
Considerations on the authenticity and nutritional aspects of green coffee products consumed in Brazil

Considerações sobre os aspectos de autenticidade e nutricionais de produtos de café verde consumidos no Brasil

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ABSTRACT

This manuscript seeks to assess the composition of commercial green coffee extracts, analyzing their authenticity, nutritional and anti-nutritional aspects, to provide technical information for a qualified discussion about the effective healthiness of the consumption of commercial green coffee extracts. Nine samples of green coffee food supplements were selected and a product was prepared to serve as a standard. It was possible to identify coffee DNA in six of the commercial samples of food supplements. The products had antioxidant activity between 33 and 2408 μmol of Trolox/g. It was possible to identify chlorogenic acid in all samples with concentrations ranging from 0.023 - 20 mg / g of coffee. Six samples presented positive values for ochratoxin A, despite being within the limits of the coffee roasted legislation, there is a certain concern about the safety of these supplements.

Keywords: Chlorogenic acid; Green coffee capsules; Ochratoxin A; Food supplement

RESUMO

Este manuscrito busca avaliar a composição dos extratos comerciais de café verde, analisando sua autenticidade, aspectos nutricionais e antinutricionais, para fornecer informações técnicas para uma discussão qualificada sobre a efetiva salubridade do consumo de extratos comerciais de café verde. Foram selecionadas nove amostras de suplementos alimentares de café verde e elaborado um produto para servir como padrão. Foi possível identificar o DNA do café em seis das amostras comerciais de suplementos alimentares. Os produtos apresentaram atividade antioxidante entre 33 e 2.408 μmol de Trolox/g. Foi possível identificar ácido clorogênico em todas as amostras com concentrações variando de 0,023 – 20 mg/g de café. Seis amostras apresentaram valores positivos para ocratoxina A, apesar de estarem dentro dos limites da legislação de café torrado, existe uma certa preocupação quanto à segurança destes suplementos.

Palavras-chave: Ácido clorogênico; Cápsulas de café verde; Ocratoxina A; Suplemento alimentar

INTRODUÇÃO

Coffee is one of the most consumed beverages in the world. Lately, the development of studies on the biological activity of the constituents of green beans and beverages has been more encouraged and explored (Lima et al., 2010). Green coffee beans differ in chemical composition according to species and origin. They are mostly composed of carbohydrates, lipids, and proteins, in addition to secondary components, such as caffeine, trigonelline, chlorogenic acids, diterpenes, melanoidins, among others (Souza et al., 2020). However, during the coffee roasting process, several chemical reactions occur at the same time, from which some compounds are degraded, such as proteins, polysaccharides, and chlorogenic acids.

Brazil is the main producer and exporter of coffee worldwide, producing in 2023 around 54.7 million bags of coffee in total, of which 37.9 and 16,8 million bags were from *Coffea arabica* and *C. canephora* respectively, mostly destined for export (ICO, 2023).

Current trends in the production and consumption of foods containing natural antioxidants serve not only the interest of providing greater protection to the components of food affected by oxidation but also the obtainment of products with functional properties that will act in the consumer's health. Different compounds present in coffee have chemoprotective effects in chemical and biological systems, justified by the presence of antioxidants, such as chlorogenic acids (Lima et al., 2010). A cup of roasted coffee beverage is rich in different and complex bioactive substances, which can have antioxidant activity, the drink being an important source of consumption of natural antioxidants (Ghaascht et al., 2015).

In addition to the consumption of coffee drink predominantly with the infusion of roasted and ground coffee, lately Green Coffee Extract (GCE) marketed in different forms, such as capsules, flours, or liquid extract, has conquered a slice of the market for the appeal of healthiness, because it contains from 6 to 12% of chlorogenic acids, of which during the process of roasting the grain undergoes degradation of a portion of them, for the preparation of the traditional

beverage (Souza et al., 2020). The consumption of GCE appeared intending to lose weight due to the thermogenic effect of coffee. Dellalibera et al. (2006) demonstrated that the consumption of chlorogenic acid contributes to weight loss. According to Shimoda et al. (2006), the consumption of chlorogenic acid as an isolated substance does not suppress weight gain in rats, that is, the study suggests that to suppress weight gain, it is necessary to have a synergy between the substances present in green coffee, such as caffeine, chlorogenic acid, and other phenolic components.

Although chlorogenic acids are present in several plants, the green coffee bean is one of the main sources, especially the species *Coffea canephora*, which can present up to three times more chlorogenic acids than *C. arabica* (Narita and Inouye, 2012). In addition, chlorogenic acid, present in coffee beans, is highly bioavailable for the human body (Crozier et al., 2009), which may contribute to the coffee extract being used successfully in food supplementation.

Despite the various nutrient compounds in coffee beans, there are also antinutrient compounds such as phytates and tannins (Nobile et al., 2010; Ramirez-Coronel et al., 2004). They bind strongly to some proteins and inhibit digestive enzymes, compromising the digestion of proteins and other macronutrients. Besides this, they interact with non-haematic iron, inhibiting the absorption of metals (Popova and Mihaylova, 2019). Moreover, several microbiological studies have shown that *Aspergillus* and *Penicillium* fungus are natural contaminants of coffee beans (Batista et al., 2003). Ochratoxin A (OTA) is a metabolite commonly produced by the fungus. This toxin is considered hepatotoxic, nephrotoxic, and carcinogenic (Schrenk et al., 2020).

Modern consumers expect food products healthy, ready to consume, and containing the same or more nutrients than natural foods. In this context, food supplements in the form of capsules or flours emerge as an alternative (Costa et al., 2020). Except for vitamins/minerals, a huge knowledge gap exists regarding the role of nutraceuticals in human health (Mahabir, 2014). However, there are few reports in the literature on the composition of green coffee commercial products, considering if coffee or their characteristics metabolites (caffeine and chlorogenic acids) are present if their antioxidant capacity was preserved, beyond other aspects.

In this way, the objective of this work was to evaluate the authenticity of commercial green coffee products available on the market as food supplements by molecular and chromatography methodology, antioxidant capacity, minerals composition, nutritional and antinutritional aspects (Phytates, tannins, and ochratoxin A). In addition, the green coffee extract was prepared for comparison with commercial samples.

MATERIAL AND METHODS

GREEN COFFEE PRODUCTS

Ten green coffee products described in Table 1, were analyzed at Embrapa Food Agroindustry. Nine food supplements based on green coffee of different commercial brands - were selected, samples 1 to 8 were purchased directly from the retail trade in Rio de Janeiro or on line in the form of capsules or flours. Sample number 9 was purchased in the United States of America and product 10, was prepared in this work.

The extraction of the bioactive compounds from commercial products was carried out, according to the methodology adapted from Liu et al., (2010) as follows: The capsule wrap was removed from the samples that were in this form, then it was weighed around 10 g of the 250 mL Becker samples and added about 150 mL of distilled water (0.067 g/mL). For the prepared sample (sample 10), Robusta coffee (*C. canephora*) from Espirito Santo (ES) was ground in an IKA grinder (A11) and sieved (480-680 µm). Then, the aqueous extract was prepared using a ratio of 0.11 g/mL (mass/volume) between coffee and distilled water.

Table 1 - Characteristics of commercial green coffee food supplements acquired for this work according to their labeling

Green coffee extract products										
	1 capsule	2 capsule	3 capsule	4 capsule	5 capsule	6 capsule	7 capsule	8 flour	9 capsule	10 soluble powder
Ingr edie nts:	sesame oil, coffee green powder, cocoa powder, soy lecithin emulsifier, gelatin gelling capsule, glycerin humectant, and colorants	acerola extract, green coffee extract, gelatin, humectant glycerin	dry green coffee extract, dry green tea extract, and gelatin capsule	Safflower oil, powder green coffee extract (<i>Coffea canephora</i> Pierre, maltodextrin, silica anti-humectant), gelatin gelling, glycerin humectant, soy lecithin emulsifier, purified water carrier, and colorants	green coffee (<i>Coffea arabica</i>), excipien t: corn starch. Anti-humecta nts: talc and silicon dioxide, Capsule composi tion: gelatin and glycerin humecta nt	capsule green coffee powder, silicon chelate, ascorbic acid, chromiu m picolinat e, biotin, microcr ystalline cellulose thickene r, lubrican ts: magnesi um silicate and magnesi um stearate, silicon dioxide	choline bitartrat e, chromiu m picolinat e, magnesi um, natural aroma: powdere d green coffee, excipien t: magnesi um silicate, anti-humecta nt: silicon dioxide, gelatin capsule, and colorant s	flours: green coffee, chia, golden flaxseed, plum, papaya, orange, grape, eggpl ant, banan a, apple, carrot, tomat o, passio n fruit, white beans, blackb erry, ginger, guaran	Green coffee extract (<i>Coffea canephora</i>). Other ingredie nts: microcr ystalline cellulose, calcium phosphat e dihydrat e, Hydrox ypropyl cellulose, croscar mellose sodium, magnesi um stearate, hydroge nated	Green coffee (<i>Coffea canephora</i>), maltode xtrin

						anti-humectant, sucralose sweetener, natural sweeteners: steviol and thaumatin glycosides, colorant		a powder, psyllium, agar-agar, chlorophyll extract, olive, and hibiscus teas	vegetable oil, silicon dioxide, vegetable base coating	
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Commercial and prepared samples were taken in Hielscher tip ultrasound with a power of 200 wats for 10 minutes with a sample immersed in an ice bath. Right after the extraction, the samples were taken to the refrigerated centrifuge at 7000 rpm for 14 minutes to separate the solid part. Then, samples were portioned in 1.5 mL aliquots and stored at $-18\text{ }^{\circ}\text{C} \pm 2$.

For concentration and drying of the bioactive compounds, extract from raw material (sample 10) was taken from spray dryer (Buchi 190 bench-top) to spray the sample into a chamber with a hot air stream at $170\text{ }^{\circ}\text{C}$ for solvent vaporization and transformation of the sample into soluble powder, being cooled up to $90\text{ }^{\circ}\text{C}$ at the outlet of the hot air chamber and with a flow rate of 0.96 liters/hour.

AUTHENTICITY ANALYSIS OF COMMERCIAL PRODUCTS

To analyze the authenticity of the commercial products were considered molecular analysis and the presence of major components such as caffeine and chlorogenic acids by chromatography.

MOLECULAR ANALYSIS

The DNA extraction of the products was carried out, followed by Polymerase Chain Reaction (PCR) for samples that presented a good DNA extraction yield, and for samples with low extraction yield, the Reaction in Real-Time Polymerase Chain (PCR-rt) was performed to identify specific DNA fragments of coffee.

DNA extraction analysis was carried out according to the methodology adapted from Murray & Thompson (1980) following the CTAB (cetyltrimethylammonium bromide) lysis buffer extraction protocol. DNA extraction required a thermal lysis step at $65\text{ }^{\circ}\text{C}$ in the presence of CTAB, followed by several extractions and purification steps, to remove contaminants such as proteins (digestion with proteinase K). Chloroform was used to separate CTAB nucleic acids and polysaccharide/protein complexes. Finally, the nucleic acids were purified by isopropanol precipitation and washed with ethanol (Couto et al., 2019).

PCR analysis was performed in GeneAmp* PCR system 9700 thermal cyclers (Applied Biosystems, USA). Reactions were conducted using CAFE1F e CAFE1R primers, developed at Embrapa. Denaturation: 95 °C/50 s; hybridization: 60 °C/50 s; 60 cycles.

For electrophoresis in agarose, gel with a 2% concentration of agarose was prepared and stained with ethidium bromide. DNA samples were stained with methylene blue to be applied to the gel, then samples were documented by photo with UV transilluminator L.PIX Molecular Imaging.

The PCR-rt analysis used the thermal cycler Abi 7500 (Applied Biosystems, USA), using the SYBR Green system (Qiagen, QuantiTect) as a dye/intercalant as a reporter and with CAFE1 primers, specific for coffee DNA, developed by Molecular Diagnostic Laboratory in Embrapa Food Agroindustry. For negative control of the analysis, Milli-Q water was used. After the end of the reaction, the data generated by the thermal cycler software, such as the graphs with the amplification and dissociation curves (melt curve) and T_m (melting temperature/dissociation temperature) of each reaction replica, were collected and analyzed (Ferreira et al., 2016).

DETERMINATION OF CAFFEINE AND CHLOROGENIC ACIDS

The caffeine and chlorogenic acids content were measured by the HPLC method with a Waters Alliance 2695 photodiode array detector (PDA), 2996 chromatograph, and Empower® software (Waters, Milford, MA, USA). An Hypersil C18 BDS (Base Deactivated Silica) column (5 cm × 4.6 mm and 2.6 µm; Thermo Scientific, Milford, MA, USA) was used.

The mobile phase for caffeine determination was composed of 10% acetonitrile in a 0.5% (v/v) acetic acid solution and was also used as a solution for sample extraction: 1 g in 25 mL volumetric flask for 10 min in an ultrasonic bath. The samples were filtered through rapid filter paper and microfiltered into disposable hydrophilic Teflon filter units with a porosity of 0.22 µm. The external caffeine standard was prepared by weighing approximately 30 mg of caffeine in 30 mL, solubilized with the mobile phase. Detection was performed at 272 nm, with a mobile phase flow rate of 0.5 mL/min and an injection volume of 20 µL (Bauer et al., 2018; Souza et al., 2020).

The mobile phase gradient for chlorogenic acid consisted of the initial composition of 5% methanol (phase A) and 95% formic acid (phase B), maintained for 6 min. After 8 min, the composition of the mobile phase reached 80% of phase A and remained there for up to 10 min. After 11 min, the composition reached the plateau of 100% of phase A, and from 12 min to 15 min, the composition returned to the initial conditions. Detection was performed at 320–325 nm, the flow rate of the mobile phase was 1 mL/min and the injection volume was 3 µL. Samples were extracted in an ultrasonic bath for 20 min with 20% acetonitrile in ultrapure water (v/v). The samples were then centrifuged, microfiltered in disposable hydrophilic Teflon filter units with a porosity of 0.22 µm. A pre-calibrated system with chlorogenic acid external standards (Sigma-

Aldrich, New York, NY, USA) was prepared by weighing about 30 mg of the standard into a solubilized 25 mL of water and pelleted with 0.5% formic acid (Bauer et al., 2018).

OXYGEN RADICAL ABSORBANCE CAPACITY (ORAC)

The determination of antioxidant activity by the ORAC method was performed according to the method described by Zuleta et al. (2009).

The samples extracts were diluted with sodium phosphate buffer 75 mM pH 7.4. Trolox solution 20 to 100 μ M were used as standards and sodium phosphate buffer 75 mM pH 7.4 as blank. Diluted extracts, standard solutions, and blank were transferred to 96 well black microplates (Greiner 96 Flat Bottom Black Polystyrol). The microplate was taken to the fluorimeter spectrum (Tecan model Infinite 200) at 37 °C programmed with an excitation wavelength of 485 nm and emission wavelength of 535 nm. Then, fluorescein solution 78 nM and AAPH [2,2'-azobis dichloride (2-amidinopropane)] radical 221 mM were added. The reaction volume proportion among samples: fluorescein: AAPH was 80 μ L: 80 μ L: 40 μ L, respectively.

Fluorescence was measured every 5 min until the relative fluorescence intensity (FI%) was lower than 5% of the initial reading value. The area under the curve (AUC) was calculated using software (Prism) by plotting the fluorescence readings overtime for all the samples, Trolox solutions, and blank. Results were expressed as μ mol Trolox Equivalent (TE) per g of sample.

PROXIMATE COMPOSITION

To certify the information on the labeling of commercial products and to provide information on the proximate composition and caloric value of the product developed, physical-chemical analyses were carried out on all products.

Moisture, protein, fat, crude fiber, and ashes were determined according to the methods described in AOAC (2010). Total carbohydrates content was calculated by difference (100—total gross chemical composition) on a dry weight basis according to AOAC (2010).

The caloric value of green coffee supplements was calculated with the following factors: protein 4 kcal/g, fat 9 kcal/g, and carbohydrates 4 kcal/g according to Brazilian regulation RDC 360 (ANVISA, 2003).

MINERALS COMPOSITION

For digestion and quantification of minerals in food samples, tests accredited to ABNT NBR / ISO 17025 (2005) were used to determine food samples.

The samples were mineralized by cavity microwaves—and quantified by plasma optical emission spectrometry following by AOAC (2005). The minerals evaluated were Na, K, Mg, Ca, Mn, Fe, Zn, Cu, P, Cr, Al, Cd, Co, and Pb.

PHYTATES AND TOTAL TANNINS DETERMINATION

Phytates determination was carried out in Plasma according to method No. 986.11 (AOAC, 2010) modified as follows: 2 g of the sample was weighed and added HCl and taken to the shaker for 3 hours to be vacuum filtered, and aliquots were removed and passed through a column filled with resin for further analysis in a 640 nm (Bel spectrophotometer).

Total tannins were determined according to the methodology described by Broadhurst & Jones (1978) based on the reaction of the sample with a 4% vanillin dissolved in methanol with 10% HCl solution. For the reaction, 1 mL of the extract was added to 5 mL of vanillin solution in test tubes. After 20 minutes of reaction at room temperature, the absorbance was measured at 500 nm (Bel spectrophotometer) against a blank sample. The blank sample was prepared by replacing the 4% vanillin solution with methanol- HCl solution. For the standard curve, a 0.1% catechin sample in 80% methanol solution in water was prepared and diluted in concentrations of 0.6 to 2.4 mg L⁻¹ of condensed tannins.

OCHRATOXIN A

Ochratoxin A of green coffee products was analyzed according to Rosa et al. (2019). The analysis was based on the extraction of ochratoxin with methanol solution: sodium bicarbonate 3% v / v, purification of the extract in immunoaffinity column (Supelco® Support), followed by detection and quantification of Ochratoxin A by HPLC with a fluorescence detector (Waters), X Terra® RP18 column, 5 µm, 4.6 x 250 mm with a mobile phase of water, acetonitrile, methanol, and acetic acid with an injection volume of 20 µL.

MYCOLOGICAL ANALYSIS

The fungus microbiota present in the green coffee samples were analyzed according to the adapted methodology of Copetti et al. (2009), 1 g of sample was weighed and added in 9 mL of peptone water (0.1%) + tween 80 (0.3%), homogenized and serial dilution was prepared. Then, a 0.1 ml aliquot of the suspension was plated in culture media Dichloran-Glycerin (DG18), Potato-Dextrose-agar (PDA), and Dichloran Rose Bengal Chloramphenicol (DRBC) and homogenized with a drigalski loop. The plates were incubated in BOD for 7 days at 25 °C, and then the growth of fungal colonies was counted. Results were expressed in CFU/g.

Representative colonies with the potential to produce Ochratoxin A (OTA) were isolated in a culture medium until the pure culture was obtained. The purified *Aspergillus* isolates were inoculated at three points in culture medium Malt Extract and Agar (MEA) and Czapek Yeast Extract Agar (CYA), before identification based on macroscopic characteristics (colony growth, colony diameter, and microscopic) using the identification scheme of Pitt and Hocking (2009), Powell, et al. (2013), and Samson et al. (2014).

RESULTS AND DISCUSSION

PRODUCTION OF GREEN COFFEE EXTRACT FROM RAW MATERIAL (GCE)

Approximately 2 liters of GCE were taken to the spray dryer equipment for atomization and transformation of the soluble powder extract, obtaining a yield of 150 g of soluble powder, which was used in the other analysis of the work.

3.2 AUTHENTICITY OF COMMERCIAL PRODUCTS

Several food source products are available on the market. The real-time PCR technique, using specific primers for a given analyte, is already established to identify DNA with high specificity (Arlorio et al., 2007; Costa et al., 2012; Ferreira et al., 2016). This technique has also been used to analyze the authenticity of food supplements (Lee et al., 2016; Pawar et al., 2017), but so far, the verification of the authenticity of food supplements based on green coffee has not been reported in the literature.

After extracting DNA from samples of dietary supplements, electrophoresis was performed. The pair of CAFE1 primers developed at the molecular diagnostic laboratory at Embrapa Food Agroindustry were used. The primer proved to be specific since only a fragment of 100 base pairs showed amplification.

Samples were applied in triplicate to wells containing negative and positive controls. The samples that showed amplification were: 2, 4, 6, and 8. The other samples were submitted to real-time PCR analysis using the SYBR Green system. Figure 1 shows the amplification curve of the samples, together with the positive control, showing the existence of coffee DNA in samples 3 and 9.

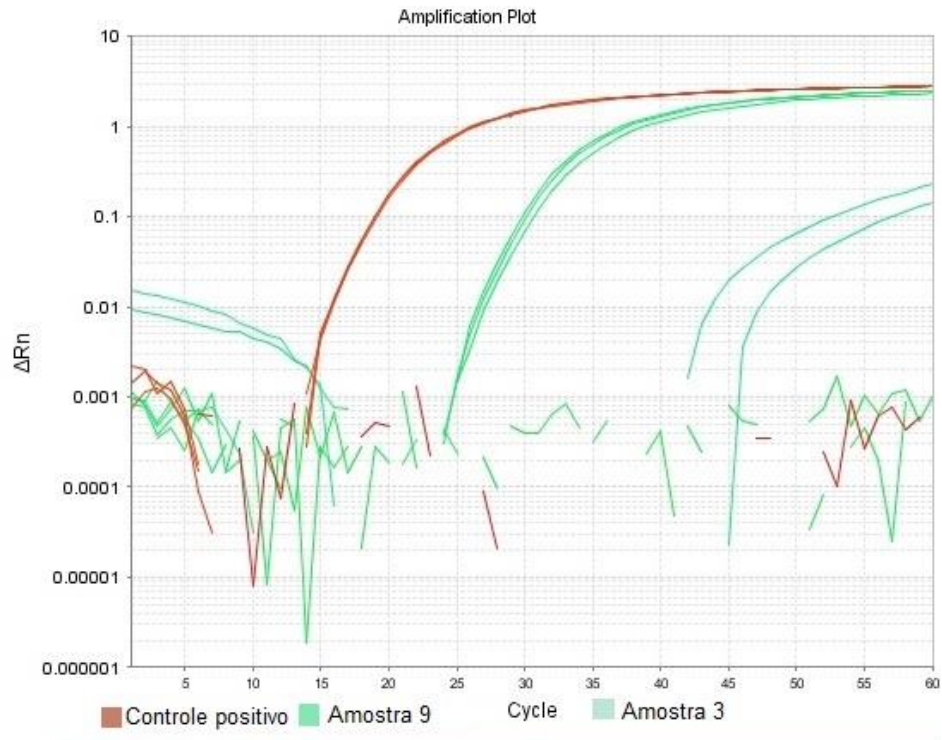


Figure 1 - Amplification curve for samples 3 and 9 and positive control

In samples 1, 5, and 7 that did not amplify using the pair of initiating oligonucleotides, was observed that the melting temperature was different from the positive control, around 78 °C, and it did not make it possible to identify the presence of coffee DNA in these samples, as shown in Figure 2.

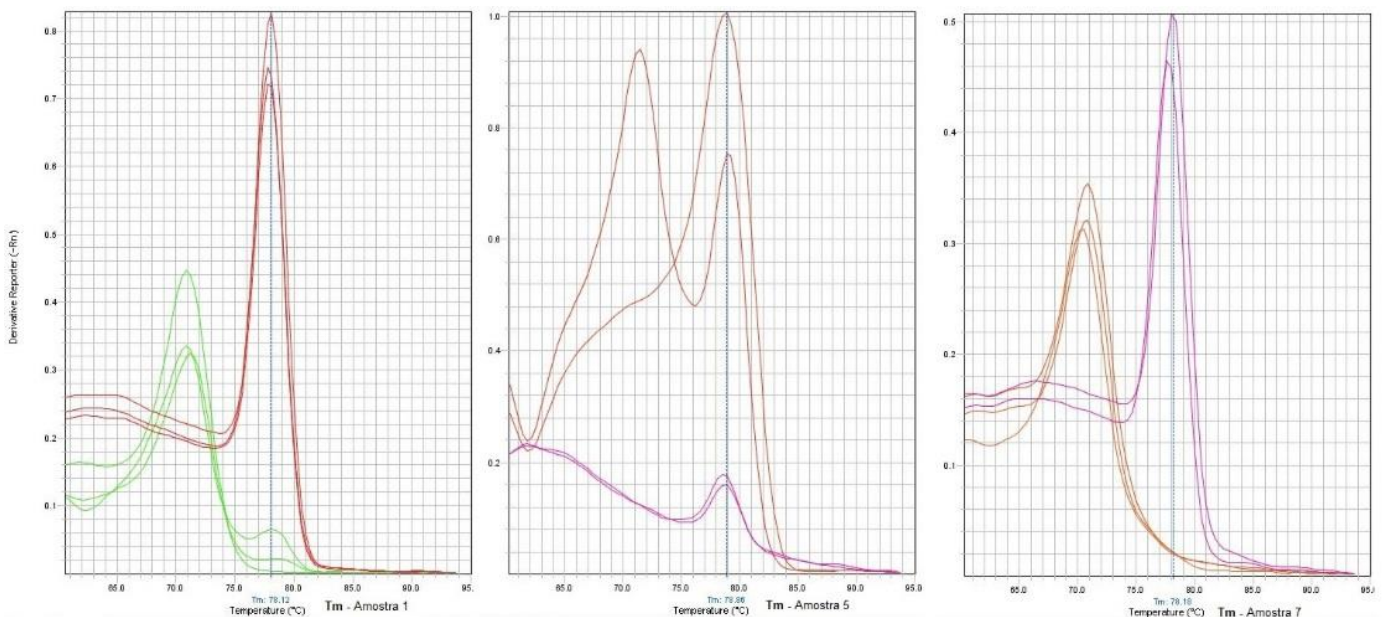


Figure 2 - Melting temperature curve for samples 1, 5, and 7, respectively.

It was possible to identify coffee DNA in 6 of the 9 samples, which shows that the technique is feasible for food supplements based on green coffee, however, the processing methods of samples 1, 5, and 7 can make PCR analysis unviable. The low yields of DNA extraction can be explained by the production process of food supplements, since they can degrade DNA in processing, in addition to the presence of polyphenols and polysaccharides that can inhibit the action of the enzyme Taq Polymerase, making PCR analysis not feasible (Manzano et al., 2003 and Martellosi et al., 2005). Other analyses, such as the quantification of chlorogenic acids and caffeine, were also used as a complementary analysis to identify the authenticity of these products (Table 2).

Table 2- Parameters considered on authenticity analysis of the green coffee food supplements.

Samples number	DNA identification	Caffeine (mg/g)	5-caffeoylquinic acid (mg/g)
1	No	9.73	17.21
2	Yes	12.77	0.45
3	Yes	1.25	0.37
4	Yes	1.41	20.55
5	No	0.57	1.33
6	Yes	0.18	0.5
7	No	3.71	0.94
8	Yes	0.24	0.023
9	Yes	5.52	14.49
10	Yes	20.00	48.8

Sample 1 presented notable quantification of caffeine (9.73) and chlorogenic acid (17.21). Despite having cocoa that is also rich in caffeine, chlorogenic acid is a typical coffee metabolite. In samples 5 and 7, chlorogenic acids and caffeine were quantified and no other ingredient rich in these compounds was found described in their label. Considering this, although samples 1, 5, and 7 did not present coffee DNA in molecular analysis, all samples analyzed were authentic, green coffee was present, even in low quantities.

The caffeine results of green coffee food supplements ranged from 0.18 to 20 mg/g. It is important to consider that in some samples, in addition to green coffee, the presence of other ingredients in the composition, such as cocoa and green tea, can influence the quantification of caffeine. The food supplement (sample 10) prepared in this study showed the highest levels of caffeine concerning commercial samples.

Jeszka-Skowron et al. (2016) reported that caffeine concentrations in 7 samples of green robusta coffee presented concentrations ranging from 68 - 80 mg/g and Belguidoum et al. (2014)

found caffeine levels in green robusta coffee between 0.25 - 27.2 mg/g, values close to green coffee food supplements evaluated in the present study.

Chlorogenic acids data are shown only as a function of the main isomer which is 5-caffeoylquinic acid (5CQA). The results varied in a range of 0.50 - 48.8 mg/g for (5CQA). The product elaborated in this work (sample 10) was the one with the highest concentrations of 5CQA acid (48.8 mg/g). Dziki et al. (2015) analyzed the profile of chlorogenic acids (5CQA) from 4 samples of Arabica coffee and obtained results of 27.64 - 39.92 mg/g.

Jeszka-Skowron et al. (2016) analyzed the levels of chlorogenic acid in 7 samples of green Robusta coffee with concentrations ranging between 56 - 144 mg/g, highlighting the levels of chlorogenic acids are higher in *C. canephora* than *C. arabica* beans and that the consumption of fresh green coffee beans can influence and contribute more to the consumption of phenolics than green coffee-based dietary supplements.

ANTIOXIDANT CAPACITY

The results of antioxidant activity determined by the ORAC methodology ranged from 33.02 μmol of Trolox/g to 2408.05 μmol of Trolox/g, which shows the heterogeneity of the products (Figure 3). The range of variation in the antioxidant activity of green coffee food supplements is probably due to the variation in the formulation of products that contain other sources of compounds that also contribute to antioxidant function (Costa et al., 2012). The GCE supplement elaborated in this work (sample 10), showed excellent antioxidant potential when compared to commercial green coffee products.

Samples with higher antioxidant activities are those with higher concentrations of chlorogenic acid (1,4,9 and 10). Sample 8 showed low concentrations of chlorogenic acid and caffeine but high antioxidant activity probably due to other components in the formulation such as fruits rich in other antioxidant compounds.

Liang et al. (2016) analyzed the antioxidant activity of green arabica coffee beans from the Dominican Republic, Peru, Sumatra, Papua New Guinea, and Ethiopia and obtained values of 410, 450, 420, 380, and 410 μg Trolox equivalent/g. Amigoni et al. (2017) analyzing the green coffee extract obtained through ultrasound extraction using water and methanol as a solvent, obtained results of 3291.7 μg equivalent Trolox/g.

The process of obtaining green coffee food supplements can be differential about the extraction of bioactive compounds, in addition to the formulation and choice of raw material, since it is known that *C. canephora* coffee beans contain more chlorogenic acids, which play a role an important factor in the antioxidant activity of a product (Hendre and Aggarwal, 2014).

PROXIMATE COMPOSITION

To compare the proximate composition of the commercial samples with the information contained on the labels and perform the physical-chemical characterization of the product elaborated in this work (sample 10), the results of the analysis were performed in Table 3.

Table 3- Proximate composition of green coffee food supplements

Proximate Composition (g /100 g)	1	2	3	4	5	6	7	8	9	10
Moisture	1.41	9.37	8.4	NQ	8	6.13	7.42	10.24	5.67	4.48
Ashes	16.22	0.35	0.46	1.62	1.97	3.54	13.35	3.05	20.6	3.71
Total Nitrogen	1.28	0.54	0.1	0.37	0.05	0.15	1.95	1.59	0.35	2.03
Protein	7.36	3.11	0.58	2.13	0.29	0.86	11.21	9.14	2.1	11.67
Fat content	61.43	0.68	0.12	81.18	0.09	0.45	0.5	2.52	2.88	0.71
Dietary fiber	NQ	2.29	NQ	NQ	NQ	68.23	10.8	20.13	39.09	3.49
Carbohydrate content	13.58	84.21	90.43	15.07	89.65	20.79	57.44	54.92	29.75	75.94
Caloric value (Kcal/100g)	626.36	355.36	365.08	799.42	360.57	90.65	279.1	278.92	152.96	356.83

NQ: Not quantified

Brazilian legislation determines that for protein, carbohydrate, fat, and fiber values above 0.5 g/portion and caloric values above 4 kcal/portion, the nutritional amount must be expressed on the label. Comparing nutritional information from Brazilian commercial samples (1 to 8) with the results obtained in the analyzes performed, 1 sample differed for fat (sample 4), 1 sample differed for protein (with a value on the label above the determined, sample 1), 1 sample differed for fiber (with value in analyses above the value expressed on the label, sample 6) and 1 sample differed for caloric value (with value in analyses above the value expressed on the label, sample 5). The divergences were more frequent in carbohydrates where 5 samples diverged from the values in label and presented values above 0.5 g/portion (sample 1, 2, 3, 5, and 7).

Sample 9, a product purchased in the United States of America, due to the country's legislation, does not specify caloric values and centesimal composition on its label.

The product elaborated in this work (sample 10) was the product that presented the highest Nitrogen and protein value. For the other analysis, the value obtained comprises the values presented for commercial samples.

MINERALS COMPOSITION

Some essential minerals for the normal metabolic functioning of an organism can be found in raw coffee (Ukers, 1976). Among these, the macro-minerals Ca, K, Mg, Na, P,

microminerals Co, Cr, Cu, Fe and Mn, Zn being mentioned as "ultra-trace" minerals, that is, elements essential to the organism in nanogram concentrations (Morgano et al., 2002). Therefore, considering the increasing consumption, the levels of toxic elements (Al, Cd, Pb) present in green coffee have to be determined and kept under control in terms of its safety (Şemen et al., 2017). The value regarding the mineral composition of green coffee food supplements proved to be very varied (Table 4), which can be explained by the variety of formulations of the products, in addition, some products such as samples 6, 7, and 9 add some minerals, as specified on the label of the products.

The results obtained in this study and the literature also demonstrated a very wide range in the concentration of minerals, as reported by Morgano et al. (2002), Santato et al. (2012), and Şemen et al. (2017). Potassium was the majority mineral in 6 samples (samples 2, 3, 4, 5, 8,10). Magnesium was the majority in 2 samples (6, 7), Phosphorus in sample 1, and Calcium in sample 9. According to Brazilian legislation, when the sodium limit exceeds 5 mg/portion, the product must be labeled. For all samples, sodium was below this limit, meaning that the labels of the brands evaluated were in agreement.

Table 3- Proximate composition of green coffee food supplements

Proximate Composition (g /100 g)	1	2	3	4	5	6	7	8	9	10
Moisture	1.41	9.37	8.4	NQ	8	6.13	7.42	10.24	5.67	4.48
Ashes	16.22	0.35	0.46	1.62	1.97	3.54	13.35	3.05	20.6	3.71
Total Nitrogen	1.28	0.54	0.1	0.37	0.05	0.15	1.95	1.59	0.35	2.03
Protein	7.36	3.11	0.58	2.13	0.29	0.86	11.21	9.14	2.1	11.67
Fat content	61.43	0.68	0.12	81.18	0.09	0.45	0.5	2.52	2.88	0.71
Dietary fiber	NQ	2.29	NQ	NQ	NQ	68.23	10.8	20.13	39.09	3.49
Carbohydrate content	13.58	84.21	90.43	15.07	89.65	20.79	57.44	54.92	29.75	75.94
Caloric value (Kcal/100g)	626.36	355.36	365.08	799.42	360.57	90.65	279.1	278.92	152.96	356.83

NQ: Not quantified

Among toxic minerals, aluminum was more frequently quantified in the samples analyzed. Sample 7 presented 353.94 µg/g, considering the recommendation of taking 2 capsules a day of the product, having as reference 1.32 g of daily consumption, the intake will be 3270.96 µg of Aluminum per week, above the limit of 1000 µg kg/wk established by CAC (2003). The same sample presented Lead but the intake will be 2.77 µg per week and this value is below to 25 µg kg/wk the established limit by CAC (2003).

PHYTATES AND TOTAL TANNINS DETERMINATION

Phytate concentrations in green coffee samples analyzed are presented in Table 5. Sample 8 showed a higher level of phytate, which can be explained by the composition of the product, which in addition to coffee contains chia and flaxseed, cereals that can be sources of phytate (Silva and Silva, 1999). The other food supplements, on the other hand, do not have cereals in their formulation, which may explain the low or absence of phytate concentration.

Table 5- Phytates and Ochratoxin A determination in samples of green coffee food supplements

Phytates and OTA determination in green coffee food supplements										
Sample number	1	2	3	4	5	6	7	8	9	10
Phytate (mg/g)	3.63	NQ	NQ	NQ	NQ	NQ	NQ	15.95	0.76	4.56
Ochratoxin (µg/kg)	158	4.80	0.61	0.26	NQ	NQ	NQ	NQ	3.19	0.26

NQ: Not Quantified

The samples of commercial green coffee supplements did not presented quantifiable concentrations of tannins, whereas the sample prepared by this work showed 0.57 mg/100 g of condensed tannins. The processing to obtain the GCE probably degraded the tannin polyphenols. Lima et al. (2012) reported tannin concentrations around 41- 64 mg/L in green and black teas. Hečimović et al. (2011) reported values of 7.36 mg / g to 12.49 mg/g of tannins in samples of green *C. canephora* coffee.

MYCOLOGICAL ANALYSIS AND OCHRATOXIN A DETERMINATION

After sowing for the growth of filamentous fungi, a low fungal diversity was observed in all analyzed samples. Fungal growth was only observed in samples 7 and 8 and the representative colonies were isolated in MEA and CYA media for later identification. The low fungal diversity can be attributed to the degree of processing and possible good manufacturing practices used in the preparation of food supplements and the production of green coffee. Potentially mycotoxin-producing fungi from the Nigri and Circumdati aspergilli sections were identified based on the macroscopic characteristics.

Considering the ten products analyzed in this work, six presented a positive result for OTA (Table 5), however, no result was above the limit of the legislation for roasted coffee, since

there is still no established limit for the consumption of mycotoxins in green coffee. The Brazilian regulation (ANVISA, 2022) established a limit for OTA in roasted coffee and soluble coffee of 10 µg/kg. However, the harm associated with OTA intake can be felt by those who consume such products.

Vaclavik et al. (2013) analyzed the levels of OTA in 50 samples of dietary supplements containing GCE and found an incidence of OTA in 36% of the samples with levels ranging from 2.7 - 136.9 µg / kg. The incidence of OTA in the dietary supplements analyzed in this study was 60%, but the contamination by this mycotoxin was much lower (0.26-4.8 µg/kg).

The contamination in “varrição” coffee is even greater, according to reports by Paulino de Moraes & Luchese (2003) who analyzed the levels of OTA in green coffee in the state of Rio de Janeiro, presenting levels from 0.3 to 160 µg / kg and in the northeast region of the country state that presented levels from 10.1 to 592 µg / kg.

Figure 4 shows the chromatogram for the ochratoxin in sample 2, which showed the highest level of contamination (4.80 µg / kg) to the retention time of the points on the standard curve (Figure 5).

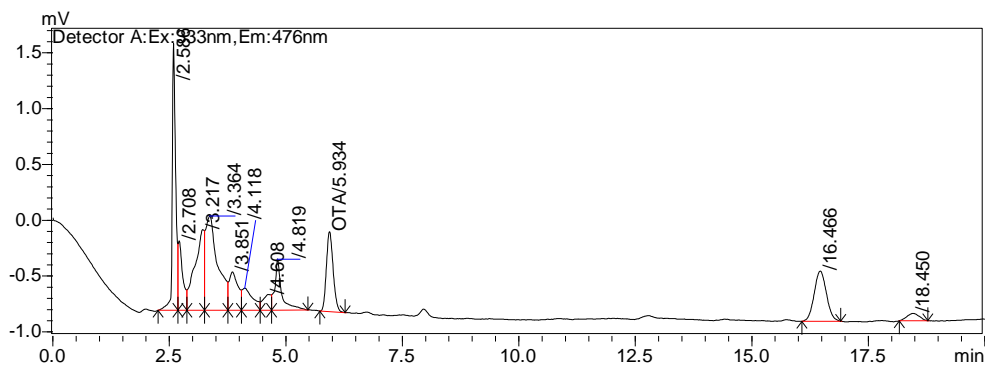


Figure 4 - Chromatogram showing contamination by OTA in sample 2

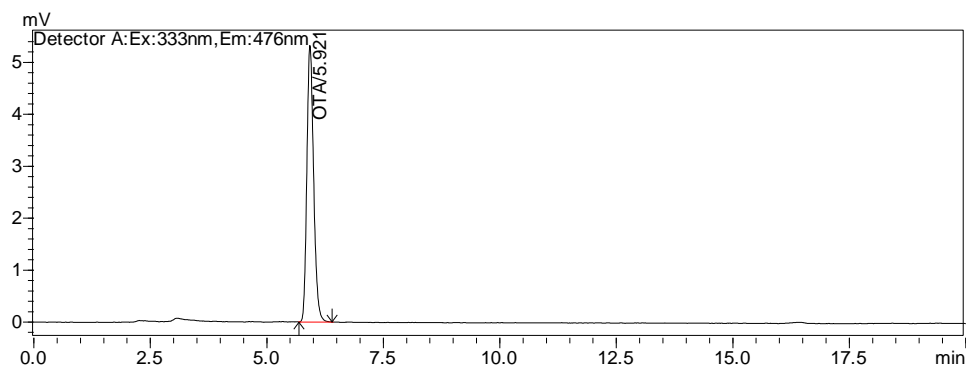


Figure 5 - Chromatogram of the standard OTA curve

CONCLUSIONS

A Green Coffee Extract (GCE) product was developed, analyzed, and compared with 9 commercial products. The evaluation of the authenticity of food supplements based on green coffee by molecular methods and quantification of caffeine and 5-caffeoylquinic acid, allowed to conclude that all the samples analyzed were authentic for green coffee. From the analysis of the centesimal composition, it was possible to compare the results obtained with the information contained on the product labels, in addition to producing information for labeling the product obtained in this work (sample 10). Discrepancies were found in the information on the labels of some food supplements acquired. The mineral composition of food supplements proved to be very varied, as some of the products are added minerals to their formulation. Samples 1; 8; 9 and 10 had phytate levels. The higher levels of phytate observed in sample 8, can be explained by its formulation. Only the sample formulated in this work showed a low concentration of condensed tannins. There was a great variation in the values of antioxidant activity in the samples, with some food supplements showing low values, despite the antioxidant appeal of these products is an important appeal for their commercialization. Although samples 7 and 8 show growth of fungi identified as possible OTA producers, it was not possible to quantify this secondary metabolite in these samples. It was possible to quantify OTA in 6 of the samples analyzed in this work, but fungi producing OTA were not identified in the analysis of the mycological diversity of the products. The production process of food supplements can lead to the destruction of fungal diversity but not of secondary metabolites such as OTA.

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Conflicts of Interest

The authors declare there are no conflicts of interest.

REFERÊNCIAS

ABNT NBR / ISO 17025: 2005

Amigoni, L., et al. “Green Coffee Extract Enhances Oxidative Stress Resistance and Delays Aging in *Caenorhabditis Elegans*.” *Journal of Functional Foods*, vol. 33, 1 June 2017, pp. 297–306, <https://doi.org/10.1016/j.jff.2017.03.056>.

ANVISA, Brazil. Instrução normativa - IN Nº 160, de 1º de julho de 2022 (Publicada no DOU nº 126, de 6 de julho de 2022) Estabelece os limites máximos tolerados (LMT) de contaminantes em alimentos. 2022. https://antigo.anvisa.gov.br/documents/10181/2718376/IN_160_2022_.pdf

ANVISA, Brazil. Resolution RDC Nº 360, 23 DE DEZEMBRO DE 2003. https://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2003/res0360_23_12_2003.html

AOAC (Association of Official Analytical Chemists). Official Methods of Analysis of AOAC International. 17th ed. Gaithersburg, 2014.

AOAC. *Association of official analytical chemists, official methods of analysis*. 18. Washington, DC: AOAC International; 2005.

AOAC. *Association of official analytical chemists, official methods of analysis - Method 986.11. Phytate in Foods*, 18 ed., 3ª rev, 2010.

Arlorio, M. et al. “Detection of Hazelnut (*Corylus Spp.*) in Processed Foods Using Real-Time PCR.” *Food Control*, vol. 18, no. 2, Feb. 2007, pp. 140–148, <https://doi.org/10.1016/j.foodcont.2005.09.005>.

Batista, L.R. et al. “Toxigenic Fungi Associated with Processed (Green) Coffee Beans (*Coffea Arabica L.*)” *International Journal of Food Microbiology*, vol. 85, no. 3, 25 Aug. 2003, pp. 293–300, [https://doi.org/10.1016/s0168-1605\(02\)00539-1](https://doi.org/10.1016/s0168-1605(02)00539-1).

Bauer, D. et al. “Effect of Roasting Levels and Drying Process of *Coffea Canephora* on the Quality of Bioactive Compounds and Cytotoxicity.” *International Journal of Molecular Sciences*, vol. 19, no. 11, 31 Oct. 2018, p. 3407, <https://doi.org/10.3390/ijms19113407>.

Belguidoum, K. et al. “HPLC Coupled to UV–Vis Detection for Quantitative Determination of Phenolic Compounds and Caffeine in Different Brands of Coffee in the Algerian Market.” *Journal of the Taiwan Institute of Chemical Engineers*, vol. 45, no. 4, July 2014, pp. 1314–1320, <https://doi.org/10.1016/j.jtice.2014.03.014>.

Broadhurst, R.B. and Jones, W.T. “Analysis of Condensed Tannins Using Acidified Vanillin.” *Journal of the Science of Food and Agriculture*, vol. 29, no. 9, Sept. 1978, pp. 788–794, <https://doi.org/10.1002/jsfa.2740290908>.

CAC (Codex Alimentarius Commission). Joint FAO/WHO Food Standards Programme, Codex Committee on Contaminants in Foods Thirty-fifth Session, Arusha, Tanzania, 17–21 March 2003, Document Reference CX/FAC 03/18.

Copetti, M.V. et al. “Comparison of Different Culture Media for Mycological Evaluation of Commercial Pet Food.” *Acta Scientiae Veterinariae*, vol. 37, no. 4, 30 Mar. 2018, p. 329, <https://doi.org/10.22456/1679-9216.16392>.

Costa, A.S.G. et al. "Teas, Dietary Supplements and Fruit Juices: A Comparative Study Regarding Antioxidant Activity and Bioactive Compounds." *LWT - Food Science and Technology*, vol. 49, no. 2, Dec. 2012, pp. 324–328, <https://doi.org/10.1016/j.lwt.2012.02.030>.

Costa, J. et al. "High Resolution Melting Analysis as a New Approach to Detect Almond DNA Encoding for Pru Du 5 Allergen in Foods." *Food Chemistry*, vol. 133, no. 3, Aug. 2012, pp. 1062–1069, <https://doi.org/10.1016/j.foodchem.2012.01.077>.

Couto, C.C. et al. "Coffea Arabica and C. Canephora Discrimination in Roasted and Ground Coffee from Reference Material Candidates by Real-Time PCR." *Food Research International*, vol. 115, Jan. 2019, pp. 227–233, <https://doi.org/10.1016/j.foodres.2018.08.086>.

Crozier, A. et al. "Dietary Phenolics: Chemistry, Bioavailability and Effects on Health." *Natural Product Reports*, vol. 26, no. 8, 2009, p. 1001, <https://doi.org/10.1039/b802662a>.

Dellalibera, O., et al. "Le Svetol®, Un Extrait de Café Vert Décaféiné, Induit Une Perte de Poids et Augmente Le Ratio Masse Maigre Sur Masse Grasse Chez Des Volontaires En Surcharge Pondérale." *Phytothérapie*, vol. 4, no. 4, Nov. 2006, pp. 194–197, <https://doi.org/10.1007/s10298-006-0181-7>.

Dziki, D. et al. "Ground Green Coffee Beans as a Functional Food Supplement – Preliminary Study." *LWT - Food Science and Technology*, vol. 63, no. 1, Sept. 2015, pp. 691–699, <https://doi.org/10.1016/j.lwt.2015.03.076>.

Ferreira, T. et al. "Using Real-Time PCR as a Tool for Monitoring the Authenticity of Commercial Coffees." *Food Chemistry*, vol. 199, May 2016, pp. 433–438, <https://doi.org/10.1016/j.foodchem.2015.12.045>.

Gaascht, François, et al. "Coffee Provides a Natural Multitarget Pharmacopeia against the Hallmarks of Cancer." *Genes & Nutrition*, vol. 10, no. 6, Nov. 2015, <https://doi.org/10.1007/s12263-015-0501-3>.

Hečimović, Ivana, et al. "Comparative Study of Polyphenols and Caffeine in Different Coffee Varieties Affected by the Degree of Roasting." *Food Chemistry*, vol. 129, no. 3, Dec. 2011, pp. 991–1000, <https://doi.org/10.1016/j.foodchem.2011.05.059>.

Hendre, P.S. and Aggarwal, R.K. "Development of Genic and Genomic SSR Markers of Robusta Coffee (*Coffea Canephora* Pierre Ex A. Froehner)." *PLoS ONE*, vol. 9, no. 12, 2 Dec. 2014, p. e113661, <https://doi.org/10.1371/journal.pone.0113661>.

ICO. International coffee organization. https://www.ico.org/trade_statistics.asp?section=Statistics, 2023 (accessed 13 June 2023).

Jeszka-Skowron, Magdalena, et al. "Relationship between Antioxidant Capacity, Chlorogenic Acids and Elemental Composition of Green Coffee." *LWT*, vol. 73, Nov. 2016, pp. 243–250, <https://doi.org/10.1016/j.lwt.2016.06.018>.

Lee, Jae-Hwang, et al. "Specific PCR Assays to Determine Bovine, Porcine, Fish and Plant Origin of Gelatin Capsules of Dietary Supplements." *Food Chemistry*, vol. 211, 15 Nov. 2016, pp. 253–259, <https://doi.org/10.1016/j.foodchem.2016.05.060>.

Liang, Ningjian, et al. "Interactions between Major Chlorogenic Acid Isomers and Chemical Changes in Coffee Brew That Affect Antioxidant Activities." *Food Chemistry*, vol. 213, Dec. 2016, pp. 251–259, <https://doi.org/10.1016/j.foodchem.2016.06.041>.

Lima, A. R., Pereira, R. G. F. A., Abrahão, S. A., Duarte, S. M. da S., & Paula, F. B. de A. (2010). Compostos bioativos do café: atividade antioxidante in vitro do café verde e torrado antes e após a descafeinação. *Química Nova*, 33(1), 20–24. <https://doi.org/10.1590/s0100-40422010000100004>

Lima, M.B., et al. “Turbidimetric and Photometric Determination of Total Tannins in Tea Using a Micro-Flow-Batch Analyzer.” *Talanta*, vol. 88, 15 Jan. 2012, pp. 717–723, <https://doi.org/10.1016/j.talanta.2011.11.076>.

Liu, Q, et al. “Optimization of Ultrasonic-Assisted Extraction of Chlorogenic Acid from Folium Eucommiae and Evaluation of Its Antioxidant Activity.” *Journal of Medicinal Plants Research*, vol. 4, no. 23, 2010, pp. 2503–2511, academicjournals.org/article/article1380705215_Liu%20et%20al.pdf. Accessed 7 Oct. 2023.

Mahabir, S. “Methodological Challenges Conducting Epidemiological Research on Nutraceuticals in Health and Disease.” *PharmaNutrition*, vol. 2, no. 3, July 2014, pp. 120–125, <https://doi.org/10.1016/j.phanu.2013.06.002>.

Manzano, M. et al. “A Molecular Method to Detect Bacillus Cereus from a Coffee Concentrate Sample Used in Industrial Preparations.” *Journal of Applied Microbiology*, vol. 95, no. 6, 1 Dec. 2003, pp. 1361–1366, <https://doi.org/10.1046/j.1365-2672.2003.02120.x>.

Martellosi, C. et al. “DNA Extraction and Analysis from Processed Coffee Beans.” *Journal of Agricultural and Food Chemistry*, vol. 53, no. 22, 4 Oct. 2005, pp. 8432–8436, <https://doi.org/10.1021/jf050776p>.

Morgano, Marcelo Antonio, et al. “Determinação de Minerais Em Café Cru.” *Ciência E Tecnologia de Alimentos*, vol. 22, no. 1, Jan. 2002, pp. 19–23, <https://doi.org/10.1590/s0101-20612002000100004>.

Murray, M.G., and Thompson, W.F. “Rapid Isolation of High Molecular Weight Plant DNA.” *Nucleic Acids Research*, vol. 8, no. 19, 1980, pp. 4321–4326, <https://doi.org/10.1093/nar/8.19.4321>.

Narita, Yusaku, and Kuniyo Inouye. “High Antioxidant Activity of Coffee Silverskin Extracts Obtained by the Treatment of Coffee Silverskin with Subcritical Water.” *Food Chemistry*, vol. 135, no. 3, Dec. 2012, pp. 943–949, <https://doi.org/10.1016/j.foodchem.2012.05.078>.

Nobile, P.M. et al. “Transcriptional Profile of Genes Involved in the Biosynthesis of Phytate and Ferritin in *Coffea*.” *Journal of Agricultural and Food Chemistry*, vol. 58, no. 6, 22 Feb. 2010, pp. 3479–3487, <https://doi.org/10.1021/jf9043088>.

Paulino De Moraes, M. H., Luchese, R.H. “Ochratoxin a on Green Coffee: Influence of Harvest and Drying Processing Procedures.” *Journal of Agricultural and Food Chemistry*, vol. 51, no. 19, 6 Aug. 2003, pp. 5824–5828, <https://doi.org/10.1021/jf026248k>.

Pawar, R. et al. “Assessment of the Authenticity of Herbal Dietary Supplements: Comparison of Chemical and DNA Barcoding Methods.” *Planta Medica*, vol. 83, no. 11, 28 Apr. 2017, pp. 921–936, <https://doi.org/10.1055/s-0043-107881>.

Pitt, John I., and Ailsa D. Hocking. *Fungi and Food Spoilage*. Boston, MA, Springer US, 2009.

Popova, A. and Mihaylova, D. “Antinutrients in Plant-Based Foods: A Review.” *The Open Biotechnology Journal*, vol. 13, no. 1, 29 July 2019, pp. 68–76, <https://doi.org/10.2174/1874070701913010068>.

Powell, K. A. et al. *The Genus Aspergillus*. Springer Science & Business Media, 29 June 2013.

Ramirez-Coronel, M. A. et al. "Characterization and Estimation of Proanthocyanidins and Other Phenolics in Coffee Pulp (*Coffea Arabica*) by Thiolytic-High-Performance Liquid Chromatography." *Journal of Agricultural and Food Chemistry*, vol. 52, no. 5, 11 Feb. 2004, pp. 1344–1349, <https://doi.org/10.1021/jf035208t>.

Rosa, J.S., Freitas-Silva, O., Campos, R.S., Castro, I.M., Teixeira, A.S. Determination of pesticides and Ochratoxin A in arabica coffee under different roasting levels. (Determinação de agrotóxicos e ocratoxina A em café arábica sob diferentes Níveis de Torrefação). Rio de Janeiro. Embrapa Agroindústria de Alimentos, 2019.18 p. – (Boletim de Pesquisa e Desenvolvimento / Embrapa Agroindústria de Alimentos, ISSN 0101-630X; 29). <https://www.infoteca.cnptia.embrapa.br/bitstream/doc/1112112/1/BPD292019ocratoxina.pdf>

Samson, R.A., et al. "Phylogeny, Identification and Nomenclature of the Genus *Aspergillus*." *Studies in Mycology*, vol. 78, June 2014, pp. 141–173, <https://doi.org/10.1016/j.simyco.2014.07.004>.

Santato, Alessandro, et al. "Using Elemental Profiles and Stable Isotopes to Trace the Origin of Green Coffee Beans on the Global Market." *Journal of Mass Spectrometry*, vol. 47, no. 9, Sept. 2012, pp. 1132–1140, <https://doi.org/10.1002/jms.3018>.

Schrenk, D. et al. "Risk Assessment of Ochratoxin a in Food." *EFSA Journal*, vol. 18, no. 5, May 2020, <https://doi.org/10.2903/j.efsa.2020.6113>.

Şemen, Sevcan, et al. "Elemental Composition of Green Coffee and Its Contribution to Dietary Intake." *Food Chemistry*, vol. 215, Jan. 2017, pp. 92–100, <https://doi.org/10.1016/j.foodchem.2016.07.176>.

Shimoda, Hiroshi, et al. "Inhibitory Effect of Green Coffee Bean Extract on Fat Accumulation and Body Weight Gain in Mice." *BMC Complementary and Alternative Medicine*, vol. 6, no. 1, 17 Mar. 2006, <https://doi.org/10.1186/1472-6882-6-9>.

Silva, M.R. and Silva, M.A.A.P. "Aspectos Nutricionais de Fitatos E Taninos." *Revista de Nutrição*, vol. 12, no. 1, Apr. 1999, pp. 21–32, <https://doi.org/10.1590/s1415-52731999000100002>.

Souza, Laurent dos Santos de, et al. "Effect of the Roasting Levels of *Coffea Arabica* L. Extracts on Their Potential Antioxidant Capacity and Antiproliferative Activity in Human Prostate Cancer Cells." *RSC Advances*, vol. 10, no. 50, 2020, pp. 30115–30126, <https://doi.org/10.1039/d0ra01179g>. Accessed 26 Nov. 2022.

Ukers, W.H. The Chemistry of the Coffee Bean. In: UKERS, W.H. (Ed.) All About Coffee. 2ed. New York: Inter-American Copyright Union, 1976. Cap. 24, p. 293.

Vaclavik, Lukas, et al. "Determination of Multiple Mycotoxins in Dietary Supplements Containing Green Coffee Bean Extracts Using Ultrahigh-Performance Liquid Chromatography-Tandem Mass Spectrometry (UHPLC-MS/MS)." *Journal of Agricultural and Food Chemistry*, vol. 61, no. 20, 22 May 2013, pp. 4822–4830, pubmed.ncbi.nlm.nih.gov/23631685/, <https://doi.org/10.1021/jf401139u>.

Zulueta, A., et al. "ORAC and TEAC Assays Comparison to Measure the Antioxidant Capacity of Food Products." *Food Chemistry*, vol. 114, no. 1, May 2009, pp. 310–316, <https://doi.org/10.1016/j.foodchem.2008.09.033>.