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Fortification of Ground Roasted Coffees with Iron, Zinc and Calcium: Evaluating the Impact of Quality and Roast Degree on Sensory Responses

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Abstract Micronutrients' deficiency is a relevant public health issue with considerable socio-economic consequences. Food fortification has been widely used as a simple low-cost strategy to increase mineral intake. Considering that coffee is among the most consumed food products worldwide, gourmet and traditional medium and dark roasted *C. arabica* and *C. canephora beans* were ground and singly or jointly fortified with ferrous bisglycinate chelate (21mg iron/kg), zinc lactate (10.5mg zinc/kg), and calcium lactate (1.5g/kg) salts. Beverages were prepared at 10%, (w/v) using electric coffee dripper with nylon filter, and mineral recoveries were evaluated by Inductively Coupled-Plasma-Optical-Emission-Spectrometry (ICP-OES). Coffee beverages' acceptance and sensory characterization were performed by 103 regular coffee consumers, using a 9-point hedonic scales and Check-all-that-apply (CATA) questions. The impact of coffee quality (gourmet or traditional), roast degree (medium and dark) and mineral fortification (singly or jointly) on the beverage were evaluated. Mineral recoveries were 51.1%, 47.6%, and 51.3% for ferrous bisglycinate chelate, zinc lactate, and calcium lactate, respectively. Mean acceptance scores varied from 6.0 to 3.4. Unfortified blends and fortified blends with the three minerals were more liked by participants and were associated with positive attributes such as caramel, characteristic flavor, and chocolate. Roast degree and quality affected acceptance results, especially in blends fortified with a single mineral. The iron-fortified dark roasted blend was the least liked due to a strong metallic flavor, while dark roast was important to mask astringency in calcium-fortified gourmet blend, which had similar acceptance to unfortified blends. The beverage fortified with zinc was more accepted in medium roasted blend, regardless of being gourmet or traditional. Therefore, when fortifying coffee, issues related to the quality of the blend, roast degree and association with other components should be considered. In addition, results showed that the association of minerals had a positive effect on the consumer acceptance.

Keywords: *coffee, fortification, calcium, minerals, consumer acceptance, CATA*

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1. Introduction

Micronutrient deficiency conditions are widespread among 2 billion people in developing and developed countries. These are silent epidemics of vitamin and mineral deficiencies affecting people of all genders and ages, as well as certain risk groups. They not only cause specific diseases, but they act as exacerbating factors in infectious and chronic diseases, greatly impacting morbidity, mortality, and quality of life [1].

Iron deficiency is one of the main factors that lead to anemia, which affects 27% of the population (1.97 billion people). It is estimated that roughly 38% (32.4 million) of pregnant women and 29% (496 million) of non-pregnant women have anemia globally [2]. Iron plays an integral role in a wide range of physiological functions; therefore, the health consequences of iron deficiency and anemia in women are extensive and potentially serious if left untreated. Symptoms are often nonspecific but can include fatigue, irritability, hair loss, poor concentration, palpitations, and dizziness. In severe cases, tachycardia, ankle edema, and heart failure may arise [3].

Zinc is one of the most important trace elements in living organisms and has three major biological functions: catalytic, structural, and regulatory. The human body mass contains 2-3 g of zinc, and approximately 57% and 29% of total body zinc exist in skeletal muscle and bone, respectively; heart and blood plasma are known to contain 0.4% and 0.1% of body zinc, respectively. This is a multifunctional metal compatible with satisfactory growth, health, and well-being. It is essential for the structure and activity of various proteins and cellular components and plays an important role in human physiology from involvement in the proper function of the immune system to its importance in cellular growth, cell proliferation, and cell apoptosis, as well as in the activity of numerous zinc-binding proteins. Based on the estimated prevalence of zinc deficiency, the global population at risk for inadequate zinc intake is up to 17%, while in South Asia, up to 30% of the inhabitants may be deficient [3].

Calcium is most commonly associated with the formation and metabolism of bone. Over 99% of total body calcium is found as calcium hydroxyapatite in bones and teeth, where it provides hard tissue with its strength. Calcium in the circulatory system, extracellular fluid, muscle, and other tissues is critical for mediating vascular contraction and vasodilatation, muscle function, nerve transmission, intracellular signaling, and hormonal secretion [4]. In developing countries, such as South Africa and Nigeria, for example, calcium deficiency is considered an important factor in the etiology of rickets [5]. Additionally, all over the world, inadequate calcium intake has been correlated with increased prevalence of diseases such as osteoporosis, systemic arterial hypertension (SAH), and colon cancer, regardless of social class [3,6].

Food fortification has been widely used by the food industry in high-, middle-, and low-income countries as a simple low-cost resource to increase mineral intake and prevent and/or correct nutritional deficiencies [7,8]. According to the 2018 Food & Health Survey of the International Food Information Council Foundation (IFIC) [9], around 35% of the consumers in USA recognize fortified foods as healthy. This is relevant as healthfulness is the third purchase driver, following taste (first) and price (second). Thanks to globalization, this trend is a worldwide phenomenon.

When choosing a matrix for fortification, consumer's eating habits must be considered, and such matrix should be well accepted. The more familiar the food is to the consumer the easier the acceptance of the fortified matrix [10]. Coffee meets these criteria. It is the most consumed beverage and food product in the world, after water. According to the International Coffee Organization, the world consumption of coffee was about 700 million tons in 2017/2018 [11]. This volume represents an average annual growth rate of 2.1% since 2016/2017 [11]. In the last decade, science has offered a completely new perspective on the use of coffee that is now considered by many as a functional food [12]. A number of caffeine-related benefits of coffee drinking, such as enhancement of mental performance, including alertness, memory, mood, cognitive functions, and physical performance, are well known [12]. Furthermore, studies have demonstrated the ability of coffee polyphenols, caffeine, and other coffee compounds to promote antioxidant and anti-inflammatory effects, protecting the body against degenerative and chronic diseases such as type 2 diabetes and Alzheimer's, cancer and liver diseases, in addition to other types of diseases [13].

The beneficial health properties of coffee, together with its high consumption due to its relatively low cost, high accessibility, and high acceptance by populations, make it an excellent option as a micronutrient fortification vehicle. In our previous study [14], coffee was shown to be an appropriate vehicle for iron and zinc fortification, since iron (as ferrous fumarate) and zinc (as zinc gluconate) added to coffee brews presented reasonable bioavailability, 58% and 78% for iron and zinc, respectively, compared to water as a vehicle for these minerals, despite the presence of a considerable amount of polyphenols in coffee matrix that could decrease the minerals bioavailability. These bioavailability values are comparable to those observed for other foods fortified with the same minerals [15,16].

Ground roasted coffee is predominantly consumed worldwide, being equivalent to about 10 million tons compared to only about 807 thousand tons of soluble coffee [17,18]. In Brazil, which is the largest coffee producer and exporter country in the world [11], about 88% of the population consumes ground roasted coffee. Among the preparation methods, filtered and espresso (the latest has grown more recently) are the two most used worldwide by different segments of populations [18].

Recently, we have also tested, after literature search and preliminary selection, the solubility and extractability of two salts of iron, zinc and calcium when preparing brews from ground roasted coffees [3]. The salts with best recovery percentages for iron, zinc and calcium were: ferrous bisglycinate chelate, zinc lactate and calcium lactate. These salts have shown good bioavailability in other food matrices [15,16,19,20,21,22,23]. However, when developing a product, consumers' acceptance and sensory aspects must be considered. Being different from a medicine, fortified foods need to be well accepted by the target consumers. The components of the fortified matrices may interact and either mask or enhance certain sensory attributes, compared to the unfortified product. Therefore, the flavor resulting from the interaction of the fortified elements should also be taken into account. The present work aimed to evaluate the consumer acceptance of brews prepared from ground roasted coffees fortified with iron bisglycinate chelate, zinc lactate and calcium lactate. Additionally, we aimed to evaluate the impact of roast degree, coffee quality (gourmet or traditional), and mineral addition (single or jointly) on the product's sensory characteristics and consumer acceptance.

2. Materials and Methods

2.1. Samples

Twenty unfortified and fortified roasted samples were evaluated in the study (Figure 1). Two blends of different qualities were used. BLEND 1 was a gourmet blend consisted of 80% specialty *Coffea arabica cv.* Mundo Novo, classified as soft beverage by the Brazilian official classification (COB) (COOXUPE, Minas Gerais - Brazil) and 20% good quality *Coffea canephora* cv. Conilon (COOABRIEL, Espírito Santo, Brazil), similar to and marketed as robusta coffee; BLEND 2 was a popular traditional blend consisted of 50% arabica coffee classified as hard beverage, 20% of a mixture of defective and non-defective arabica beans (PVA) (COOXUPE), and 30% good quality conilon coffee (COABRIEL).The beans were roasted to reach two color degrees: medium (Agtrom - SCA # 55; 210ºC for 15 minutes) and dark (# 35; 210ºC for 18 minutes) in an electric lab scale drum roaster (CAEL LTDA, Brazil) and ground in a disc mill (Gourmet M-50, LEOGAP, Curitiba, PR, Brazil), to reach medium particle sizes in a 20 mesh screen.

2.2. Mineral Salts

A number of salts of iron, zinc and calcium were initially selected and tested, taking into account solubility, bioavailability and sensory aspects reported in previous fortification studies, using different food matrices [14,15,16,19,20,21,22,23,24,25]. After a preliminary study based on extractability from the ground roasted coffee to the brew and on sensory aspects [3], three mineral salts were finally chosen: ferrous bisglycinate chelate (Infiniti, São Paulo, Brazil), zinc lactate (Purac, Rio de Janeiro, Brazil), and calcium lactate (Purac, Rio de Janeiro, Brazil). Salts were analyzed by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) for verification of compliance of salts with their label information regarding the amount of target element.

2.3. Fortification

Based on the salts chemical structures and on the preliminary results from ICP-OES analyses, iron bisglycinate quelate, zinc lactate and calcium lactate were weighted to obtain the concentration of each mineral, corresponding to 15% of the Brazilian Health Surveillance Agency (ANVISA) Dietary Reference Intake (DRI) for adults (2.1mg of iron, 1.05mg of zinc and 150.0 mg of calcium per 100 g of ground roasted coffee, as the DRI for adults is 14mg for iron, 7mg for zinc and 1000 mg for calcium) [26]. Considering the USDA Nutrient Database for Standard Reference, the amount of salts added to 100g of ground roasted coffee would correspond to 11.7% and 26.2% of iron RDI for women and men respectively, 13.1% and 9.5% of zinc, respectively, and 15% of calcium RDI for men and women equally. Fortification of ground roasted coffees was performed using geometric dilution for homogenization of the salts in the coffee powders [27]. Six aliquots of each fortified coffee were collected randomly at different occasions for spectrometric analysis. The final mean SD was <2% of results per 100g, indicating that fortification and homogenization were well performed. The coffee blends (unfortified-control and fortified) were vacuum-packaged in metal packages and stored at -20°C. They were used according to sensory analysis demand, which occurred within two months.

2.4. Brews Preparation

Brews from the unfortified and fortified ground roasted coffee blends were prepared in accordance with the hygiene and safety requirements of ANVISA, in electric coffee drippers (Britânia® CB30), with paper filter (Melitta® nº 103), at 10% (10g coffee per 100mL 90-95 °C spring water), in line with the most traditionally used method in Brazil [18]. Extraction time was 110 seconds for each batch.

2.5. Minerals Analysis

For determination of mineral contents in coffee powder, 250mg of coffee powder were digested with 2.5mL of 65% nitric acid (VETEC, Brazil), in a 90°C water bath for 4 hours. One milliliter of ultra-pure hydrogen peroxide 30-32% (VETEC) was added to stop the reaction. For brews, 1mL of brew was digested with 1mL of nitric acid, with no addition of hydrogen peroxide [28]. Analyses were performed in triplicate by an inductively coupled plasma optical emission spectrometer (ICP-OES), model 4300 DV (Perkin Elmer-Sciex, Norwalk, CT, USA). The simultaneous operation mode was applied and the optimized parameters for the quantification of Fe, Zn and Ca elements were: plasma generator power 1.5 kW; auxiliary air flow 0.2 L/min; cooling air flow 15.0 L/min; air mist flow 0,45L/min; pump speed 1,50mL/min. The wavelengths (λ) applied for readings were 259.94 nm for Fe; 206.20 nm for Zn and 317.93 nm for Ca [28]. Quantitative calibration mode was used. Analytical curves were built using suitable dilutions of a multi-element aqueous standard solution of 1000 mg/L (Merck- IV; 23 elements, Merck, Darmstadt, Germany). Four calibration solutions with the following concentrations were used: 0.050, 0.100, 0.200, 0.500 mg/L ($r = 0.99999$ for Fe, 0.99999 for Zn and 0.99998 for Ca). Samples were introduced through a conical concentric nebulizer with cyclonic chamber (Glass Expansion, Australia) without previous filtration. Readings were performed in the automatic background correction mode. All reagents were of analytical grade. Ultra pure water (resistivity of 18.2 MΩ, Milli Q system, Millipore, USA) was used for solutions preparation. The multi-elements aqueous standard solution was also used for spectral interference tests. Limits of detection (LOD) calculated as 3 times the sample SD of ten blank readings of calibration curve (ultra pure water acidified with nitric acid) were 0.00014mg/L for Fe,0.0009mg/L for Zn and 0.0048 mg/mL for Ca. The limits of quantification (LOQ) calculated as 3.3 times the LOD value for the respective elements were: 0.00046mg/mL for Fe; 0.00297 mg/mL for Zn and 0.01584 mg/mL for Ca [3].

2.6. Consumer Acceptance and Sensory Characterization

The present study (# 32291913.2.0000.5257) was previously approved by the Research Ethics Committee of Clementino Fraga Filho University Hospital. All participants in the sensory tests were informed about the products and procedures and expressed their agreement, signing the Informed Consent Form.

Check-all-that-apply (CATA) method was developed in the last decade to investigate the consumer's perception of sensory characteristics of food products [29]. This method consists of a list of words or phrases obtained in a preliminary test, from which the participants are required to select all terms that they consider appropriate to describe the product. It has been widely used to describe the sensory attributes in several food matrices [30,31,32].

One hundred and three coffee consumers, aged from 18 to 66 years-old (59% women), students, teachers, visitors and employees of two academic/research institutions (Brazilian Agriculture Research Corporation-EMBRAPA Food Technology, and Federal University of Rio de Janeiro- UFRJ), both located in Rio de Janeiro/RJ, Brazil, were invited to take part in the study. All participants consumed at least one cup of black coffee/day. They evaluated the coffee beverages using nine-point hedonic scales followed by check-all-that-apply (CATA) questions. The hedonic scales ranged from 1: extremely disliked to 9: extremely liked, and the CATA questions comprised 28 sensory attributes related to appearance, aroma, flavor and mouth feel, which were identified in preliminary session performed with 10 assessors. The evaluated attributes used in the study were as follows: almond, astringent, bitter, brackish, burnt, burnt rubber, calcareous, caramel, characteristic flavor, chocolate, clove, cucumber, earth, fermented, grass, iodine, medicine, metallic, peanut, popcorn, rancid, roasted cereal, salty, slime, smoke, sour, sweet, vanilla. Demographic information of participants was also collected, including gender, age, educational level, occupation, family income and frequency of coffee consumption.

The sensory data were collected at EMBRAPA and UFRJ, in individual sensory booths. The assessors were instructed to drink the coffee beverage as they normally do, i.e. they had the option of not sweetening, or to add sugar or artificial sweeteners such as saccharin and aspartame, which were available. They were advised (and monitored) to use the same type and amount in all samples. Crackers and spring water at home temperature were offered between samples to clean the mouth. After preparation (item 2.4), the brews were kept up to 20 minutes, to ensure the temperature of 68°C ± 2°C [33,34,35]. After that time, they were discarded. Approximately 30 mL of beverages were served in coded porcelain cups with random three-digit numbers and presented in monadic and balanced order. Participants were asked to rate the whole set of samples. The presentation order of CATA terms was balanced among participants.

2.7. Statistical Analyses

Analysis of Variance (ANOVA) was performed on consumer's overall liking scores, considering consumer and sample as fixed source of variation, followed by the Fisher's test ($p \leq 0.05$). Multiple Factor Analysis (MFA) was performed to analyze the frequency response table of CATA questions, considering preference data as a supplementary variable. Cluster analysis was used to segment participants with similar coffee beverage acceptance. All analyses were performed using XLASTAT 2014 program (Addinsoft, version 2104.2.07).

Figure 1 contains the schematic representation of the fortification of coffee samples used in the sensory evaluation. UF = unfortified (control); Fe = iron; Zn = zinc; Ca = calcium; FeZnCa = iron, zinc and calcium.

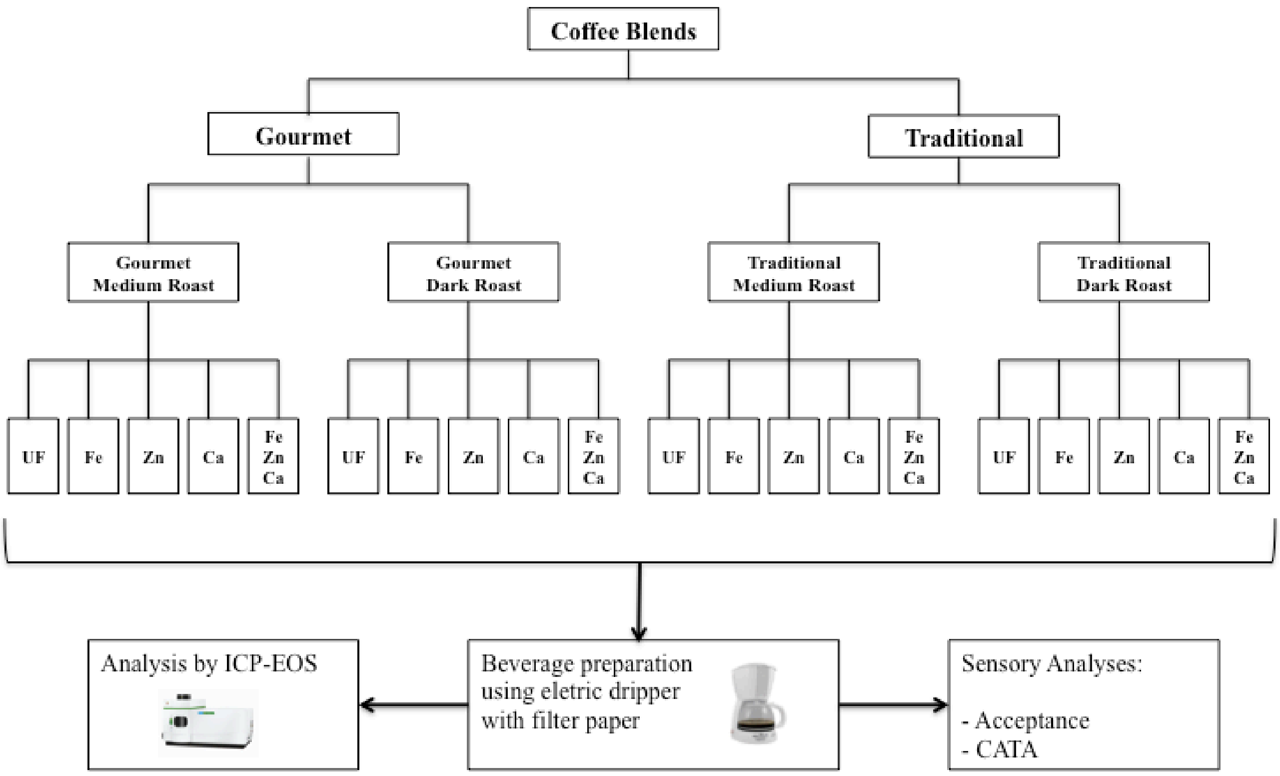

Figure 1. Schematic representation of the fortification of coffee samples used for the sensory evaluation. UF = unfortified (control); Fe = iron; Zn = zinc; Ca = calcium; FeZnCa = iron, zinc and calcium. (UF: unfortified; Fe: Iron; Zn: Zinc; Ca: Calcium)

Table 1. Concentration of minerals in the coffee powders and beverages prepared by electric coffee maker for the sensory test.

Mineral (mg/100 mL)	SAMPLES																			
	Gourmet blend										Traditional blend									
	GMUF	GDUF	GMFe	GDFe	GMZn	GDZn	GMCa	GDCa	GMFeZnCa	GDFeZnCa	TMUF	TDUF	TMFe	TDFe	TMZn	TDZn	TMCa	TDCa	TMFeZnCa	TDFeZnCa
Iron	0.33 ± 0.02	0.33 ± 0.03	4.93 ± 0.02	4.93 ± 0.04	0.32 ± 0.04	0.33 ± 0.02	0.33 ± 0.05	0.32 ± 0.05	4.93 ± 0.05	4.92 ± 0.03	0.32 ± 0.03	0.33 ± 0.01	4.94 ± 0.05	4.93 ± 0.04	0.32 ± 0.01	0.33 ± 0.02	0.32 ± 0.02	0.32 ± 0.01	4.92 ± 0.02	4.92 ± 0.05
Zinc	0.26 ± 0.02	0.25 ± 0.04	0.26 ± 0.01	0.25 ± 0.02	1.45 ± 0.03	1.46 ± 0.04	0.26 ± 0.03	0.26 ± 0.01	1.45 ± 0.02	1.46 ± 0.05	0.26 ± 0.01	0.25 ± 0.05	0.26 ± 0.02	0.25 ± 0.02	1.45 ± 0.04	1.46 ± 0.05	0.26 ± 0.05	0.26 ± 0.05	1.45 ± 0.04	1.46 ± 0.05
Calcium	37.09 ± 0.05	36.09 ± 0.05	37.10 ± 0.05	36.10 ± 0.04	37.10 ± 0.05	36.09 ± 0.03	222.00 ± 0.04	221.08 ± 0.04	222.00 ± 0.01	221.10 ± 0.02	37,10 ± 0.01	36.09 ± 0.03	37.09 ± 0.04	36.10 ± 0.02	37.10 ± 0.01	36.09 ± 0.04	222.00 ± 0.03	221.08 ± 0.03	222.00 ± 0.01	221.10 ± 0.05

Results from triplicate extraction and analysis are expressed as mean ± standard deviation. Coefficient of variation < 5% for all analyzes; Note: T: Traditional; G: Gourmet; M: Medium; D: Dark, F: Fortified; UF: Unfortified; Fe: Iron; Zn: Zinc; Ca: Calcium

3. Results and Discussion

3.1. Mineral Contents in Coffee Brews Used in Sensory Analysis

The mineral content of coffee beverages (unfortified and fortified) is presented in Table 1. There was no difference between the mineral contents in samples of different qualities or roast degrees offered in the sensory tests of the present study, which was expected, given that the intrinsic minerals are resistant to heat and that the added minerals were equally weighted and mixed in all blends. Mean mineral percent recoveries during brewing (evaluated by ICP-OES), considering the initial amounts in the coffee powders were: 51.1%, 47.6%, and 51.6% for iron, zinc, and calcium, respectively. In our previous study [3], espresso machine was able to extract 82.6.8% of iron, 90.0% of zinc, and 70.2% of calcium. However, considering that electric dripping is still the most used extraction method for coffee, especially in the classes that most need fortification, we opted for electric dripper. Considering that Brazilians consume, on average, about 220mL of coffee per day [36] and taking into account the percentage of the mineral extraction during brewing, consumers would be drinking 1.7% of iron RDI, 1.6% of zinc RDI and 1.7% of calcium RDI, according to ANVISA. Considering the USDA Nutrient Database for Standard Reference, the amount of minerals provided by 220mL of coffee consumed daily would be 1.3% and 3.0% of iron RDI for women and men respectively, 1.4% and 1.0% of zinc RDI, respectively, and 1.7% of calcium RDI for men and women equally.

3.2. Sensory Evaluation Results

3.2.1. Acceptance Results

Substantial differences were found among the individual acceptance scores (from 9 to1) and mean acceptance scores (from 6.0 to 3.4) for all products. The mean scores are shown in Figure 2. Considering a 9-point hedonic scale, such mean acceptances may be considered medium to low.

Unfortified medium roasted (6.0 ± 0.2, and 5.8 ± 0.2) and dark roasted (5.8 ± 0.3, and.7 ± 0.3) traditional and gourmet blends, respectively, obtained the highest mean scores (Figure 2).Gourmet blends were intentionally prepared with specialty arabica coffee, which is more aromatic and flavorful and contained only 20% of good quality conilon beans, which are often used to increase body and highlight arabica positive attributes, resulting in a good quality beverage [37]. Despite the higher technical quality of the beans, the overall acceptance was similar to traditional blends. According to Yue Liu et al. [38], many of human consumption behaviors, including food consumption preferences, exhibit ambiguous characteristics such as novelty-seeking and repetitive nature. In the last decades, when Brazilians did not have high availability of gourmet coffee in the market as they currently do, higher scores were often given to low quality coffees because they were habitually drank. The present results support the fact that the massive (and still increasing) gourmet coffee penetration and dissemination in the market of the large cities and coffee producing regions of the country in the last ten years [39] is raising the Brazilians quality standard, especially in medium and high socio-economical classes.

The fact that the highest mean score was only 6.0-5.7 can most probably be attributed to a strong carry over effect caused especially by iron-fortified samples and, to a lesser extent, to zinc-fortified samples. This effect can help explain the wide range of scores given to the same samples that were presented to participants in a balanced order. Probably, the highest scores for the good quality samples were given when they were offered in the beginning of the sessions. Even though water and crackers were used for cleaning the palate between samples, they were not sufficiently effective given the strong residual taste of these minerals. The large number of samples also probably helped perpetuate the carry over effect. Alternatively, Giacalone, Degn, Yang, Liu, Fisk & Münchow [40] used plain white toast bread, milk and tepid water before the first and between each sample, when evaluating coffee roast defects (‘dark’, ‘light’, ‘scorched’, ‘baked’ and ‘underdeveloped’). Probably, this alternative would have been more indicated for samples that have strong carry over effect, as the ones used in the present work.

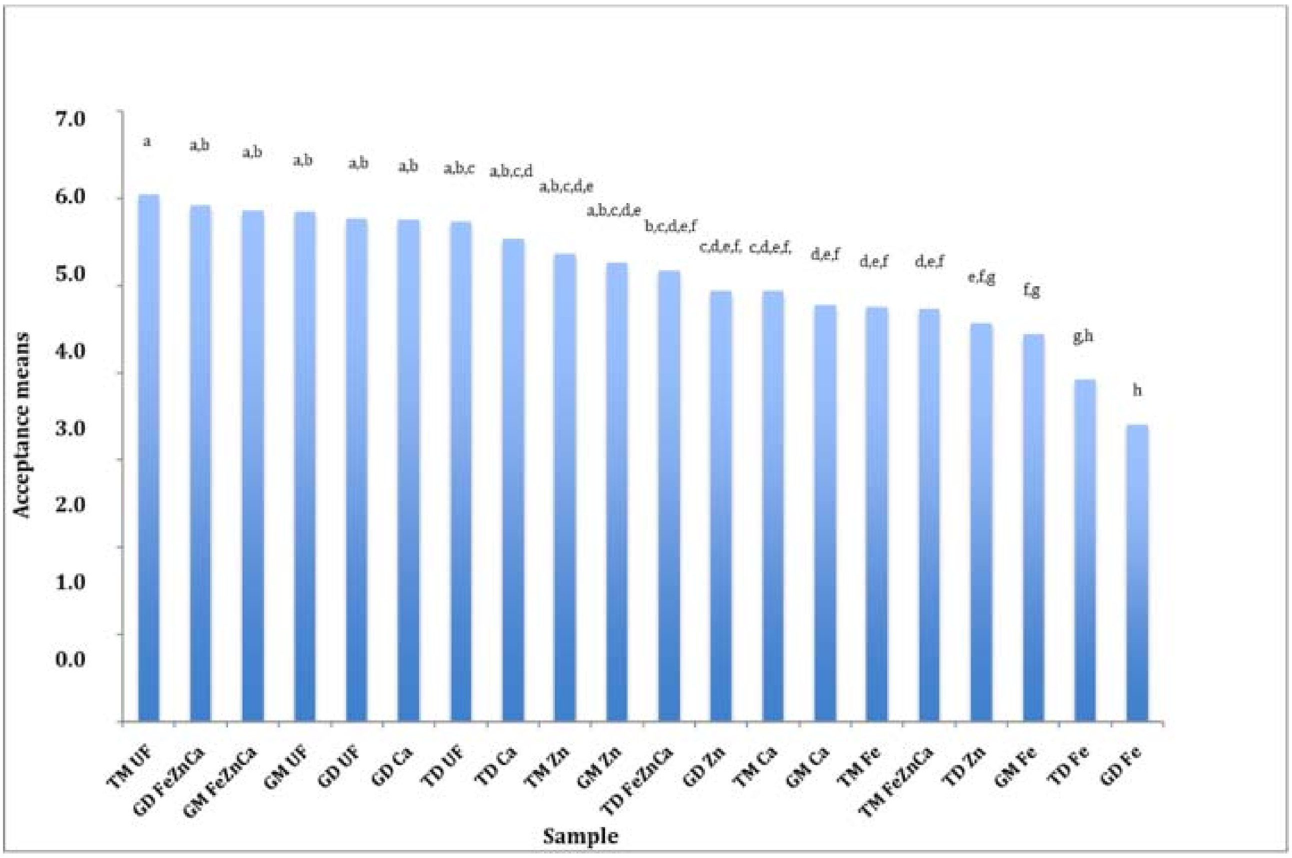

Figure 2. Acceptance means of control (unfortified) and fortified coffees with iron, zinc and calcium salts. (Different letters over the bars indicate that the values differ statistically by Fisher's test (p <0.05). T: Traditional; G: Gourmet; M: Medium; D: Dark, F: Fortified; UF: Unfortified; Fe: Iron; Zn: Zinc; Ca: Calcium. Samples were evaluated in 9-point hedonic scales varying from 1: disliked extremely to 9: liked extremely.)

When gourmet and traditional coffee blends were fortified with the three minerals, they presented similar acceptance to unfortified coffees, regardless of the roast degree (mean score of 5.9 for both) (Figure 2). The fact that samples fortified with the three minerals were the most accepted samples, together with control samples, receiving statistically similar scores, is a positive finding, taking into account that the aim of the study was to select mineral salts presentation that would not affect the original acceptability of the matrix.

Interestingly, when the minerals were added singly, the acceptance decreased for zinc and iron fortified samples, independently on coffee quality. The acceptance of iron-fortified blends was the lowest among all evaluated ones. In both medium roasted gourmet and traditional blends, iron-fortified coffees (4.4 ± 0.2, respectively and 4.7 ± 0.2) had higher ($p \leq 0.05$) acceptance scores than dark ones (3.4 ± 0.3 and 3.9 ± 0.3 and, respectively) (Figure 2). A greenish tonality was also observed in iron-fortified coffee brews, but not in the brews from coffees fortified with the three minerals. For zinc-fortified coffees, medium roasted gourmet and traditional coffees were also more accepted, with similar scores for both types of blends (5.2 ± 0.2 and 5.3 ± 0.2), compared to dark roasts. Even though dark roasts are usually very well accepted by Brazilians compared to medium roasts [18], this roast may have further emphasized unpleasant attributes related to iron and zinc, such as the metallic perception. In zinc-fortified dark roasted coffees, gourmet blend (4.9 ± 0.2) and traditional blend (4.5 ± 0.2) were similarly accepted (Figure 2).

Calcium-fortified dark roasted gourmet (5.8 ± 0.3) and traditional (5.5 ± 0.3) blends had a better performance in terms of liking. They were similar to the counterpart non-fortified samples. Calcium-fortified medium roasted blends received lower scores (4.8 ± 0.3 and 4.9 ± 0.3 for gourmet and traditional, respectively) ($p < 0.05$) (Figure 2). Considering that calcium is a macronutrient and, therefore, the amount used for fortification was much higher than those of iron and zinc in the fortified samples, associated with the fact that this mineral has a neutral taste, it is most probable that it masked the unpleasant taste of iron and zinc when they were offered together, leading to similar acceptance to control samples.

Taking into account that people can differ considerably in relation to food acceptance, cluster analysis was carried out to identify segments of consumers with similar liking. It was performed based on the hierarchical grouping of acceptance scores. This is relevant for distinguishing different market niches. Three groups of consumers were identified both for gourmet and traditional samples:

- For the gourmet blends, members of segment 1 (n=15) assigned higher acceptance mean scores to dark roasted unfortified coffee (7.9), medium roasted unfortified coffee (7.3) and dark roasted coffee fortified with calcium (7.2). In this group, the lowest scores were attributed to medium roasted coffee with zinc (6.2), and medium roasted coffee with calcium (6.6). In segment 2 (n=34), the highest mean score (4.3) was given to dark roasted coffee fortified with three minerals, and the lowest scores to medium and dark roasted coffees fortified with iron (average 2.8 and 3.2, respectively). Segment 3 (n=54), the largest one, appreciated more the unfortified medium roasted coffee (6.0), the zinc-fortified medium roasted coffee (5.8), the calcium-fortified dark roasted coffee(5.8) and dark roasted coffee fortified with three minerals (5.8). The lowest score was, again, attributed to dark coffee with iron (4.1).

- Regarding the traditional blends, participants of segment 1 (n=32) gave higher scores to medium roasted unfortified blend (5.3) and dark roasted fortified blend with the three minerals (5.1). Segment 2 (n=20) did not enjoy any sample, with the highest score (3.8) attributed to dark roasted with calcium and the lowest to dark roasted with iron and zinc, (2.9 and 2.7 respectively). Segment 3 (n=47), the largest one, attributed higher mean acceptance to unfortified dark (6.5) and medium (6.4) roasted coffees, and to medium roasted fortified with three minerals (6.3). They also liked less the dark roasted blend with iron (5.0).

It is interesting to see that although scores varied considerably for the same samples in different clusters, the most and least accepted samples remained about the same. In most clusters, no statistical difference in relation to age, level of education, family income and frequency of consumption of the beverage was observed. There was only significant difference in relation to gender, with higher ($p \leq 0.05$) acceptance of gourmet coffee by women.

3.2.2. CATA Questions

The frequency of mention of CATA question terms for gourmet and traditional roasted and ground coffee samples investigated in the study is shown in Table 2. It is known that depending on the roast degree different chemical changes occur in the compounds present in coffee seeds, directly influencing the perceived flavors in the sensory evaluation [37]. Traditionally, while nuts and peanuts are perceived in lighter roasts, bitter, smoke and tobacco attributes are perceived in darker roasts, as observed by Giacalone et al. [40]. In our study, we did not have lighter roasts, only medium and dark. The most marked attributes for traditional and gourmet medium roasted unfortified coffees were: characteristic, roasted cereal and bitter, and for dark roast: characteristic, burnt and bitter. These are also typical coffee attributes [24] and the only important difference between medium and dark roasts was the roasted cereal note identified in medium roasted coffees only.

Iron-fortified blends received a high frequency of mention for the attributes medicinal, grass, and sweet; and the attributes astringent, medicinal, salty, caramel were highly used to describe the zinc-fortified blends. Coffees fortified with iron and zinc had lower frequency of “characteristic” attribute. On the other hand, calcium-fortified blends had higher frequency of characteristic but also astringent, sour, and sweet notes. A few mentions of calcareous were observed for these samples (Table 2).

Figure 3A contains the sensory attributes in the first and second dimensions of MFA applied to the frequency of use of CATA terms for evaluation of traditional and gourmet coffee beverages; Figure 3B shows the position of the samples. The left side of Figure 3A contains coffee characteristic and pleasant sensory attributes. In the left quadrants of Figure 3B, one can observe the association of pleasant attributes with the samples that received the highest mean acceptance scores (unfortified and fortified with all three minerals). The red circle include the samples related to the preference area (in red) in Figure 3A.The right side of Figure 3A contains the unpleasant sensory attributes, which were mostly associated with all iron fortified samples. Mostly, the figure shows that coffee fortification with ferrous bisglycinate quelate alone was unsuccessful, and confirmed that the fortification using the three minerals jointly achieved better performance. Indeed, fortification of food with iron salts is considered technically one of the most difficult challenges and, according to Morales et al [41] the most bioavailable forms are usually the most reactive ones, often producing undesirable effects when added to food.

Table 2. Frequency of mention of the check-all-that-apply (CATA) question terms for gourmet and traditional roasted and ground coffee samples investigated in the study.

SAMPLES	ROASTED CEREAL	CHARACTERISTIC	CUCUMBER	PEANUT	CARAMEL	ALMOND	CHOCOLATE	GRASS	POPCORN	VANILLA	CLOVE	MEDICINE	FERMENTED	BURNT RUBBERY
GMUF	17	30	0	4	7	2	0	3	2	2	0	4	1	2
GDUF	11	25	0	2	5	4	2	4	1	4	1	3	0	5
GMFe	14	15	2	2	5	3	1	9	0	1	1	4	3	2
GDFe	10	9	0	2	3	2	0	10	0	1	0	6	0	6
GMZn	14	21	1	5	11	2	2	3	1	4	1	6	2	2
GDZn	6	15	1	7	3	0	1	1	2	0	2	4	1	4
GMCa	13	24	0	6	3	0	1	1	2	4	2	5	0	2
GDCa	10	31	0	3	6	3	1	5	1	4	1	4	2	3
GMFeZnCa	10	25	1	6	8	4	3	3	1	2	2	2	0	2

GDFeZn Ca	13	30	1	1	5	4	4	1	2	1	1	2	1	4
TMUF	17	29	0	4	5	2	2	3	1	4	0	6	1	0
TDUF	11	20	0	4	3	3	1	5	0	2	2	3	2	3
TMFe	10	16	1	4	7	3	1	10	1	3	0	9	1	2
TDFe	8	13	0	3	2	2	0	6	0	0	1	5	1	7
TMZn	15	22	0	6	9	6	0	6	0	5	3	1	3	2
TDZn	9	7	2	5	1	1	1	4	1	0	0	9	3	1
TMCa	12	26	0	2	3	0	1	1	0	5	1	2	3	3
TDCa	14	26	0	8	6	5	2	4	0	1	1	3	1	2
TMFeZn Ca	9	18	0	5	2	6	0	6	0	3	1	6	2	3
TDFeZn Ca	13	23	0	3	2	2	2	1	1	1	1	4	2	0

SAMPLES	EARTH	SMOKE	IODINE	BURNT	RANCID	SOUR	SALTY	SWEET	BITTER	SLIME	CALCAREOUS	BRACKISH	METALLIC	ASTRINGENT
GMUF	2	1	1	17	2	3	2	7	31	1	1	0	3	4
GDUF	2	1	0	12	3	0	4	5	23	1	0	2	3	2
GMFe	7	6	1	12	4	4	5	10	14	3	0	5	9	5
GDFe	8	3	1	13	1	10	5	2	17	5	1	9	20	4
GMZn	5	2	2	9	1	4	8	10	13	1	4	2	5	1
GDZn	2	6	0	24	1	2	7	7	23	1	0	2	3	1
GMCa	5	4	1	18	1	6	0	6	19	1	0	2	3	8
GDCa	2	2	0	13	3	3	4	15	15	1	2	2	2	3
GMFeZnCa	2	3	1	14	1	1	3	9	23	0	0	1	2	2
GDFeZnCa	3	5	1	13	0	1	2	5	22	1	1	2	2	3
TMUF	2	3	0	11	0	5	3	7	31	0	0	0	1	4
TDUF	1	3	0	14	1	4	5	11	21	0	0	4	5	5
TMFe	7	7	1	12	0	5	2	13	15	0	1	3	8	5
TDFe	6	2	3	9	2	12	4	4	26	5	0	8	15	4
TMZn	5	2	0	5	1	2	3	14	16	1	1	3	3	5
TDZn	1	4	3	16	3	1	6	9	23	2	5	6	7	11
TMCa	4	2	0	12	1	10	3	6	23	1	2	3	6	8
TDCa	4	5	1	8	2	6	3	9	24	0	1	2	3	6
TMFeZnCa	4	6	1	13	1	2	2	6	26	1	2	3	5	3
TDFeZnCa	2	3	0	13	0	5	4	4	21	0	3	0	6	4

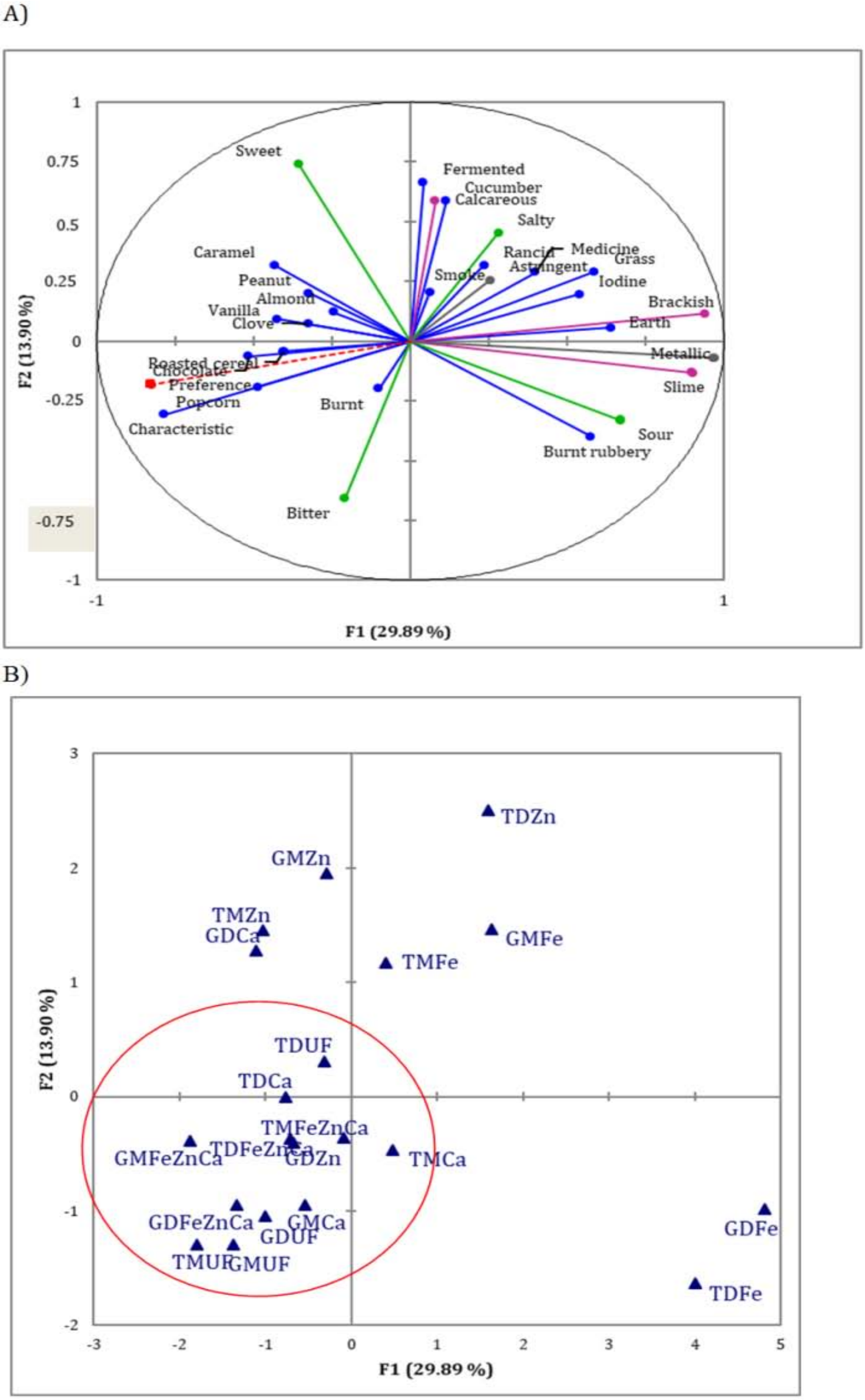

Figure 3. First and second dimensions of Multiple Factor Analysis (MFA) applied to frequency of use of terms of CATA questions for the evaluation of coffee beverages: (A) Sensory attributes, and (B) Coffee beverage samples. (T: Traditional; G: Gourmet; M: Medium; D: Dark, F: Fortified; UF: Unfortified; Fe: Iron; Zn: Zinc; Ca: Calcium)

4. Conclusive Remarks

In the present study, the quality of the blend contributed to improve the acceptance, particularly among women; roast degree also affected results, especially when minerals were offered singly. Fortification with ferrous bisglycinate quelate (and possibly with other iron salts) singly is not recommended unless they are offered in an encapsulated form, due to the strong impact on flavor and appearance of the beverage, which caused negative effect on consumer's acceptance. Medium roast seemed to be more appropriate to mask the zinc metallic flavor and astringency, both in gourmet and traditional blends. Calcium affected less coffee flavor compared to other minerals, and dark roast was more efficient to mask calcium slight astringency. Finally, unfortified blends, blends fortified with the three minerals, and dark gourmet blend fortified with calcium had the highest acceptance among participants; showing that calcium was beneficial in the mixture of minerals to mask their off-flavor and increase consumer's acceptance.

As already mentioned, coffee largely meets the prerequisites for use as a food fortification vehicle, as it is widely consumed by the world population, at all socioeconomic levels. Considering the small quantities used, fortification with ferrous bisglycinate chelate, zinc lactate and calcium lactate would not change the final cost of a cup of coffee for consumer, and it can be easily implemented by coffee producers. This would be still considered a low cost food that could be consumed by all socioeconomic classes, including popular restaurants and government supplementation programs. The fortification of soluble coffee would also be an option, since the technological process does not cause loss of salts. However, given the very low cost of these mineral salts and the higher cost and lower acceptability of soluble coffee by Brazilian consumers, fortification of ground roasted coffee seems to be a better and promising option.

Considering that nutrition deficiencies do not reach only low income populations. for the health conscious consumers niches, gourmet products containing either calcium or calcium and other minerals can also be produced. For higher income population, espresso capsules could be offered considering the higher extraction efficiency. Women in their menopausal period, for example, could largely benefit from calcium fortification. Regarding the possibility of toxicity, the tolerable upper intake level (UL) can be defined as the highest average daily nutrient intake that is likely to pose no risk of adverse health effects to almost all individuals in the general population [42]. For iron, zinc, and calcium, these limits would be 45 mg, 40 mg, and 2500 mg, respectively. Considering a consumption of for example, 500 mL/day, individuals could consume, on average, 2.5 mg of iron, 0.7 mg of zinc, and 111.4 mg of calcium through filtered brew from fortified coffee, or about 4.0 mg of iron, 1.1 mg of zinc, and 155.6 mg of calcium in the espresso brew [3]. Therefore, considering filtered or espresso brews, even heavy coffee consumers would not reach the UL for these minerals [43].

In future sensory studies, the effect of different iron salts on acceptance, should be evaluated. The positive or negative effects of different salts associations on the brew´s sensory attributes and acceptance should also be evaluated. With the development of micro- and nano-encapsulation technologies, one may ask why traditional salts were used for ground coffee fortification (especially iron) since they have the disadvantage of possibly interacting with the food matrix and alter its sensory properties. Nonetheless, considering that filtered coffee is the most used preparation method by those who are in need of fortification, the authors hypothesized that in addition to being costlier, such particles, especially microparticles, could present higher retention in the paper or nylon filter. However, new experiments should be performed using such technologies for coffee fortification, especially nanoparticles, which can be very fine and may pass through these filters. Considering that product development must include not only consumer acceptance and microbiological safety, but also stability during storage, this aspect should be approached in future studies.

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