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Green coffee extract increases the functional and sensory appeal of brownie

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Abstract

In this study, a brownie formulation enriched with green coffee extract was developed, aiming to combine the typical sensory characteristics of the dessert with functional appeal and nutritional profile. Brownies with formulations containing 0%, 2%, 3.5%, and 5% green coffee extract were prepared and subjected to physicochemical, microbiological, sensory, and phenolic compound analyses. The results showed that the addition of 5% extract increased moisture content and phenolic compounds, reaching 186.78 mg of gallic acid equivalent per 100 g, indicating a higher quantity of phenolic compounds. Microbiological analysis indicated the absence of contamination by coliforms, fungi, and Bacillus cereus, highlighting the product's safety. In the sensory evaluation, the brownies with extract were well received, maintaining desirable appearance, aroma, flavor, and texture attributes, such as shine, dense texture, and chocolate and coffee aroma. The results suggest that green coffee extract may be a viable alternative to enrich desserts such as brownies, promoting a healthier food product without compromising its sensory characteristics.

Keywords: Total phenolic content; Proximate composition; Coffee; Chocolate.

1. Introduction

Green coffee refers to coffee beans that have not undergone the roasting process, thus retaining their characteristic color regardless of the fruit's maturation stage (Ag Eldin, 2021). The absence of roasting preserves a range of bioactive compounds that would otherwise be partially or completely degraded under high thermal conditions (ElGamal et al., 2023). Among these compounds, chlorogenic acids stand out, such as caffeoylquinic acid (CQA), dicaffeoylquinic acid (diCQA), and feruloylquinic acid (FQA), in addition to caffeine, a widely studied alkaloid (Pimpley et al., 2020). These substances are known for their antioxidant potential and have been associated with a variety of health benefits (Pimpley et al., 2020).

Chlorogenic acid has attracted significant attention due to its ability to reduce glucose absorption in the intestine, which can aid in the regulation of blood sugar levels and contribute to the prevention and management of metabolic disorders such as type 2 diabetes (Sosa et al., 2023). Furthermore, it possesses antioxidant and anti-inflammatory properties that help protect against oxidative cellular damage and play a role in the prevention of chronic diseases, including cardiovascular and neurodegenerative disorders (Altanam et al., 2025).

Given its promising functional properties, green coffee extract

has been increasingly used in the development and enrichment of functional food products. Notable examples include its incorporation into chocolates (Zohreh, 2020), instant coffee (Corso and Benassi, 2015), sausages (Fetsch et al., 2024), cereal bars (Conceição, 2018), among others. The addition of green coffee extract not only enhances the sensory characteristics of these products but also adds nutritional value due to the presence of health-promoting bioactive compounds.

An alternative use for green coffee extract in food enrichment could be in desserts, especially those that use chocolate, such as brownies. Among the most popular desserts is brownie, a dense cake of North American origin, traditionally made with wheat flour, eggs, and chocolate. Although widely appreciated for its flavor and texture, the conventional brownie has limited nutritional appeal, being typically high in refined sugars and saturated fats. According to the report *Brownie Mix Market Size and Forecasts* (2025, https://www.cognitivemarketresearch.com/brownie-mixes-market-report), the global brownie mix market is experiencing notable growth, with a projected compound annual growth rate (CAGR) of 5.6% between 2024 and 2032 (BUSINESS RESEARCH INSIGHTS, 2024) highlighting the potential for healthier innovations in this segment (See the Report in File S1).

An effective strategy to improve the nutritional quality of this type of dessert is to reduce the number of common sugars and saturated fats. In addition, enriching it with natural ingredients rich in phenolic compounds and other bioactive compounds, such as whole-wheat flours, dried fruits, and especially green coffee extract, is beneficial. This approach aims not only to enhance the nutritional profile of the final product but also to preserve or even improve sensory attributes such as flavor, aroma, and texture.

In this context, the aim of the present study was to develop a brownie enriched with green coffee extract, seeking to maintain the dessert's typical sensory characteristics while promoting a more balanced and functional nutritional profile. This work innovates in the development of brownies enriched with green coffee extract, a material with great potential, but there are still few studies about its applicability.

2. Methodologies

2.1. Green coffee extract

Green coffee syrup was obtained according to the methodology described (Fernandes et al., 2024). Ethanol was used as the solvent to prepare the green coffee extract through solid-liquid reflux extraction. The coffee was reflux extraction for 4 hours at 78°C, with a liquid-to-solid ratio of 5:1. The extracts were then filtered and placed in a Buchi R-144 Rotavapor for 2 hoethanolinate the ethanol. The extracts were left for 24 hours in a fume hood at room temperature for complete evaporation. Subsequently, the extracts were stored in hermetically sealed glass jars and stored in a freezer (−10°C).

2.2. Brownie development

To prepare the brownies, the following ingredients were used: flour, sweetener, cocoa powder, coconut oil, coconut milk, chocolate, and a nut mix (Table 1) Subsequently, green coffee extract was added, in different concentrations (0%, 2%, 3.5% and 5%), the calculation was based on the total mass of the ingredients in the control formulation. For the preparation, the dry ingredients were weighed and placed into a container, followed by the addition of the wet ingredients, and the mixture was homogenized. The mixture of the chocolate and the nut mix were then added. The brownies were baked at approximately 180 °C for 25 minutes.

Table 1. Ingredients used for brownie development

Ingredients	Quantity (g)
wheat flour	115
Xylitol	90
Coconut oil	42
Coconut milk	107
Chocolate 70%	33
Cocoa powder	29
Mix of nuts and raisins*	52
Baking powder	1.4

*Walnuts, Brazil nuts, cashew nuts and raisins.

2.3. Experimental design

The experiment was carried out in a completely randomized experimental design built with four treatments up (levels of enrichment of brownie with green coffee extract: 0, 2.5, 3.5 and 5%) and three repetitions.

2.4. Analyses

2.4.1. Proximate composition

The moisture, protein, and fiber content of the brownie were determined using official AOAC protocols (AOAC, 2016), in three independent replicates.

2.4.1.1. Moisture

Moisture content was quantified gravimetrically by drying the samples in an oven at 105 ± 1 °C until a constant mass was obtained. To ensure complete removal of residual moisture, the mortars used were previously dried in an oven for 3 hours and, after weighed, received 10 g of the brownie sample, and were subsequently dried until the mass stabilized.

2.4.1.2. Lipid

Lipid determination was performed using the Bligh & Dyer method, employing cold extraction with chloroform, methanol, and sodium sulfate solution (Bligh and Dyer, 1959). Initially, 2.5 g of the previously dehydrated sample were transferred to a hermetically sealed container, to which 10 mL of chloroform, 20 mL of methanol, and 8 mL of distilled water were added. The mixture was stirred in a rotary stirrer for 30 min. Then, 10 mL of chloroform and 10 mL of 1.5% sodium sulfate solution were added, the system was sealed again and stirred for 2 min. After filtration, the supernatant was transferred to a separatory funnel, discarding the upper phase. To the lower phase, 1 g of anhydrous sodium sulfate

Table 2. Proximal composition and total phenolic compounds content of brownie for 5% green coffee extract (5% C.E.), 3.5% green coffee extract (3.5% C.E.), 2% green coffee extract (2% C.E.) and for the 0% C.E. control

Formulations	Moisture (%)	Lipids (%)	Proteins (%)	Fiber (%)	Phenolic compounds content (GCE 100 g^{-1})
5 % E.C	21.48[a]	23.2[a]	15.72[a]	8.86[a]	188.78[a]
3.5 % E.C	16.59[b]	24.29[a]	12.41[a]	8.26[a]	136.28[b]
2 % E.C	15.39[b]	22.46[a]	17.04[a]	10.62[a]	128.45[b]
0 % E.C	13.51[b]	22.39[a]	10.28[a]	8.30[a]	88.36[b]

Means followed by the same letters do not differ statistically from each other according to the Tukey test (p>0.05).

was added, followed by further stirring and filtration. A 5 mL aliquot was transferred to a previously tared beaker and placed in an oven at 80 °C for complete evaporation of the solvent, allowing for the quantification of lipids.

2.4.1.3. Protein

The protein content was determined by the Kjeldahl method, based on the quantification of total nitrogen after digestion, distillation, and titration. For digestion, 100 mg of the sample wrapped in parchment paper were added to digestion tubes, along with approximately 600 mg of K_2SO_4, 300 mg of $CuSO_4$, and 5 mL of concentrated H_2SO_4. Digestion was carried out at 400 °C until a clear solution was obtained. Distillation was performed in a Kjeldahl apparatus, using an Erlenmeyer flask containing 10 mL of H_3BO_3 at the condenser outlet. 15 mL of NaOH were added to the digestion tube, allowing the release of ammonia, which was transferred to the Erlenmeyer flask by steam distillation. Approximately 75 mL of distillate were collected. Titration was conducted with a standardized 0.2 N HCl solution, allowing the calculation of the protein content of the sample.

2.4.1.4. Crude fiber

Crude fiber was determined by the Weende method, which involves acid digestion. For this analysis, 0.5 g of the dry, defatted sample was transferred to a digestion tube, to which 17.5 mL of 70% acetic acid, 0.5 g of trichloroacetic acid, and 1.2 mL of nitric acid were added. The tubes were stopped with reflux rods and kept warm for 30 min. The mixture was vacuum filtered in glass wool-lined porous-bottom crucibles, previously dried and tared, and the residue was washed with hot distilled water. The crucibles containing the residue were dried in an oven at 105 °C until constant mass, cooled in a desiccator, and weighed. The crude fiber content was calculated from the residual mass obtained.

2.4.2. Total phenolic content

The total phenolic content of the brownie was evaluated using the Folin-Ciocalteu method (Waterhouse, 2002). For this, 5 g of the sample was weighed into 50 mL Falcon tubes, and 20 mL of 50% methanol was added. The mixture was homogenized and subjected to agitation for 30 minutes in an ultrasonic bath, followed by centrifugation at 15,000 rpm for 15 minutes. The supernatant was transferred to an amber bottle, and 20 mL of 70% acetone was added to the residue. The sample was homogenized again and subjected to ultrasonic bath treatment for 30 minutes. It was then centrifuged once more and filtered. The obtained extract was used for the quantification of total phenolic by adding the Folin-Ciocalteu reagent. A calibration curve was prepared using gallic acid as the standard for phenolic compound quantification (see Table 2 for the result).

2.4.3. Antimicrobial activity

Microbiological analyses were carried out to assess the quality of the developed brownies, ensuring reliability. All analyses followed the methodology described by Da Silva et al (2017). Samples were handled in a laminar flow hood, previously sanitized with a 70% (v/v) alcohol solution. Initially, 25 grams of each sample were weighed and homogenized in 0.1% peptone water using a Stomacher Metroderm. Serial decimal dilutions from 10^{-1} to 10^{-3} were then prepared. For coliform analysis, the first step was the presumptive test. Aliquots of 1 mL from the dilutions were transferred to a series of three tubes containing LST broth (lauryl sulfate tryptose) and incubated at 37 °C for 24 to 48 hours to verify gas formation. After this period, and upon confirmation of acid and gas production, aliquots were transferred from these tubes to two new sets of three tubes: one containing brilliant green broth (for coliforms at 35 °C) and another containing Escherichia coli broth. These were incubated at 37 °C and 45 °C, respectively. Escherichia coli was confirmed only when turbidity and gas formation were observed in the specific tubes. For the analysis of molds and yeasts, the surface plating technique was used. Aliquots of 0.1 mL from the appropriate dilutions were transferred to plates containing DRBC agar (Dichloran Rose Bengal Chloramphenicol), from the Himedia brand (India). Plates were incubated at 25 °C for 5 to 7 days. To count Bacillus cereus, Polymyxin agar supplemented with egg yolk was used. Aliquots of 0.1 mL of the proper dilutions were spread onto the plates using a Drigalski spatula. Plates were incubated at 37 °C for 24 h. The results for coliforms and Escherichia coli were expressed using the Most Probable Number (MPN) table. The counts of molds, yeasts, and Bacillus cereus were expressed in colony-forming units per gram (CFU/g) of brownie, and the colonies were counted using a colony counter.

2.4.4. Sensory analysis

Authorization for the sensory analysis was obtained from the research ethics committee (CEP) (n° 6,675,568). The sensory evaluation was conducted in two stages. In the first stage, a focus group with 11 panelists was held to identify sensory attributes for use in the Check-All-That-Apply (CATA) test. Attributes related to the appearance, aroma, flavor, and texture of the brownies were identified (Table 3). In the second stage, the sensory evaluation involved 81 panelists, who assessed the attributes generated in the previous stage and provided their overall acceptance of the products. Four brownie samples (each one corresponding to a different formulation in terms of green coffee extract concentration) were provided, coded in a mo-

Table 3. Attributes related to the sensory characteristics of brownies in the focus group

Appearance	Aroma	Texture	Flavor
Shiny	Coffee aroma	Dense	Sweet flavor
Matte	Chocolate aroma	Dry	Flour flavor
Moist	Sweet aroma	Soft	Raisin flavor
Dry	Roasted aroma	Brittle	Bitter aftertaste
Crumbly	Chestnut aroma	Crunchy texture	Bitter flavor
Oily	Rancid odor	Light	Coffee flavor
Brown	Flour aroma	Crumbled	Chocolate flavor
Airy		Heterogeneous	Cocoa flavor
Characteristic brownie appearance		Homogeneous	No flavor
			Vanilla flavor
			Oil flavor
			Astringent flavor

nadic and balanced order. Water was offered to cleanse the palate between evaluations. Prior to starting the evaluation, consumers were instructed to read through the list of attributes, then taste each sample and check the terms on the CATA scale that best described each product. Data collection was carried out using the Compusense software. Sensory acceptance was assessed using a nine-point hedonic scale, ranging from 1 ("dislike extremely") to 9 ("like extremely") (Dutcosky, 2019. The participants in the sensory evaluation were recruited randomly, without prior targeting. For the focus group phase, 11 participants were selected, characterized as untrained tasters, all regular consumers of brownies. The inclusion criterion established considered exclusively individuals who reported consuming the product, excluding those who did not have this habit, to guarantee a minimum familiarity with the characteristics of the food analyzed. The age range of the participants varied between 18 and 60 years. In total, 83 tasters participated in the sensory evaluation, of which 24 were male and 59 were female.

2.4.5. Statistics

Statistical analysis was performed using the SigmaPlot software, and Tukey's test was applied to evaluate significant differences among the means ($p < 0.05$).

3. Results and discussion

The green coffee extract used in this study was previously evaluated by liquid chromatography to verify the chemical compounds present in its composition. The amounts of the major constituents found were: caffeine 73.86 mg g^{-1}, caffeic acid 43.75 mg g^{-1}, and chlorogenic acid 15.1 mg g^{-1} (Fernandes et al., 2024).

3.1. Proximate composition

The proximate composition of the brownies is summarized in Table 2. It was observed that the moisture content of the brownies varied according to the concentration of green coffee extract. Moisture values of 21.48%, 16.59%, 15.39%, and 13.51% were recorded for the brownies containing 5% GCE, 3.5% GCE, 2% GCE, and 0% (control, without green coffee extract), respectively. The 5% GCE treatment was the only one statistically different from the control, according to Tukey's test. Since the extracts were prepared with water, the higher moisture observed in brownies enriched with the extracts is attributed to this water. However, the addition of 5% of extract was the only treatment able to promote a significant difference in the moisture of brownies in comparison to the control or even to the other treatments.

The literature presents various types of brownies developed with the aim of improving their physicochemical properties, making them healthier. The addition of sweet potato puree also influenced the moisture content, as demonstrated in this study. Without the addition of puree, the moisture content was 14.42%, while with 25% puree added, the moisture increased to 19% (Selvakumaran et al., 2019).

Regarding the other proximate compounds analyzed, no difference was detected among treatments. Therefore, the adding of extracts did not affect the ether extract, protein and crude fiber of brownies. On average, the brownies presented 23.08%, 13,86% and 9.01% of these compounds, respectively.

3.2. Total phenolic content

The enrichment of brownies with green coffee extract increased the averages of total phenolic compounds. However, in the assay performed using the Folin-Ciocalteu method, an increase in gallic acid equivalent (GAE) content per 100 g of sample was observed. The control group presented a value of 88.36 mg GAE/g. In the samples enriched with green coffee extract (GCE), the values were 115.49 mg GAE/g for the 2% GCE formulation, 136.28 mg GAE/g for the 3.5% GCE, and 186.78 mg GAE/g for the 5% GCE treatment (Table 2).

Although an increase in total phenolic content was observed with the addition of GCE, indicating a higher concentration of bioactive compounds, only the 5% GCE treatment exhibited a statistically significant difference when compared to the control, or even to other treatments. This result suggests that while phenolic content tends to rise with increasing GCE concentration, only the highest concentration was sufficient to produce a meaningful sta-

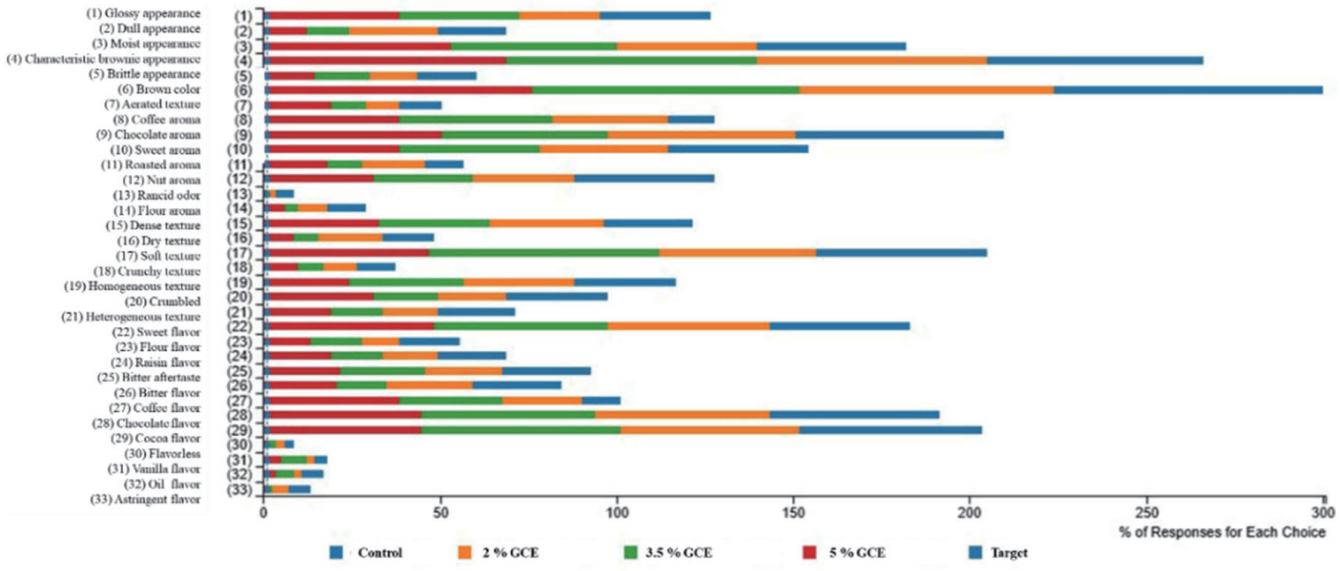

Figure 1. Percentage of Attributes obtained in the focus grup related to the sensory, visual and quality characteristics of brownies for each treatment, brownie for 5% green coffee extract (5% C.E.), 3.5% green coffee extract (3.5% C.E.), 2% green coffee extract (2% C.E.) and for the 0% C.E. control.

tistical impact.

Similar trends have been reported in the literature. For instance, enrichment of brownies with chia flour extract led to an increase in phenolic compounds, with a value of 52.77 ± 2.0 mg GAE/g compared to the control group. This supports the potential of functional ingredients such as chia to enhance the antioxidant profile of bakery products (Moreira et al., 2023).

Therefore, the incorporation of higher levels of green coffee extract in brownie formulations contributes to the enhancement of phenolic compound content. This enrichment may offer health benefits due to the antioxidant properties of these bioactive compounds, potentially aiding in cellular protection and promoting overall well-being through improved functional appeal of the product.

3.3. Antimicrobial activity

The microbiological analysis conducted encompassed the detection of fungi and bacteria potentially present in the product, including total coliforms, *Escherichia coli*, molds, yeasts, and *Bacillus cereus*. Total coliforms are a widely used indicator group for assessing the sanitary quality of foods, as their presence may indicate fecal or environmental contamination. Within this group, *Escherichia coli* is a thermotolerant coliform whose detection suggests recent fecal contamination and, therefore, a higher risk to consumer health. Molds and yeasts, on the other hand, are fungi that can cause food spoilage, affecting its taste, aroma, and safety. *Bacillus cereus* is a pathogenic bacterium capable of producing toxins responsible for foodborne intoxications, making its analysis essential in bakery products.

After the appropriate incubation period, no growth of any of these microorganisms was observed in the brownie samples analyzed. This result demonstrates the effectiveness of the production processes employed, with a baking temperature of 180 °C, as well as the quality of the ingredients used, ensuring that the final product is free from relevant microbiological contamination.

In addition to the confirmed microbiological safety, it is noteworthy that the developed brownie is completely egg-free, an ingredient commonly associated with the presence of pathogenic microorganisms such as *Salmonella* spp., one of the main agents responsible for food poisoning related to bakery products. The absence of eggs not only reduces the microbiological risk of the product but also broadens its suitability for specific groups, such as allergic or intolerant individuals, or those following vegan diets.

Thus, the brownie presents an important differential both from a food safety standpoint and nutritional accessibility, potentially meeting a growing market that values safe, healthy, and inclusive food.

3.4. Sensory analysis

In the focus group, four attributes related to the sensory characteristics of the brownies were selected, as presented in Table 3, which provides a detailed description of the attributes discussed with the panelists during the evaluation of each sample. The definition of these attributes was based on relevant criteria for product assessment, encompassing aspects such as texture, flavor, appearance, aroma, and other factors that influence consumer perception. The careful selection of these parameters aimed to ensure a comprehensive and in-depth analysis of the target audience's preferences and expectations regarding the developed product.

The sensory analyses revealed that, regarding visual attributes (Figure 1, the samples were predominantly associated with a glossy appearance, moist texture, brown coloration, and the typical look of a brownie. These characteristics reinforce both the authenticity and the visual appeal of the product. Concerning aroma, the evaluators described predominant notes of coffee and chocolate, accompanied by sweet nuances and a subtle hint of nuts, resulting in a complex and harmonious olfactory profile. In terms of texture, panelists highlighted a dense, soft, and homogeneous structure, features that contributed to a pleasant sensory experience and were consistent with expectations for this type of product. Flavor analysis indicated sweetness, coffee nuances, a slight bitter aftertaste, and pronounced notes of chocolate and cocoa as the main gustatory descriptors. This combination added depth and balance to the sensory profile of the samples, reflecting alignment with consumer

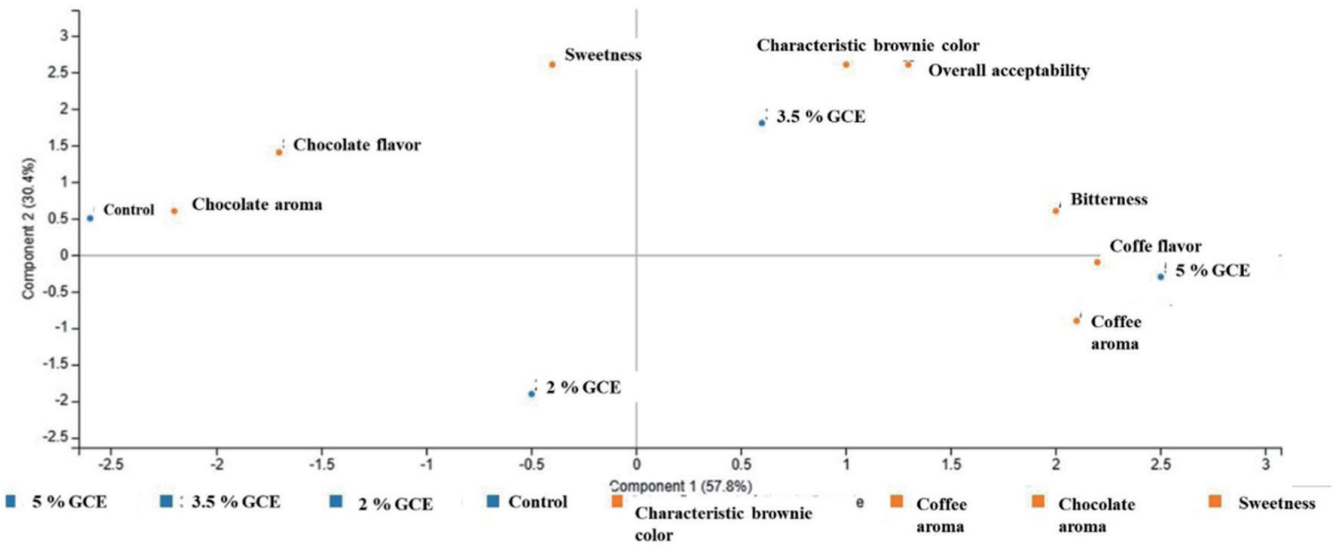

Figure 2. Principal component analysis in relation to the attributes for sensory evaluation and the different brownie treatments.

expectations and careful formulation development.

Based on the collected data, a Principal Component Analysis (PCA) was conducted to explore correlations between different treatments and the sensory attributes evaluated (Figure 2). This multivariate approach enabled the identification of association patterns between samples and attributes, highlighting those that contributed most to differentiating the treatments. The PCA results indicated that the control sample showed a stronger correlation with chocolate aroma and flavor, reflecting adherence to the traditional sensory profile of brownies. In contrast, the sample containing 3.5% green coffee extract was more closely associated with typical brownie color and overall acceptance, suggesting that this level of addition positively contributed to the aesthetic and sensory value of the product. The sample with 5% extract showed greater correlation with bitterness, coffee flavor, and coffee aroma, reinforcing the influence of increasing extract concentration on intensifying specific sensory notes, particularly those related to coffee, which may appeal to consumers who enjoy more intense and distinctive flavors.

The average acceptance scores for the formulations with 0% (control), 2%, 3.5%, and 5% green coffee extract were 6.5, 6.4, 6.9, and 6.7, respectively. These results indicate good overall acceptance of the brownies, regardless of the level of extract added, with the 3.5% concentration achieving the highest mean score (Figure 3). However, there was no statistical difference between the treatments, according to Tukey's test with a 0.05 level of significance, indicating that the addition of coffee extract did not influence overall acceptance.

The analysis of score distributions showed that most responses were concentrated in the mid-range categories of the scale, such as "like slightly," "like moderately," and "like very much," suggesting broad acceptance among the participants (Figure 4. Only one panelist gave the minimum score (1), and this response was associated with the samples containing higher extract concentrations, possibly due to the more pronounced bitterness, the taster not liking the flavor of the coffee and reduced perceived sweetness. On the other hand, the control brownie was preferred by 8 panelists, who rated it with the maximum score ("like extremely"), while the formulations containing 2%, 3.5%, and 5% extract received 13, 13, and 11 maximum scores, respectively. This indicates a slight preference for formulations with moderate extract concentrations. Formulations with extracts also improved brownie acceptance compared to the control.

Overall, the developed brownies demonstrated satisfactory sensory performance, indicating market potential. In addition to receiving positive sensory evaluations, the product presents a notable advantage by being diet and vegan, thus meeting the growing demand for healthier and more inclusive food options. The incorporation of green coffee extract not only contributed to the sensory profile of the brownies but also added functional value to the product. This ingredient is widely recognized for its high content of phenolic compounds, particularly chlorogenic acid, with antioxidant properties associated with reduced oxidative stress and general health benefits. Thus, the results demonstrate that green coffee extract can be successfully used as a functional ingredient in brownie formulations, enhancing their added value without compromising sensory acceptance. The combination of functional appeal, satisfactory sensory attributes, and suitability for contem-

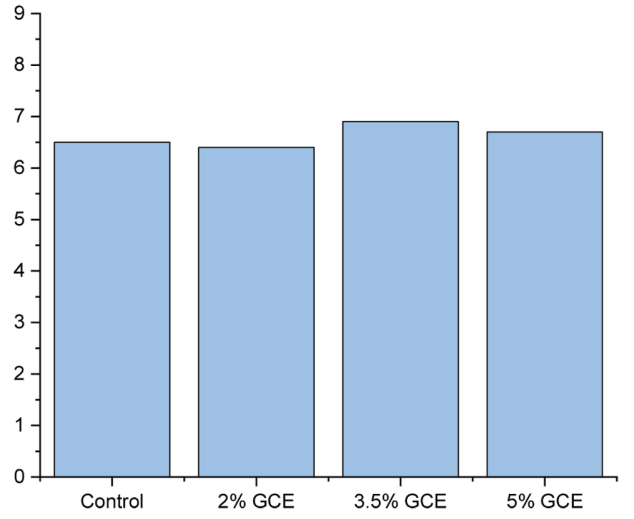

Figure 3. Overall acceptability of the brownies by the tasters in the brownies 5% green coffee extract (5% C.E.), 3.5% green coffee extract (3.5% C.E.), 2% green coffee extract (2% C.E.) and for the 0% C.E. control.

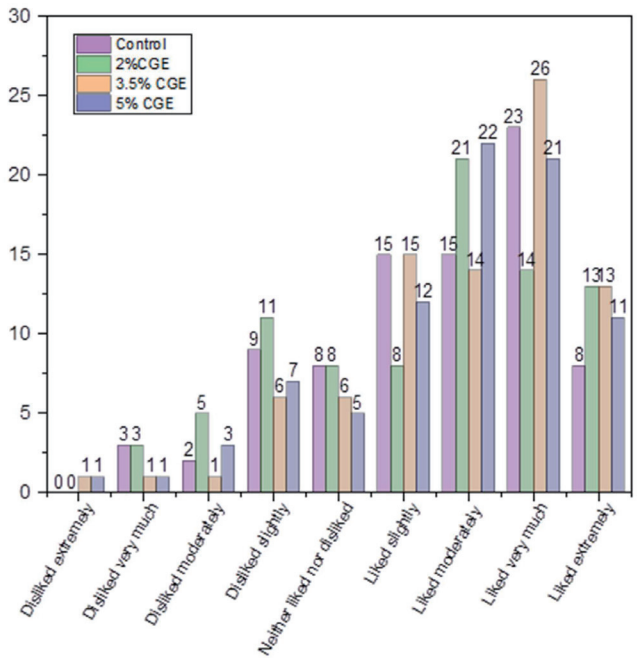

Figure 4. Limits of hedonic acceptability provided by tasters for brownies with 5% green coffee extract (5% C.E.), 3.5% green coffee extract (3.5% C.E.) and 2% green coffee.

porary dietary demands makes this product a promising alternative in the healthy and functional foods market.

4. Conclusion

The results obtained in this study demonstrate that the incorporation of green coffee extract into brownie formulations is a promising strategy for the development of functional foods that combine health benefits with sensory acceptability. The enrichment of the brownies with green coffee led to a significant increase in total phenolic content, especially at the 5% concentration, without causing undesirable changes in appearance, aroma, flavor, or texture. This indicates that it is possible to enhance the nutritional profile of commonly consumed foods through the addition of natural bioactive compounds, while maintaining their consumer appeal.

Furthermore, the absence of microbial contamination across all samples confirms the microbiological safety of the formulations, supporting their potential application in the food industry. These findings contribute to the growing body of research focused on the development of healthier alternatives to conventional sweets and desserts, aligning with current consumer trends that seek products that are both indulgent and beneficial to health. Future studies may explore the long-term stability of these compounds during storage, their bioavailability after ingestion, and their potential effects on human health, further reinforcing the use of green coffee as a valuable functional ingredient in food innovation.

Acknowledgments

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Supplementary material

File S1. Report of Standard Coffee Brownie.

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