Chemistry, 130*(4), 1036-1043. https://doi.org/10.1016/j.foodchem.2011.07.127

Moon, J-K., Yoo, H. S., & Shibamoto, T. (2009). Role of roasting conditions in the level of chlorogenic acid content in coffee beans: Correlation with coffee acidity. *Journal of Agricultural Food and Chemistry*, *67*(12), 5365-5369. https://doi.org/10.1021/jf900012b

Muñoz, A. E., Hernández, S., Tolosa, A. R., Pérez-Burillo, S., & Olalla-Herrera, M. (2020). Evaluation of differences in the antioxidant capacity and phenolic compounds of green and roasted coffee and their relationship with sensory properties. *LWT - Food Science and Technology*, *128*, 1-7. https://doi.org/10.1016/j.lwt.2020.109457

Ormaza-Zapata, A. M., Diaz-Arango, F. O., & Rojano, B. A. (2022). Analysis of gravity- filtration preparation methods' antioxidant content, capacity, and coffee beverage acceptance. *Ciencia y Tecnología Agropecuaria*, *23*(2), e2438. https://doi.org/10.21930/rcta.vol23_num2_art:2438

Osorio-Tobón, J. F. (2020). Recent advances and comparisons of conventional and alternative extraction techniques of phenolic compounds. *Journal of Food Science and Technology*, *57*(12), 4299-4315. https://doi.org/10.1007/s13197-020-04433-2

Patriche, S., Boboc, M., Leah, V., & Dinica, R. M. (2015). Extraction and evaluation of bioactive compounds with antioxidant potential from green arabica coffee extract. *The Annals of the University Dunarea de Jos of Galati Fascicle VI – Food Technology*, *39*(2), 88-95. https://www.researchgate.net/publication/298842597

Pérez, V. O., Usaquén, J. P. P., Gallego, C. P., & Echeverri-Giraldo, L. F. (2021). Efecto de las temperaturas y tiempos de tueste en la composición química del café. *Revista Cenicafé*, *72*(1), e72103-e72103. https://doi.org/10.38141/10778/72103

Perrone, D., Farah, A., & Donangelo, C. M. (2012). Influence of coffee roasting on the incorporation of phenolic compounds into melanoidins and their relationship with antioxidant activity of the brew. *Journal of Agricultural Food and Chemistry*, *60*, 4265-4275. https://doi.org/10.1021/jf205388x

Reichal, P., & Rajeshkumar, S. (2022). Anticariogenic and anti-inflammatory activity of coffee bean powder ethanolic extract. *International Journal of Early Childhood Special Education*, *14*(5), 5318-5324.

Rojas-González, A., Figueroa-Hernández, C. Y., González-Rios, O., Suárez-Quiroz, M. L., González-Amaro, R. M., Hernández-Estrada, Z. J., & Rayas-Duarte, P. (2022). Coffee chlorogenic acids incorporation for bioactivity enhancement of foods: A review. *Molecules*, *27*(11), 1-23. https://doi.org/10.3390/molecules27113400

Runti, G., Pacor, S., Colomban, S., Gennaro, R., Navarini, L., & Scocchi, M. (2015). Arabica coffee extract shows antibacterial activity against *Staphylococ*cus *epidermidis* and *Enterococcus faecalis* and low toxicity towards a human cell line. *LW-Food Science and Technology*, *62*(1), 108-114. https://doi.org/10.1016/j.lwt.2014.12.039

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, *299*, 152-178. https://doi.org/10.1016/S0076-6879(99)99017-1

Smith, P., Ho, C. K., Takagi, Y., Djaballah, H., & Shuman, S. (2016). Nanomolar inhibitors of *Trypanosoma brucei* RNA triphosphatase. *mBio*, *7*(1), 1-16. https://doi.org/10.1128/mBio.00058-16

Somporn, Ch., Kamtuo, A., Theerakulpisut, P., & Siriamornpun, S. (2011). Effects of roasting degree on radical scavenging activity, phenolics and volatile compounds of Arabica coffee beans (*Coffea arabica* L. cv. Catimor). *International Journal of Food Science and Technology*, *46*, 2287-2296. https://doi.org/10.1111/j.1365-2621.2011.02748.x

Spiro, M., & Chong, Y. Y. (1997). The kinetics and mechanism of caffeine infusion from coffee: the temperature variation of the hindrance factor. *Journal of the Science of Food and Agriculture*, *74*, 416-420. https://doi.org/10.1002/(SICI)1097-0010(199707)74:3<416::AID-JSFA868>3.0.CO;2-0

Tarigan, E. B., Wardiana, E., Hilmi, Y. S., & Komarudin, N. A. (2022). The changes in chemical properties of coffee during roasting: A review. *IOP Conf. Series: Earth and Environmental Science*, *974*, 1-8. https://doi.org/10.1088/1755-1315/974/1/012115

Tian, X., An, L., Gao, L. Y., Bai, J. P., Wang, J., Meng, W. H., Ren, T. S., & Zhao, Q. C. (2015). Compound MQA, a caffeoylquinic acid derivative, protects against NMDA-induced neurotoxicity and potential mechanisms in vitro. *CNS Neuroscience & Therapeutics*, *21*(1), 575-584. https://doi.org/10.1111/cns.12408

Vandeponseele, A., Draye, M., Piot, C., Bernard, D., Fanget, P., & Chatel, G. (2022). Supercritical carbon dioxide in presence of water for the valorization of spent coffee

grounds: Optimization by response surface methodology and investigation of caffeine extraction mechanism. *Foods*, *11*, 4089. https://doi.org/10.3390/foods11244089

Villaño, D., Fernández-Pachón, M.S., Moyá, M. L., Troncoso, A. M., & García-Parrilla, M. C. (2007). Radical scavenging ability of polyphenolic compounds towards DPPH free radical. *Talanta*, *71*(1), 230-235. https://doi.org/10.1016/j.talanta.2006.03.050

Yahayu, M., Sawal Hamid, Z. B., El Adawi, H., Dailin, D. J., Malek, R, A., Hanapi, S. Z., Boumehira, A., Ngadiran, S., Yusoff, I. M., Ho, T., & Enshasy, H. E. (2020). Antioxidant activity in green and roasted coffee: A critical review. *Bioscience Research*, *17*(3), 2249-2263. https://www.researchgate.net/publication/346110317