Journal of Food Quality

Coffee Quality: Cultivars, Blends, Processing, and Storage Impact

Lead Guest Editor: Gabriel H. H. De Oliveira
Guest Editors: Ana P. L. R. De Oliveira, Fernando M. Botelho, Pedro C. Treto, and Silvia C. C. Botelho

Coffee Quality: Cultivars, Blends, Processing, and Storage Impact

Journal of Food Quality

Coffee Quality: Cultivars, Blends, Processing, and Storage Impact

Lead Guest Editor: Gabriel H. H. De Oliveira
Guest Editors: Ana P. L. R. De Oliveira, Fernando M. Botelho, Pedro C. Treto, and Silvia C. C. Botelho

Editorial Board

Contents

Coffee Quality: Cultivars, Blends, Processing, and Storage Impact
Gabriel Henrique Horta de Oliveira, Ana Paula Lelis Rodrigues de Oliveira, Fernando Mendes Botelho, Pedro Casanova Treto, and Silvia de Carvalho Campos Botelho
Editorial (1 page), Article ID 9805635, Volume 2018 (2018)

Boron, Copper, and Zinc Affect the Productivity, Cup Quality, and Chemical Compounds in Coffee Beans
Junia Maria Clemente, Herminia Emilia Prieto Martinez, Adriene Woods Pedrosa, Yonara Poltronieri Neves, Paulo Roberto Cecon, and John Lonfover Jifon
Research Article (14 pages), Article ID 7960231, Volume 2018 (2018)

Quality of Commercial Coffees: Heavy Metal and Ash Contents
Mariana Teixeira Pigozzi, Flávia Regina Passos, and Fabrícia Queiroz Mendes
Research Article (7 pages), Article ID 5908463, Volume 2018 (2018)

Effect of Yeast Fermentation of Green Coffee Beans on Antioxidant Activity and Consumer Acceptability
Han Sub Kwak, Yoonhwa Jeong, and Misook Kim
Research Article (8 pages), Article ID 5967130, Volume 2018 (2018)

Shade Trees Spatial Distribution and Its Effect on Grains and Beverage Quality of Shaded Coffee Trees
Francisco José da Silva Neto, Kátia Priscilla Gomes Morinigo, Nathalia de França Guimarães, Anderson de Souza Gallo, Maicon Douglas Bispo de Souza, Rubismar Stolf, and Anastácia Fontanetti
Research Article (8 pages), Article ID 7909467, Volume 2018 (2018)

Propositions on the Optimal Number of Q-Graders and R-Graders
Lucas Louzada Pereira, Rogério Carvalho Guarçoni, Gustavo Soares de Souza, Dério Brioschi Junior, Taís Rizzo Moreira, and Carla Schwengber ten Caten
Research Article (7 pages), Article ID 3285452, Volume 2018 (2018)

Influence of Solar Radiation and Wet Processing on the Final Quality of Arabica Coffee
Lucas Louzada Pereira, Rogério Carvalho Guarçoni, Wilton Soares Cardoso, Renato Côrrea Taques, Taís Rizzo Moreira, Samuel Ferreira da Silva, and Carla Schwengber ten Caten
Research Article (9 pages), Article ID 6408571, Volume 2018 (2018)

Hindawi
Journal of Food Quality
Volume 2018, Article ID 9805635, 1 page
https://doi.org/10.1155/2018/9805635

Editorial

Coffee Quality: Cultivars, Blends, Processing, and Storage Impact

Gabriel Henrique Horta de Oliveira,[1] **Ana Paula Lelis Rodrigues de Oliveira**,[1] **Fernando Mendes Botelho**,[2] **Pedro Casanova Treto**,[3] **and Silvia de Carvalho Campos Botelho**[4]

[1]*Instituto Federal de Educação, Ciência e Tecnologia do Sudeste de Minas Gerais, Campus Manhuaçu, Manhuaçu, MG, Brazil*
[2]*Agrarian and Environmental Sciences Institute, Universidade Federal de Mato Grosso, Sinop, MT, Brazil*
[3]*Research Institute of Engineering, Universidad de Costa Rica, San Pedro de Montes de Oca, San José, Costa Rica*
[4]*Embrapa Agrossilvipastoril, Sinop, MT, Brazil*

Correspondence should be addressed to Gabriel Henrique Horta de Oliveira; gabriel.oliveira@ifsudestemg.edu.br

Received 9 May 2018; Accepted 9 May 2018; Published 19 June 2018

Coffee is one of the most important agricultural products in the world, being produced in different countries and under several conditions, which leads to an enormous variety of beverages, according to cultivar selection, types of blends, processing technologies, and storage procedures, among other features. To spread and foment the discussion regarding these sources of variations to coffee drink among producers, industry, and consumers, some important issues must be addressed such as analysis of residues and micronutrients in coffee grain, emerging technologies like controlled fermentations, intercropping and solar radiation, methods of sensory analysis, and so on. In this special issue on coffee quality, we have invited a few papers that address such issues.

The first paper of this special issue aimed at quantifying the ash content and determining the concentration of heavy metals in roasted ground coffee. These parameters are important due to their persistency in the environment, becoming an indicator for coffee quality. The second paper presents the study on the yeast fermentation of green coffee beans, which consumers indicated that these coffees did not present negative aroma or flavor and presented higher antioxidant activity than coffee without fermentation. The third paper is on the influence of different distances of shading coffee trees on plant height, canopy diameter, plagiotropic branches' length, yield, coffee fruits' phenological stage, ripe cherries' Brix degree, percentage of black, unripe, and insect damaged beans, bean size, and beverage quality. The best cup quality was obtained in coffee beans coming from coffee trees closer to shaded trees.

The fourth paper of this special issue analyzed the optimal number of Q-graders and R-graders on the sensory analysis consistency for specialty coffees. The authors indicated that the use of 6 tasters is sufficient to conduct sensorial analysis following SCA and BSCA protocol for coffees in the Arabica group, as well as 6 tasters for coil and Conilon coffees. Additional tasters did not improve the sensorial analysis. The fifth paper researched the influence of solar radiation and wet processing on the final quality of arabica coffee, being indicated that water fermentation and shaded region are more likely to provide coffee with higher grades. The final paper investigated, for two consecutive seasons, the effect of two different applications of boron, copper, and zinc over productivity and cup quality. Application via foliar spray presented better results than trunk injections, leading to higher productivity and cup quality.

Gabriel Henrique Horta de Oliveira
Ana Paula Lelis Rodrigues de Oliveira
Fernando Mendes Botelho
Pedro Casanova Treto
Silvia de Carvalho Campos Botelho

Hindawi
Journal of Food Quality
Volume 2018, Article ID 7960231, 14 pages
https://doi.org/10.1155/2018/7960231

Research Article

Boron, Copper, and Zinc Affect the Productivity, Cup Quality, and Chemical Compounds in Coffee Beans

Junia Maria Clemente,[1] Herminia Emilia Prieto Martinez,[1] Adriene Woods Pedrosa,[2] Yonara Poltronieri Neves,[2] Paulo Roberto Cecon,[3] and John Lonfover Jifon[4]

[1]*Department of Plant Science, Universidade Federal de Viçosa, Avenue PH Rolfs, 36.570-000 Viçosa, MG, Brazil*
[2]*Empresa de Pesquisa Agropecuária de Minas Gerais, EPAMIG Sudeste, Campus UFV–Cx. P. 216, 36.571-000 Viçosa, MG, Brazil*
[3]*Department of Statistic, Universidade Federal de Viçosa, Avenue PH Rolfs, 36.570-000 Viçosa, MG, Brazil*
[4]*Texas A&M Agrilife Research and Extension Center, Environmental Plant Physiology, 2415 East Business 83, Weslaco, TX 78596, USA*

Correspondence should be addressed to Herminia Emilia Prieto Martinez; herminia@ufv.br

Received 8 December 2017; Accepted 8 April 2018; Published 14 May 2018

Academic Editor: Ana P. L. R. De Oliveira

Micronutrients perform specific and essential functions in plant metabolism, and their deficiency may lead to metabolic disturbances that affect coffee production and quality beverage. In Brazil, the B, Cu, and Zn are the main micronutrients, and these are provided by soil or foliar fertilization, frequently with low recovery efficiency. This work objected verifying the feasibility of supplying of B, Cu, and Zn via insertion of tablets in the orthotropic branch of *Coffea arabica*, as well as to evaluate the coffee plant response in terms of productivity and quality of the beverage. Adult plants received B, Cu, and Zn, each micronutrient alone or combined with the other two, by foliar fertilization or by tablets inserted in the trunk base. The productivity, cupping quality, and some chemical indicators of beans quality were evaluated in two crop seasons. Boron, copper, and zinc supplied by foliar spray or solid injections in the trunk influenced the chemical composition and quality of the coffee beans, characterized by the cupping test and the levels of caffeine, trigonelline, sucrose, glucose, arabinose, mannose, 3-caffeoylquinic acid, 5-caffeoylquinic acid, polyphenol oxidase activity, and total phenolic compounds. Copper and zinc were equivalent in either form of supply regarding the production and quality of coffee.

1. Introduction

Brazil is the largest world producer of coffee. As a world leader in production and exportation, the country needs to attend market requirements, innovating and adopting technologies to produce good-quality types of coffee. The necessity of offering good coffees is rising due to an increase in coffee consumers looking for refined tastes and aromas, which are related to the chemical composition of the coffee beans. Flavor and aroma are the main criteria to evaluate beverage quality and also constitute the most important attributes for consuming coffee [1]. The cup test is the standard approach to evaluate the flavor and aroma of coffee; however, quite often, it is criticized because of its subjective nature [2]. Therefore, it is essential to explore alternative methods to accurately assess the chemical characteristics and the quality of beverage.

The production of bioactive compounds related to desirable flavor and aroma involves extremely complex chemical reactions, in which some mineral nutrients could have a key role. However, until now, little is known about the effect of the mineral nutrients B, Cu, and Zn on the production of chemical compounds that define the good quality of coffee. Most of the studies about mineral nutrition and coffee fertilization are focused on sources and doses of these nutrients, in order to optimize the productivity.

Boron deficiency is common in most of the Brazilian soils [3]. In coffee plants, its deficiency has been attributed to the natural loss of soil fertility, as well as to the wide use of highly demanding varieties. Boron deficiency in coffee plants

can reduce the root system growth and cause the death of thin root tips and consequently the decrease in water and mineral absorption. In consequence of that, the plants become sensible to drought and less responsive to fertilizations. As B is highly immobile in the phloem sap [4], its supply via soil is desirable, although been supplied many times by foliar sprays, mixed with Cu and Zn.

The Cu deficiency compromises the activity of several enzymes that catalyze oxidative reactions of several metabolic routes, especially the plastocyanin, superoxide dismutase, and polyphenoloxidase [5]. In the soil, Cu is strongly complexed by organic matter [6], so the common technique used for supplying Cu to coffee plants is through foliar sprays. The Cu deficiency can cause irreversible metabolic disturbances in coffee plants and possibly compromise the production of chemicals related to the beverage quality.

According to Fageria et al. [7], the lack of Zn impairs the world agriculture production, as well as the nutritional quality of grains. The main symptoms of Zn deficiency are related, in a still unclearly way, to disturbance in auxin metabolism [5, 8], which plays an essential role in the synthesis of tryptophan, an amino acid precursor of IAA [9]. The photosynthetic activity of coffee plants is hugely diminished under lack of Zn, given its importance on enzymes involved in carbon fixation [10]. Besides this, Zn regulates or makes part of the structure of several other enzymes involved in protein synthesis and in nitrogen metabolism [5, 11]. In clayey acid soils, several reactions are responsible for the low Zn availability, which together with the low mobility of Zn in the coffee plant phloem drives to foliar fertilizations in such conditions [12, 13]. Also, like other Brazilian coffee producer regions, Zona da Mata is a mountain region in which manually done foliar sprays are time-consuming and costly.

Coffees of superior quality are those that have chemical compounds responsible for the flavor and aroma such as caffeine, trigonelline, aldehydes, furans, ketones, sugars, proteins, amino acids, pyrroles, pyridines, pyrazines, oxazoles, carboxylic acids, fatty acids, and phenolic compounds in an equilibrated proportion to obtain good body, acidity, and smoothness of the beverage.

When in the presence of microorganisms or under anaerobic conditions, the sugars present in the coffee mucilage can be fermented and produce alcohols that may be broken down successively in acetic, lactic, propionic, and butyric acids. Pinto et al. [14] studying the quality of beans used to prepare espresso coffee observed that the low-quality ones, such as the types "rio" and "rioysh," presented higher acidity than the types "strictly soft" and "soft."

Electrical conductivity (EC) or the leached potassium (LK) has been used in researches as consistent indicators of cellular membrane integrity. They are accessory attributes used preferentially to differentiate beverages of the same class, having little adequacy as a unique mean of differentiation. Grains of bad quality commonly present higher EC and less organization and cellular structuring than the good ones, showing that EC is a strong indicator of membrane and cell wall damage [15].

Polyphenoloxidase (PPO) is a cupric enzyme linked to the cellular membranes, and as discussed in the literature, it is directly involved with the quality of the coffee beverage [2, 16, 17]. It is established that coffee beans that are strongly damaged or Cu deficient may have low PPO activity and low quality. Carvalho et al. [2] performed pioneering works, with physical and chemical evaluations of processed coffee beans previously classified as "strictly soft," "soft," "softish," "hard," "rioysh," and "rio," and verified that the coloration index and PPO allow the separation of beans with different coffee quality types.

Chlorogenic acids are the main nonvolatile phenolic compounds found in coffee beans and account for 6 to 12% of their dried mass [18]. They are formed by means of esterification of *trans*-cinnamic acids, such as caffeic acid, ferulic acid, and *p*-coumaric acid with quinic acids [19]. During the roast process, they are strongly degraded generating acids, lactones, and volatile compounds such as the phenil, guaiacol, and 4-vinyl guaiacol [20, 21], which contribute to the flavor and aroma of coffee, especially for the astringency of the beverage; the proanthocyanidins along with the polyphenols also provide an astringent flavor [22]. Within acceptable limits, chlorogenic acids have a positive effect on the beverage body.

Caffeine, commonly known as 1,3,7-trimethylxanthine, belongs to the methylxanthine class and gives bitterness to the coffee taste [19].

Trigonelline corresponds to around 1% of raw beans, and it is one of the precursors of aroma in coffee and undergoes degradation of up to 90% during roasting, forming mainly niacin, pyridines, and some pyrroles; the lower the trigonelline content in the beans, the lower the quality of the coffee beverage [23]. It is worth to highlight that the more drastic one is the roast process in which the lower levels of trigonelline will be found in the samples [24].

Production of secondary metabolism metabolites, such as polyphenols, caffeine, trigonelline, alcohols, and aldehydes, depends on the primary metabolism and its catabolic reactions that produce energy and the carbonic skeleton, such as sucrose. Therefore, if any factor affects photosynthates production during the fruits development, then it can also affect negatively the quality of beverage [25].

This work is aimed at evaluating the production of bioactive compounds and the quality of raw coffee beans harvested from plants fertilized with B, Cu, and Zn via foliar sprays or solid injections of salts in the trunk and correlates these variables with the nutritional status of the plants.

2. Materials and Methods

Three experiments were performed in July, in a field crop area of the Universidade Federal de Viçosa, Viçosa, Minas Gerais State, Brazil, using an adult orchard of *Coffea arabica* L. cv. Catuaí IAC-99 and have been conducted and evaluated during two crop seasons. The experimental field is located at 20°45 south and 42°51 west, at 541 m above the sea level. The soil of the experimental field is classified as a red-yellow latosol and the climate is classified as Cwa, according to the Köppen classification, with annual temperature and precipitation averages of 19.4°C and 1221.4 mm, respectively.

2.1. Treatments and Experimental Design. In the first experiment, the following treatments were performed to evaluate the boron effect: control without B supply, foliar sprays with boric acid at 0.4%, injection of tablets containing B salts at the base of the trunk, injection of tablets containing B + Cu salts at the base of the trunk, injection of tablets containing B + Zn salts at the base of the trunk, and injection of tablets containing B + Cu + Zn salts at the base of the trunk.

The second experiment, to evaluate the copper effect, was performed in the same way as the first, receiving the following treatments: control without Cu supply, foliar sprays with copper sulphate at 0.4%, injection of tablets containing Cu salts at the base of the trunk, injection of tablets containing Cu + B salts at the base of the trunk, injection of tablets containing Cu + Zn salts at the base of the trunk, and injection of tablets containing Cu + B + Zn salts at the base of the trunk.

The third experiment, to evaluate the zinc effect, was performed like the other two, receiving the following treatments: control without Zn supply, foliar sprays with zinc sulphate at 0.4%, injection of tablets containing Zn salts at the base of the trunk, injection of tablets containing Zn + B salts at the base of the trunk, injection of tablets containing Zn + Cu salts at the base of the trunk, and injection of tablets containing Zn + B + Cu salts at the base of the trunk.

All the experiments were assigned as randomized blocks with 5 replications. Each plot was composed of 18 plants distributed in three rows 3 m apart and with 1 m between plants in a row. The four central plants constituted the useful plot. We worked with 30 total plots in each experiment.

The tablets of B and Zn were prepared at the Laboratory of Civil Engineering, Universidade Federal de Viçosa, using a hydraulic press with a force of 0.5 tons. The copper was supplied through capsules without any compression of the salts, due to the difficulty of these in forming compact mass with excipient agents. The tablets were implanted into the orthotropic branch of the coffee tree at 10 cm above the ground.

Considering that usually coffee foliar sprays are done with a volume of 400 $L \cdot ha^{-1}$ and that the orchard had 3333 plants per hectare, we used 120 mL of spraying solution per plant, totaling 480 mL per plot. All nutrients were sprayed by means of handheld sprayers with cone-filled nozzles, and we added 1 $mL \cdot L^{-1}$ of the adhesive adjuvant to the solution. Plastic curtains were placed between the rows to prevent drift.

In each crop season, three foliar sprays were applied between September and February. The liming and fertilization with nitrogen, phosphorus, and potassium were performed based on soil analysis, and the expected productivity, following the recommendations of [26].

In order to determine the nutritional status of the plants subjected to the different treatments, in the two crop seasons, coffee leaves were taken from the third or fourth nodes and counted from the apex to the base of plagiotropic branches, at a median height in the canopy and in the period between flowering and the first rapid expansion of the fruits. The leaves were washed in deionized water and dried in an oven with forced air at 70°C, until constant weight.

Boron content was determined using the azomethine-H method after dry digestion of the plant material [27].

Table 1: Numerical scores for the coffee cupping test.

Taste	Nota
Strictly soft (specialty coffee)	≥87
Soft	80–86
Softish	74–79
Hard	≤74

The content of Cu and Zn was determined by atomic absorption spectrophotometry [28] in the extract of the nitric-perchloric acid digestion [29].

2.2. Evaluations

2.2.1. Production. The harvesting of the four usable plants of the plot was carried out, when the plants had approximately 95% coffee cherries. The coffee cherries were handpicked and dried on a bench in a greenhouse until achieving 11% moisture content. After drying, they were hulled and used for the chemical analysis.

2.2.2. Cupping Quality. The cupping test was performed by professional tasters, using the CoE (cup of excellence) method (Table 1). Each attribute (clean beverage, sweetness, acidity, body, taste, flavor, and reminiscent taste) received a score based on the taste intensity exhibited by the samples, according to the Brazilian official method plus the grades described by the SCAA method for specialty coffees [30].

2.2.3. Chemical Analysis of the Coffee Beans. Total sugars, nonreducing sugars, coloration index, total titratable acidity, pH, electrical conductivity, and leached potassium were evaluated in beans harvested in the crop season 2010/2011. Coloration index, leached potassium, total titratable acidity, pH, electrical conductivity, caffeine, trigonelline, total phenolic compounds, sucrose, glucose, mannose, arabinose, galactose, proanthocyanidin, 3-caffeoylquinic acid, 4-caffeoylquinic acid, 5-caffeoylquinic acid, and PPO activity were evaluated in beans harvested in the crop season 2011/2012 as described below. All the extractions and readings were performed in duplicates.

Total sugars and reducing sugars were extracted by the Lane–Enyon method as described by the Association of Official Analytical Chemists [31] and determined by the Somogyi technique, adjusted by Nelson [32]. The nonreducing sugars were determined by the difference between total and reducing sugars. The coloration index was determined by Singleton [33], adapted for coffee. The results of total sugar were expressed in percentage (%) and the coloration index in DO 425 nm, respectively.

Total titratable acidity and the pH were determined as described by the Association of Official Analytical Chemists [31]. Already, electrical conductivity was determined according to the method described by Loeffler et al. [34], and the leached potassium was determined using a flame photometer, as described by Prete [35]. The results of total titratable acidity, electrical conductivity, and leached potassium were expressed

Table 2: Contents of B, Cu, and Zn ($mg \cdot kg^{-1}$) of index leaves of coffee plants that received B, Cu, and Zn as solid injections or foliar sprays (FSs).

Crop season	2010/2011	2011/2012
Boron		
WB	24.08*	27.46
FS (control)	35.04+	30.35
B	35.51+	50.41*,+
B + Cu	35.04+	64.65*,+
B + Zn	33.26+	72.31*,+
B + Cu + Zn	23.94*	78.64*,+
CV (%)	6.59	13.19
Copper		
WCu	9.64*	5.24*
FS (control)	13.51+	13.14+
Cu	17.06*,+	18.62*,+
B + Cu	14.94+	15.79*,+
Cu + Zn	18.96*,+	19.41*,+
B + Cu + Zn	16.46*,+	16.52*,+
CV (%)	8.25	6.15
Zinc		
WZn	6.5*	4.68*
FS (control)	10.06+	7.42+
Zn	10.85+	11.58*,+
B + Zn	10.63+	10.89*,+
Cu + Zn	11.75*,+	13.66*,+
B + Cu + Zn	9.85+	9.98*,+
CV (%)	6.25	16.31

WB, WCu, and WZn: control treatments, without application of B, Cu, and Zn, respectively; FS: foliar spray with boric acid, copper sulphate, and zinc sulphate (0.4%); B: trunk injection of tablets containing B salts; Cu: trunk injection of tablets containing Cu salts; Zn: trunk injection of tablets containing Zn salts; B + Cu: trunk injection of tablets containing B and Cu salts; B + Zn: trunk injection of tablets containing B and Zn salts; Cu + Zn: trunk injection of tablets containing Cu and Zn salts; B + Cu + Zn: trunk injection of tablets containing B, Cu, and Zn salts; *mean values are statistically different from those of the control treatment (FS) at the 10% significance level, according to Dunnett's test; +mean values are statistically different from those of the control treatment (WB-WCu-WZn) at the 10% significance level, according to Dunnett's test.

in mL of NaOH 100 g^{-1} of the sample, $\mu S \cdot cm^{-1} \cdot g^{-1}$, and $g \cdot kg^{-1}$, respectively.

The polyphenoloxidase (PPO) activity was determined as described by Ponting and Joslyn [36], using the sample extract without DOPA as the blank, and the results were expressed in U/min/g of the sample. Chlorogenic acids were extracted according to Farah et al. [37] and Trugo and Macrae [18], and the results were expressed in percentage (%).

Caffeine was determined according to the method described by Mazzafera et al. [38] with additional modifications as described by Vitorino et al. [39], the trigonelline was determined according to the method described by Vitorino et al. [39], and phenolic compounds were determined by the Folin–Denis method as described by the Association of Official Analytical Chemists [31]. The results were expressed in percentage (%).

The proanthocyanidins were determined as described by Hagerman et al. [40], and sucrose, glucose, mannose, galactose, and arabinose were determined as described by Sluiter et al. [41]. The results were expressed in percentage (%).

2.2.4. Statistics. Data were submitted to variance analysis, and the means were compared by Dunnett's test at 10% of probability in the program SAEG 9.1 [42]. The treatment without B, Cu, and Zn was considered as the first control because it is the control of all treatments, and the sprayed treatment was considered as the second control because it is the usual form to supply B, Cu, and Zn to coffee plants.

3. Results and Discussion

3.1. Boron Content in Index Leaves. In the crop season 2010/2011, the index leaves of the coffee plants that received B as foliar spray or solid injections with B, B + Cu, or B + Zn presented higher contents of B than the control treatment without boron (WB). For this same crop season, the experiments evaluating tablets of Cu and Zn behaved the same way compared to the control treatments without Cu and without Zn (WCu-WZn; Table 2).

The content of B in the leaves of the treatments with B, B + Zn, and B + Cu was statistically similar to that in the sprayed control treatment, and the treatment with B + Cu + Zn was statistically lower in the first crop season (Table 2).

The content of Cu of the treatments with Cu, Cu + Zn, and B + Cu + Zn was statistically higher than that observed in the sprayed control, suggesting fast release of the nutrient in the treatments that received Cu as solid injections. For the Zn content in leaves, only the Cu + Zn treatment was significantly higher than the sprayed control (Table 2).

Considering the sufficiency ranges of 29 to 52 $mg \cdot kg^{-1}$ for B, 13 to 29 $mg \cdot kg^{-1}$ for Cu, and 6 to 12 $mg \cdot kg^{-1}$ for Zn as determined by Martinez et al. [43] for the region of Viçosa, only the plants of the control treatment (WB-WCu) were deficient in B and Cu and the plants of the treatment with B + Cu + Zn were deficient in B.

In the crop season 2011/2012, solid injections of B, Cu, and Zn in the trunk, pure or combined with two or three elements, resulted in higher contents of these nutrients in leaves than the treatments WB, WCu, and WZn. In the case of B, the concentrations attained could be considered toxic to the plants, while in comparison to the sprayed treatment, it can be noted that the content of all treatments that received solid injections of B, Cu, and Zn was significantly higher (Table 2).

In all treatments, except the treatments WB, WCu, and WZn, the concentrations of Cu and Zn were considered adequate according to the method described by Martinez et al. [43]. The results suggest that both forms, in both crop seasons, were efficient to increase the contents of B, Cu, and Zn in index leaves of coffee plants, even with the necessity to review the composition and doses of B salts.

3.2. Production. In the first crop season, there were no statistically significant differences in coffee production. Plants in the B experiment yielded 3.59 kg of cherries per plant (2992 $kg \cdot ha^{-1}$ of processed coffee), while plants in the Cu and Zn experiments produced on average 3.61 and 3.54 kg of coffee cherries per plant (3.005 $kg \cdot ha^{-1}$ and 2947 $kg \cdot ha^{-1}$ of processed coffee, resp.). This result could be

Table 3: Coffee cherry production of coffee plants submitted to the fertilization via solid salts injections in the trunk and foliar sprays with B, Cu, and Zn.

Treatments	Production	
	2010/2011	2011/2012
Boron		
WB	3.47	4.55
FS	3.57	5.47
B	3.83	4.30
B + Cu	3.74	6.15*,+
B + Zn	3.47	5.59*,+
B + Cu + Zn	3.45	3.93
Means	3.59	5.00
CV (%)	22.88	26.66
Copper		
WCu	3.47	4.55
FS	3.57	5.47
Cu	3.99	5.56
B + Cu	3.74	6.15*,+
Cu + Zn	3.42	5.15
B + Cu + Zn	3.45	3.93
Means	3.61	5.13
CV (%)	22.85	20.38
Zinc		
WZn	3.47	4.55
FS	3.57	5.47
Zn	3.83	5.24
B + Zn	3.47	5.59
Cu + Zn	3.42	5.15
B + Cu + Zn	3.45	3.93
Means	3.54	4.99
CV (%)	28.56	27.03

WB, WCu, and WZn: control treatments, without application of B, Cu, and Zn, respectively; FS: foliar spray with boric acid, copper sulphate, and zinc sulphate (0.4%); B: trunk injection of tablets containing B salts; Cu: trunk injection of tablets containing Cu salts; Zn: trunk injection of tablets containing Zn salts; B + Cu: trunk injection of tablets containing B and Cu salts; B + Zn: trunk injection of tablets containing B and Zn salts; Cu + Zn: trunk injection of tablets containing Cu and Zn salts, B + Cu + Zn: trunk injection of tablets containing B, Cu, and Zn salts; *mean values are statistically different from those of the control treatment (FS) at the 10% significance level, according to Dunnett's test; +mean values are statistically different from those of the control treatment (WB-WCu-WZn) at the 10% significance level, according to Dunnett's test.

due to the fact that fruits are produced in nodes formed in the previous growing season; therefore, nodes in which the fruiting occurred were already formed prior to treatment applications in this study (Table 3).

In the crop season 2011/2012, there was a significant difference in coffee production among the treatments at 10.9 % of probability (Table 3), with the productions of the treatments containing B (B + Cu and B + Zn) 35.01% and 22.82% greater than that in the treatment WB. Such differences correspond to 1328 kg·ha^{-1} and 866 kg·ha^{-1} of processed coffee, respectively, even with the index leaves presenting excessive concentrations of the nutrient (Tables 2 and 3).

For the Cu experiment, the production of the treatment with B + Cu was statistically different, considering the treatment without Cu or the sprayed treatment as controls (Table 3). These results suggest that tablets containing B + Cu salts supplied Cu in adequate amounts, but the most interesting finding was the good combination with B and Cu in the same tablet, since the treatments containing only Cu, Cu + Zn, and B + Cu + Zn and the sprayed treatment were statistically equal compared to the treatment WCu. Later, maybe the plants were slightly sensitive to high concentrations of Cu, as can be concluded taking into account the Cu content in the index leaves.

For the Zn experiment, there was no significant effect of Zn on production, in the two crop seasons, even with the variations in the content of Zn in index leaves, with the mean values of the production in the second crop year being 4.99 (4157 kg·ha^{-1} of processed coffee) (Table 3).

The high level of probability used can be considered acceptable for coffee experiments conducted in commercial orchards because the experimental conditions are very heterogeneous and each coffee plant of the plant population has great variability.

According to Brown and Shelp [44], the B moves in the form of complexes with polyols (sugar-alcohol), and coffee has a large amount of mannitol, but there is little information on the distribution of these polyols. Brown and Hu [45] observed that, in coffee plants, the B is immobile in phloem because of the low capacity of forming stable complexes with sucrose; therefore, the foliar sprays correct the deficiency only in the leaves that received the fertilizers, and the leaves that grow after fertilizers application will present low B concentrations, demanding a greater number of applications.

Santinato et al. [46] working with high doses of boric acid applied in the soil observed that, despite the plant did not present toxicity symptoms, excessive doses caused reduction of 600 kg·ha^{-1} of processed coffee.

Positive correlations between B availability in soil and the harvest index were found by Lima Filho and Malavolta [47] for *Coffea arabica* cv. Catuaí Amarelo. They have observed a great correlation among the harvest index and B content in leaves, branches length, and number of leaves and branches and low correlation among the harvest index and dry matter of the roots, stem, branches, and leaves. These variables are important for coffee production because, according to Rena and Maestri [48], the vertical growth of coffee plant determines the formation of nodes, and from buds of these nodes emerge plagiotropic branches, in which nodes will develop leaves and inflorescences. Therefore, the flowering depends on the branches growth, the number of nodes, and numbers of leaves, since it is to verify that many nodes without leaves do not flower.

According to Santinato et al. [49], 6 to 12 applications per year with solutions at the concentration of 0.25% organic boron (10% B) not coinciding with the flowering provided high productivity and maintained good correlation between B content in leaves and production, although there was no significant difference between the treatments regarding the B content in leaves. Barros et al. [50] observed that boric acid application at 0.3% twice a year resulted in productions only 8% higher than the control treatment without B application. On the other hand, Lima Filho and Malavolta [47] and Barros et al. [50] reported that the increase in B

concentration not always provides an increase in coffee productivity.

According to Andrade [51], in adult coffee plants receiving sprays containing Cu, high Cu content present in leaf did not reduce production possibly because Cu is located on the leaf surface or even because the element remains largely in the cuticle not reaching the cytoplasm. Loneragan [52] states that Cu movement into the plants depends on its concentration. During the initial stages of growth, Cu in excess causes reduction on the branching, thickening, and abnormal coloration of rootlets [53].

Regarding Zn in a field experiment, Guimarães et al. [54] observed an increase of 60 to 360 kg·ha^{-1} of processed coffee with Zn supplementation by foliar spray, followed by the increase in the concentration of Zn from 8 to 21 mg·kg^{-1} in index leaves. In turn, Lima Filho and Malavolta [55] proved the positive interaction between B and Zn studying the dry matter production in seedlings of coffee varieties, and when the nutrients were supplied together, the dry matter production was 21% higher.

In a field experiment performed in the same conditions of that of the experiment reported here, Martinez et al. [56] studied the effect of Zn on the production and on some quality attributes of coffee beans and did not observe effect of the nutrient on the production; however, there was an effect of Zn on beans size. Moreover, plants supplemented with Zn had the highest percentage of exportable grains, retained in the sieves 17 and 18. Still according to these authors, there was no significant effect of Zn on the cup quality of coffee beans; however, the score related to the cup quality of the beans harvested from plants that did not receive Zn was 60, while in the beans harvested from plants that received Zn, it was 72.5.

In the same orchard, Neves et al. [57] studied the effect of Zn, supplied by trunk injection and foliar sprays, on the production and on some attributes of quality. The cumulative production of two crop seasons for treatments that received tablets of Zn inserted in the trunk and the treatment without Zn was 11,292 and 7810 kg·ha^{-1} of processed coffee, respectively. The difference among them was 3482 kg·ha^{-1}, which corresponds at 30.9%. Still according to the authors, the beans were classified as "hard" type, and there was no significant effect of Zn on coffee bean quality evaluated by the cup test.

3.3. Cupping Quality. There was no significant effect of B on the cupping test in both years, with the overall mean grades of 83.73 and 80.4 in the respective assessed years; in general, the coffee beans were classified as "soft" (Table 4).

Comparing tablets with the treatment WCu and the sprayed treatment, only the treatment containing Cu + Zn had statistically low scores in the crop season 2011/2012, evidencing the effect of the way of Cu supply and the effect of the nutrient on the cupping quality. As previously reported, the index leaves of the plants subjected to this treatment had slightly high Cu content. In case of Cu and Zn tending to the excess quantity, there is a negative interaction with other cationic micronutrients that when in appropriate concentrations certainly would influence positively the route of production of compounds associated with desirable flavors and aromas (Tables 2 and 4).

Table 4: Cupping test of coffee beans harvested from plants submitted to fertilization via solid salts injections and foliar spray with B, Cu, and Zn.

Treatments	2010/2011	2011/2012
Cupping test		
Boron		
WB	82.4	82.7
FS	84.8	83.9
B	84.4	72.8
B + Cu	83.4	83.25
B + Zn	83.6	79.35
B + Cu + Zn	83.8	80.4
CV (%)	3.76	8.20
Copper		
WCu	82.4	82.7
FS	84.8	83.9
Cu	85.0	81.5
B + Cu	83.4	83.25
Cu + Zn	82.0	74.6$^{*,+}$
B + Cu + Zn	83.8	80.4
CV (%)	4.27	5.81
Zinc		
WZn	82.4	82.7
FS	84.8	83.9
Zn	84.2	78.9
B + Zn	83.6	79.35
Cu + Zn	82.0	74.6
B + Cu + Zn	83.8	80.4
CV (%)	4.34	10.17

WB, WCu, and WZn: control treatments, without application of B, Cu, and Zn, respectively; FS: foliar spray with boric acid, copper sulphate, and zinc sulphate (0.4%); B: trunk injection of tablets containing B salts; Cu: trunk injection of tablets containing Cu salts; Zn: trunk injection of tablets containing Zn salts; B + Cu: trunk injection of tablets containing B and Cu salts; B + Zn: trunk injection of tablets containing B and Zn salts; Cu + Zn: trunk injection of tablets containing Cu and Zn salts; B + Cu + Zn: trunk injection of tablets containing B, Cu, and Zn salts; *mean values are statistically different from those of the control treatment (FS) at the 10% significance level, according to Dunnett's test; $^{+}$mean values are statistically different from those of the control treatment (WB-WCu-WZn) at the 10% significance level, according to Dunnett's test.

There was no effect of Zn on the cupping test, reaching scores of 83.46 and 79.97 in the two crop seasons, respectively, and the beans would be classified as "soft" (Table 4). In spite of the fact that the precision and validity of the cupping are much discussed because of its subjective nature and the limitation of tasters abilities, this result points out to the major importance of the postharvest procedures than the mineral nutrition of the plant on coffee quality.

3.4. Electrical Conductivity, Leached Potassium, and Coloration Index in the Crop Season 2010/2011. In the first crop season, there was no effect of B, Cu, and Zn supplied as tablets on CI, TTA, pH, EC, KL, TS, and RS of the beans

Table 5: Coloration index (CI, DO 425 nm), total titratable acidity (TTA, mL NaOH 100 g^{-1}), pH, electrical conductivity (EC, $\mu S \cdot cm^{-1} \cdot g^{-1}$), leached potassium (KL, $g \cdot kg^{-1}$), total sugars (TS, %), and reducing sugars (RS, %) in coffee beans of *Coffea arabica* treated with different forms of B, Cu, and Zn supply, in the crop season 2010/2011.

Crop season 2010/2011							
Boron							
Treatments	CI	TTA	pH	EC	KL	TS	RS
WB	0.81	11.2	5.61	41.39	1.29	11.73	0.216
FS	0.89	10.4	5.60	42.71	1.40	11.41	0.197
B	0.84	10.0	5.60	41.36	1.30	11.63	0.212
B + Cu	0.93	10.4	5.58	41.84	1.29	12.09	0.199
B + Zn	0.91	10.8	5.50	41.09	1.23	9.70	0.279
B + Cu + Zn	0.93	11.2	5.61	38.87	1.23	11.28	0.264
CV (%)	12.03	20.05	1.35	14.26	12.67	13.91	34.39
Copper							
Treatments	CI	TTA	pH	EC	KL	TS	RS
WCu	0.81	11.2	5.61	41.39	1.29	11.73	0.216
FS	0.89	10.4	5.61	42.71	1.40	11.41	0.197
Cu	0.87	10.4	5.60	41.98	1.36	11.48	0.221
Cu + B	0.93	10.4	5.58	41.84	1.29	12.09	0.199
Cu + Zn	0.88	11.6	5.58	38.14	1.18	12.20	0.211
B + Cu + Zn	0.93	11.2	5.61	38.87	1.23	11.28	0.264
CV (%)	10.76	24.48	0.75	13.8	13.67	14.16	20.22
Zinc							
Treatments	CI	TTA	pH	EC	KL	TS	RS
WZn	0.80	11.2	5.61	41.39	1.29	11.73	0.216
FS	0.89	10.4	5.60	42.71	1.40	11.41	0.197
Zn	0.90	11.2	5.62	41.09	1.32	12.13	0.209
Zn + B	0.91	10.8	5.50	41.09	1.23	9.70	0.279
Zn + Cu	0.88	11.6	5.58	38.14	1.18	12.20	0.211
B + Cu + Zn	0.93	11.2	5.61	38.87	1.23	11.28	0.264
CV (%)	11.06	17.27	1.45	16.7	14.13	17.24	35.64

WB, WCu, and WZn: control treatments, without application of B, Cu, and Zn, respectively; FS: foliar spray with boric acid, copper sulphate, and zinc sulphate (0.4%); B: trunk injection of tablets containing B salts; Cu: trunk injection of tablets containing Cu salts; Zn: trunk injection of tablets containing Zn salts; B + Cu: trunk injection of tablets containing B and Cu salts; B + Zn: trunk injection of tablets containing B and Zn salts; Cu + Zn: trunk injection of tablets containing Cu and Zn salts; B + Cu + Zn: trunk injection of tablets containing B, Cu, and Zn salts; *mean values are statistically different from those of the control treatment (FS) at the 10% significance level, according to Dunnett's test; +mean values are statistically different from those of the control treatment (WB-WCu-WZn) at the 10% significance level, according to Dunnett's test.

Table 6: Coloration index (CI, DO 425 nm), total titratable acidity (TTA, mL of NaOH 100 g^{-1}), pH, electrical conductivity (EC, $\mu S \cdot cm^{-1} \cdot g^{-1}$), and leached potassium (KL, $g \cdot kg^{-1}$) of coffee beans of *Coffea arabica* treated with different forms of B, Cu, and Zn supply, in the crop season 2011/2012.

Crop season 2011/2012					
Boron					
Treatments	CI	TTA	pH	EC	KL
WB	0.51	49.41	5.72	57.52	2.15
FS (control)	**0.76**	**48.42**	**5.63**$^{+}$	**50.18**	**2.01**
B	0.74	41.50	5.63^{+}	56.77	2.27
B + Cu	0.37*	40.52	5.68	60.98	2.05
B + Zn	0.66	45.46	5.69	58.83	1.86
B + Cu + Zn	0.41*	50.40	5.74*	59.27	2.02
CV (%)	31.89	31.12	0.86	13.47	14.28
Copper					
WCu	0.51	49.41	5.72	57.52	2.15
FS (control)	**0.76**$^{+}$	**48.42**	**5.63**	**50.18**	**2.01**
Cu	0.50*	59.29	5.64	56.92	1.93
Cu + B	0.37*	40.52	5.68	60.98	2.05
Cu + Zn	0.50*	48.42	5.70	62.73	2.12
B + Cu + Zn	0.41*	50.40	5.74	59.27	2.02
CV (%)	29.25	31.61	1.26	12.79	14.15
Zinc					
WZn	0.51	49.41	5.71	57.52	2.15
FS (control)	**0.76**	**48.42**	**5.63**	**50.18**	**2.01**
Zn	0.52	42.49	5.65	58.29	2.36
Zn + B	0.66	45.46	5.69	58.83	1.86
Zn + Cu	0.50	48.42	5.70	62.73	2.12
B + Cu + Zn	0.41	50.40	5.74	59.27	2.02
CV (%)	31.29	30.14	1.17	15.86	15.91

WB, WCu, and WZn: control treatments, without application of B, Cu, and Zn, respectively; FS; foliar spray with boric acid, copper sulphate, and zinc sulphate (0.4%); B: trunk injection of tablets containing B salts; Cu: trunk injection of tablets containing Cu salts; Zn: trunk injection of tablets containing Zn salts; B + Cu: trunk injection of tablets containing B and Cu salts; B + Zn: trunk injection of tablets containing B and Zn salts; Cu + Zn: trunk injection of tablets containing Cu and Zn salts; B + Cu + Zn: trunk injection of tablets containing B, Cu, and Zn salts; *mean values are statistically different from those of the control treatment (FS) at the 10% significance level, according to Dunnett's test; +mean values are statistically different from those of the control treatment (WB-WCu-WZn) at the 10% significance level, according to Dunnett's test.

compared to the treatments WB, WCu, and WZn or the sprayed treatments as controls (Table 5).

In the second crop season, there was no effect of B on the TTA, EC, and KL. Only the treatments with B + Cu + Zn and B + Cu differed from the sprayed treatment but did not differ from the treatment without B, indicating the effect of the way of B supply on this variable but not the effect of the nutrient (Table 6).

There was no effect of Cu on TTA, pH, EC, and KL. The treatments that received Cu, Cu + B, Cu + Zn, and B + Cu + Zn via solid injections differed from the sprayed treatment, and considering the treatment WCu as control, only the sprayed treatment was different, indicating the effect of Cu and the way of Cu supply on CI (Table 6).

Zinc did not influence the CI, TTA, pH, EC, and KL in this crop season (Table 6).

According to Carvalho et al. [2], the coloration index allows separation of different types of coffees, such as "rioysh" and "rio," that are not acceptable drink with coloration indexes lower than 0.650 DO 425 nm. For those classified as "hard" (acceptable), "soft," "softish" (fine), and "strictly soft" (extra fine), the coloration indexes would be equal or greater than 0.650 DO 425 nm.

Results obtained during the second crop season of evaluation suggest that the coloration index may not be a good indicator of quality as assessed by the cupping test. Although color indices were very similar, cup quality scores for beans from trees with spray treatments were 15.24% higher than those with trunk insertion of B tablets (Tables 4 and 6).

The coloration index of the sprayed treatment is in agreement with that reported by [2] in which the coffees of

best quality were darker and the dark coloration attributed to the formation of essential compounds to develop desirable flavors and aromas (Table 6).

On the other hand, the treatments containing B + Cu and B + Cu + Zn presented the coloration index quite low (0.368 and 0.411 DO 425 nm, resp.) and high cupping quality scores (83.25 and 80.4, resp.), being, in this case, classified as "softish," probably because of the positive effects of Cu and Zn, contradicting the findings of Carvalho et al. [2]. Corrêa et al. [58] also reported that higher CI could be attributed to the occurrence of biochemical alterations and oxidative reactions caused by the dry conditions or inadequate storage.

Martinez et al. [56] studying Zn effect on the production and cupping quality of coffee did not observe Zn effect on the coloration index, with the mean values being 0.95 DO 425 nm, which is in agreement with the results found by Lima et al. [59] for good coffees and, also, with the means found in this work.

Cellular membrane damage and the subsequent loss of permeability control were proposed by Heydecker Vigour [60] and Harrington [61] as the early step in the seed deterioration process. According to Amorim [16], since the leached potassium is proportional to the loss of bean quality, it can be observed that the loss of membrane permeability caused damage in coffee beans.

Malta et al. [62] studying some attributes related to the quality of different coffee varieties noted that Catuaí Vermelho presented an electrical conductivity of 104 $\mu S \cdot cm^{-1} \cdot g^{-1}$, considerably higher than that reported in the present experiment, even for the treatments with no micronutrient supply.

Lima et al. [63] determined the electrical conductivity of beans subjected to B doses and verified that both the absence and toxic concentrations of the nutrient are harmful to the quality of bean seeds, enhancing the electrical conductivity. Moreover, high physiological quality of bean seeds was obtained in a consortium with beans and castor beans, when supplied with adequate doses of B.

Evaluating the physiological quality of bean seeds over different doses of Mn and Zn, Teixeira et al. [64] did not observe the effect of Zn on the electrical conductivity; however, adequate doses of Mn improve seed quality resulting in low electrical conductivity (65.8 $\mu S cm^{-1} \cdot g^{-1}$). The seed quality was the lowest in the control treatment, without application of Zn and Mn. Amorim [16], Prete [35], and Lima et al. [59] observed inverse correlation between leached potassium, electrical conductivity, and cupping quality of coffee.

According to Amorim [65] and Chagas et al. [66], good coffees have high contents of sugars, around 8% according to Navellier [67] and around 5 to 10% according to Prete [35]. In general, the results of the first crop season were above the mean values reported by the literature.

It can also be noted that total acidity remained below 211.2 g NaOH 100 mL^{-1} of the sample, considered by Carvalho et al. [2] as a parameter for good coffees. With repect to the pH, it can also be observed that the mean of both years of assessment is quite close to that found by Barrios [68] which was between 5.73 and 5.88. According to Sivetz and Desrosier [69], roasted beans of palatable coffees, without bitter or acidity, must have pH between 4.95 and 5.2. The results of the present work are slightly above the range established by this author.

Neves et al. [57] studying the effect of different doses of Zn supplied to coffee plants by trunk injections of tablets containing Zn salts observed that the electrical conductivities of the control treatment, without Zn application, were 22.61 and 88.42 $\mu S \cdot cm^{-1} \cdot g^{-1}$ in two consecutive crop seasons, respectively. For the treatments with Zn inserted into the trunk, the means of the electrical conductivities were 16.84 and 66.16 $\mu S \cdot cm^{-1} \cdot g^{-1}$. The average values of leached potassium were 1.13 and 0.95 $g \cdot kg^{-1}$, in two consecutive crop seasons, for the treatments without Zn application and 0.83 and 0.65 $g \cdot kg^{-1}$ for the treatments with Zn application.

Table 7: Caffeine (Caf, %), trigonelline (Trig, %), sucrose (Suc, %), glucose (Glu, %), galactose (Gal, %), arabinose (Ara, %), and mannose (Man, %) of coffee beans harvested from plants submitted to the fertilization via solid salts injections in the trunk or foliar sprays with B, Cu, and Zn, in the crop season 2011/2012.

Treatments	Caf	Trig	Suc	Glu	Gal	Ara	Man
Boron							
WB	1.01*	0.83*	5.14*	0.18*	0.10	0.03	0.14
FS	**1.51**+	**0.96**+	**6.45**+	**0.26**+	**0.14**	**0.03**	**0.15**
B	1.51+	0.98+	6.61+	0.28+	0.11	0.03	0.15
B + Cu	1.46+	0.96+	6.45+	0.28+	0.11*	0.05*+	0.17*,+
B + Zn	1.50+	0.99+	6.81+	0.35*+	0.16*	0.04*,+	0.15
B + Cu + Zn	0.93*	0.85*	5.10*	0.24+	0.11	0.03	0.14
CV (%)	5.85	5.54	6.04	14.32	42.61	19.23	6.59
Copper							
WCu	1.01*	0.83*	5.14*	0.18*	0.10*	0.03	0.14*
FS	**1.51**+	**0.96**+	**6.45**+	**0.26**+	**0.14**+	**0.03**	**0.15**+
Cu	1.50+	0.99+	6.01+	0.29+	0.10*	0.02	0.16*,+
B + Cu	1.46+	0.96+	6.45+	0.28+	0.11*	0.05*+	0.17*,+
Cu + Zn	1.46+	0.97+	6.53+	0.30+	0.12	0.03	0.16+
B + Cu + Zn	0.93*	0.85*	5.10*	0.24	0.11*	0.03	0.14*
CV (%)	6.16	4.63	6.14	16.78	17.06	19.39	4.05
Zinc							
WZn	1.01*	0.83*	5.14*	0.18*	0.10	0.03	0.14*
FS	**1.51**+	**0.96**+	**6.45**+	**0.26**+	**0.14**	**0.03**	**0.15**+
Zn	1.55+	0.99+	6.62+	0.31+	0.10	0.04*,+	0.16+
B + Zn	1.50+	0.99+	6.81+	0.35*,+	0.16	0.04*,+	0.15
Cu + Zn	1.46+	0.97+	6.53+	0.30+	0.12	0.03	0.16+
B + Cu + Zn	0.93*	0.85*	5.10*	0.24+	0.11	0.03	0.14*
CV (%)	5.95	5.03	6.41	13.35	49.26	12.54	4.14

WB, WCu, and WZn: control treatments, without application of B, Cu, and Zn, respectively; FS: foliar spray with boric acid, copper sulphate, and zinc sulphate (0.4%); B: trunk injection of tablets containing B salts; Cu: trunk injection of tablets containing Cu salts; Zn: trunk injection of tablets containing Zn salts; B + Cu: trunk injection of tablets containing B and Cu salts; B + Zn: trunk injection of tablets containing B and Zn salts; Cu + Zn: trunk injection of tablets containing Cu and Zn salts; B + Cu + Zn: trunk injection of tablets containing B, Cu, and Zn salts; *mean values are statistically different from those of the control treatment (FS) at the 10% significance level, according to Dunnett's test; +mean values are statistically different from those of the control treatment (WB-WCu-WZn) at the 10% significance level, according to Dunnett's test.

The difference among the treatments was attributed to the Zn functions on the cell membrane integrity of coffee beans.

Corrêa et al. [57] have also not observed variations in acidity of beans harvested from plants that received Zn by injection of tablets in the trunk, with the average values being 156.3 mL of NaOH $100\,g^{-1}$, in coffees that were classified as "hard" in the cup test. In the same orchard, Martinez et al. [56] did not observe the effect of the nutrient on the total titratable acidity and pH, with the average values being 14.7 mL of NaOH $100\,g^{-1}$ and 5.4 mL of NaOH $100\,g^{-1}$, respectively.

3.5. Caffeine, Trigonelline, Glucose, Galactose, Arabinose, and Mannose. In this study, the effect of B, Cu, and Zn on the caffeine, trigonelline, sucrose, and glucose productions was evident by the significant differences between the sprayed treatment and the treatments that received the nutrients via solid injections compared to the control treatments WB, WCu, and WZn (Table 7).

It is possible to observe the effect of the different ways of B, Cu, and Zn supply on the contents of caffeine, trigonelline, and sucrose by the difference between the treatments with B + Cu + Zn and the sprayed treatment (Table 7). The results suggest that the lack of B, Cu, and Zn influenced the route of caffeine and trigonelline synthesis, but probably the excessive concentrations of B, like in the treatment containing B + Cu + Zn, also did.

There was no effect of B on galactose production. The arabinose of the treatments containing B + Cu and B + Zn presented means statistically higher than those presented by the control treatment (WB). Regarding mannose, only the treatment containing B + Cu was statistically different from the control treatment WB, suggesting the effect of B on its production (Table 7).

The effect of Cu on mannose levels in the coffee beans was evidenced by the significant difference among the beans produced from the plants of the sprayed treatment and those receiving tablets of Cu, Cu + B, and Cu + Zn inserted in the trunk compared to the treatment WCu. Regarding the levels of galactose, only the sprayed treatment differed from the treatment WCu, and arabinose was significantly greater only in the treatment containing Cu + B inserted in the trunk (Table 7).

With regard to the galactose, significant differences were not observed between the treatments with Zn applications. The arabinose content in beans of the treatments containing Zn and Zn + B was statistically greater than that of the treatment WZn. Mannose of the sprayed treatment and of those receiving Zn and Cu + Zn inserted in the trunk differed from that of the treatment WZn, showing the Zn effect on monosaccharides synthesis and the close relationship between its production and the content of Zn in index leaves (Table 7).

Mazzafera [70], working with nutritive solution and young coffee plants, did not found significant effects of B, Cu, and Zn deprivation on the caffeine production by coffee leaves. The author states that the effect of mineral nutrients on the activity of methyltransferases involved in caffeine synthesis is still unclear.

The contents of trigonelline observed in this work, in general, are in agreement with those established by the literature that vary from 0.6 to 1.2% for *Coffea arabica* [71], and the caffeine contents are close to those determined by Screenath [72] of about 1.2% for *Coffea arabica*.

Within the monosaccharides and oligosaccharides, sucrose is a nonreducing sugar in greater quantity in coffee beans, varying from 1.9 to 10% of the dry matter [73, 74]. According to Knopp et al. [75], sucrose represents more than 90% of the total low molecular weight carbohydrates and corresponds to 7.07% of the dry matter of coffee beans; this value is very close to that found in this experiment.

According to Camacho-Cristobal et al. [76], glucose 6-phosphate, an enzyme involved in the glycolysis route, in conditions of B sufficiency appears complexed with borate anion and thus restricts the flow of the respiratory substrate to the pentose phosphate pathway; therefore, when B is adequate, the sucrose production is greater.

Brown and Clark [77] reported that Cu-deficient wheat plants had significantly lower soluble carbohydrate contents than well-fertilized plants. The lower levels of plastocyanin, as a consequence of Cu deficiency, may decrease the efficiency of photosynthetic electron transport in photosystem I and thus impair the CO_2 fixation rate, in such a way that starch and soluble sugars content (especially sucrose) are reduced.

Coffee bean contents of specific sugars such as mannose, gallactose, glucose, and arabinose were slightly lower than those previously reported by Fischer et al. [78] for *Coffea arabica*.

3.6. Caffeoylquinic Acids (3-CQA, 4-CQA, and 5-CQA), PPO Activity, and Phenolic Compounds. Among the phenolic compounds, caffeoylquinic acids, dicaffeoylquinic acids, and feruloylquinic acids are the main chlorogenic acid subgroups present in coffee. In general, these compounds react during the roasting process producing free phenolic acids and therefore volatile phenolic compounds that contribute to the aroma of coffee beans [37].

The effects of B, Cu, and Zn on 3-CQA and 5-CQA contents were evidenced by the significant difference between the treatments that received the nutrients via solid injections or foliar sprays and the treatments WB, WCu, and WZn. Compared to the sprayed treatment, only the treatment with B + Cu + Zn differed significantly, for 5-CQA content, responding to the different ways of B, Cu, and Zn supply (Table 8).

The 4-CQA, proanthocyanidin, and total phenolic compounds were not affected by B treatments. The effect of Cu, also, was not significant for the contents of 4-CQA and proanthocyanidin. For Zn, 4-CQA and proanthocyanidin were not significant (Table 8).

The effect of B on PPO activity was evidenced by the significant difference of the treatments with B + Cu and the sprayed treatment compared to the treatment WB, even when B concentration in the index leaves of the treatment that received B by trunk injections was above the adequate range established by Martinez et al. [43].

Table 8: 3-Caffeoylquinic acid (3-CQA, %), 4-caffeoylquinic acid (4-CQA, %), 5-caffeoylquinic acid (5-CQA, %), proanthocyanidin (Pro, %), polyphenol oxidase activity (PPO, $U \cdot min^{-1} \cdot g^{-1}$), and phenolic compounds (TP, %) of coffee beans harvested from plants submitted to the fertilization via solid salts injections in the trunk or foliar sprays with B, Cu, and Zn, in the crop season 2011/2012.

Treatments	3-CQA	4-CQA	5-CQA	Pro	PPO	TP
Boron						
WB	0.65*	0.73	1.69*	6.36	74.52*	6.37
FS	0.44+	0.72	1.41+	5.70	85.93+	5.15
B	0.44+	0.58	1.35+	5.53	82.07	4.21
B + Cu	0.47+	0.66	1.40+	6.78	85.50+	4.61
B + Zn	0.45+	0.64	1.38+	7.14	78.38	5.50
B + Cu + Zn	0.52	0.61	1.58*	6.41	74.68*	6.13
CV (%)	19.89	22.27	6.36	38.15	9.01	24.59
Copper						
WCu	0.65*	0.73	1.69*	6.36	74.53*	6.38
FS	0.44+	0.72	1.41+	5.70	85.93+	5.15
Cu	0.47+	0.75	1.40+	6.23	85.15+	4.06+
B + Cu	0.47+	0.66	1.39+	6.78	85.51+	4.61
Cu + Zn	0.44+	0.62	1.30+	7.59	85.19+	4.87
B + Cu + Zn	0.52+	0.61	1.58*	6.41	74.68*	6.14
CV (%)	16.10	14.37	5.45	37.40	6.25	22.57
Zinc						
WZn						
T	0.65*	0.73	1.69*	6.36	74.52*	6.37*
FS	0.44+	0.72	1.41++	5.70	85.93+	5.15+
Zn	0.48+	0.74	1.41+	7.21	79.08	5.70
B + Zn	0.45+	0.64	1.38+	7.14	78.38	5.50
Cu + Zn	0.44+	0.61	1.30+	7.59	85.19+	4.87+
B + Cu + Zn	0.52+	0.60	1.58*	6.41	74.68*	6.13
CV (%)	16.25	18.28	6.08	31.93	7.11	14.01

WB, WCu, and WZn: control treatments, without application of B, Cu, and Zn, respectively; FS: foliar spray with boric acid, copper sulphate, and zinc sulphate (0.4%), B: trunk injection of tablets containing B salts, Cu: trunk injection of tablets containing Cu salts, Zn: trunk injection of tablets containing Zn salts, B + Cu: trunk injection of tablets containing B and Cu salts, B + Zn: trunk injection of tablets containing B and Zn salts, Cu + Zn: trunk injection of tablets containing Cu and Zn salts, B + Cu + Zn: trunk injection of tablets containing B, Cu, and Zn salts; *mean values are statistically different from those of the control treatment (FS) at the 10% significance level, according to Dunnett's test; +mean values are statistically different from those of the control treatment (WB-WCu-WZn) at the 10% significance level, according to Dunnett's test.

It is suggested that the content of B that is good for great growth and production is below the content that maximizes the PPO activity, a feature that has been directly related to cupping quality (Tables 2 and 8).

A significant difference was observed between the sprayed treatment and the treatments with Cu, Cu + B, and Cu + Zn compared to the treatment WCu for the PPO activity, with a particular focus on the clear inverse relationship between the PPO activity and the concentration of 5-CQA in the beans (Table 8).

Even though average leaf Cu concentrations of sprayed treatments were somewhat lower than those of trunk injection treatments, they were still within the sufficiency range reported in the literature of Martinez et al. [43]. It suggests that the composition of caffeoylquinic acids and PPO activity remained constant within the range of Cu concentration in the index leaves, which indicates adequate nutrition (Tables 2 and 8).

Regarding the Zn nutrition, the PPO activity, and contents of total phenolic compounds, only the treatment with Zn + Cu and the sprayed treatment differed from the treatment WZn, with the highest activity being observed when phenolic compounds concentrations were low (Table 8).

For the PPO activity, compared with the sprayed treatment, all treatments that received B, Cu, and Zn via solid injections, except B + Cu + Zn, are statistically similar, confirming the equal effect of different ways of supply of the studied micronutrients (Table 8).

Several works, in the literature, relate the accumulation of caffeoylquinic acids to B deficiency. Camacho-Cristobal et al. [76] reported that the main effect of B deficiency is the accumulation of glucose, fructose, and starch, followed by an increase in the 3-CQA, 4-CQA, and 5-CQA contents in tobacco leaves. Therefore, the high concentration of phenolic compounds, in B-deficient plants, could be a result of the soluble sugars accumulation [5].

Camacho-Cristobal et al. [79] attributed this effect to the enhancement of the phenylalanine ammonia lyase activity and consequent increase in the phenolic compounds synthesis. Additionally, according to this author, when in high concentration, B and glucose 6-phosphate form complexes and therefore restrict the flow of the respiratory substrate for the pentose phosphate pathway. Such a behavior may explain the enhanced concentrations of the 3-CQA and 5-CQA in the control treatment WB, WCu, and WZn.

In addition, phenolic compounds accumulation is a feature of B-deficient plants because of the formation of borate complexes with some phenols that can be involved in the regulation of free phenol concentration and in the alcohol phenol synthesis, which are direct precursors of the lignin [80].

A strong relationship between caffeoylquinic acid contents and cupping quality was not observed in the present study. Coffee beans from the control treatment (WB-WCu-WZn) had an average score of 82.7 and were hence classified as "soft."

3.7. PPO. Hajiboland and Farhanghi [81] studying the effect of adequate and low doses of B in turnip plants observed that PPO activity and phenolic compounds increased in roots and shoot when the B supply was low. The PPO activity in leaves and roots of deficient plants was 6.3 and 4.6 folds higher, respectively, than that of the control plants receiving sufficient B.

According to Karabal et al. [82], excess of B alters the cell membrane integrity; thus, a progressive increase in PPO activity is observed initially followed by falling, because of quinones production that inhibits the enzyme, which may explain the low PPO activity of the treatment containing B + Cu + Zn inserted into the trunk.

Carvalho et al. [2] proposed a way to assess the coffee quality using PPO activity levels. According to them, "rio" and "rioysh" types are well correlated to PPO activities below 55.99 $U \cdot min^{-1} \cdot g^{-1}$ of the sample; hard type is correlated to

activities between 55.99 and 62.99 $U \cdot min^{-1} \cdot g^{-1}$ of the sample; soft type is correlated to activities between 62.99 and 67.66 $U \cdot min^{-1} \cdot g^{-1}$ of the sample, and strictly soft type is correlated to activities above 67.99 $U \cdot min^{-1} \cdot g^{-1}$ of the sample.

In the present work, it is not possible to establish a good relationship between cupping quality and PPO activity, since the treatments WB, WCu, WZn, and with B + Cu + Zn had high cupping test scores (82.7 to controls and 80.4 to combination: "soft" type) followed by low PPO activity. It can be highlighted, however, that low PPO activity in these treatments was accompanied by high concentrations of 3-CQA and 5-CQA.

According to Mazzafera and Robinson [83], the 5-CQA is likely the main substrate of the PPO. Farah et al. [23] stated that there is an increase in the coloration intensity of coffee beans in response to the PPO action on 5-CQA; thus, the authors associated the oxidation products with the low quality of coffee beans rich in caffeoylquinic acid. Amorim et al. [84] reported that PPO action, along the structural changes of the membrane, is a possible cause for the formation of beans classified as "rioysh" type.

It is often mentioned by the literature that Cu is the PPO catalyst [85]; thus, the evaluation of PPO activity can be a good indicator of the nutritional status in Cu. Taking into account the importance of Cu on the PPO structure and because of the enzyme involved in the control of the concentration of free phenolic compounds, the efficiency in Cu supplementation to the plants is, therefore, a requirement of great importance in order to obtain coffees with higher quality.

In a nutritive solution experiment [86], it was observed that Zn doses affected the contents of chlorogenic acids of *Coffea arabica* beans. Total phenolic compounds, 5-CQA, and 4-CQA reached minimum points when the index leaves presented 10 $mg \cdot kg^{-1}$ of Zn, that is about in the center of the sufficiency range established by Martinez et al. [43]. The grains produced in conditions of deficiency or excess of Zn presented higher values of these compounds. The curves for PPO and 3-CQA presented exactly an inverse shape, reaching the maximum points in grains of plants with 10 $mg \cdot kg^{-1}$ of Zn in index leaves. Due to the direct relationship between 3-CQA and PPO, the author questioned if the content of 3-CQA, in a different way from that of 5-CQA, could be related to good quality of coffee beans.

Although, in this work, there was no good agreement between the cupping test and chemical attributes of coffee quality, it should be emphasized that the cupping test is subjective and new methods must be studied in order to evaluate properly the coffee bean quality.

4. Conclusions

Boron, copper, and zinc supplied by foliar sprays or solid trunk injections influence the chemical composition and quality of the coffee beans, characterized by the contents of caffeine, trigonelline, sucrose, glucose, arabinose, mannose, 3-caffeoylquinic acid, 5-caffeoylquinic acid, polyphenol oxidase activity, and total phenolic compounds.

Copper and Zn supplied by solid trunk injections give equivalent results to foliar sprays, both in production and quality.

Trunk injections of tablets containing B salts resulted in toxicity and affected negatively the production, while some attributes related to the quality of the grains were higher with high B supply, capable of limiting the growth and yield of coffee plants.

Disclosure

This paper is part of the Ph.D. thesis presented to the Universidade Federal de Viçosa, Viçosa, Brazil, by the first author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The authors would like to thank the financial support of the Brazilian government agencies Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

References

[1] E. Cantergiani, H. Brevard, Y. Krebs, A. Feria-Morales, R. Amadò, and C. Yeretzian, "Characterization of mouldy/earthy defect in green Mexican coffee," in *Colloquium of International Coffee Science Association*, Vol. 18, ASIC, Helsinki, Finland, 1999.

[2] V. D. Carvalho, S. M. Calfoun, S. J. R. Chagas, N. Botrel, and E. S. G. Juste Júnior, "Relações entre a composição físico-química e química do grão beneficiado e da qualidade de bebida do café. Pesquisa Agropecuária Brasileira," *Brasília*, vol. 29, no. 3, pp. 449–454, 1994.

[3] E. Malavolta, "Nutrição, adubação e calagem para o cafeeiro," in *Cultura do Cafeeiro: Fatores que Afetam a Produtividade*, A. B. Rena, E. Malavolta, M. Rocha, and T. Yamada, Eds., pp. 136–274, Potafos, Piracicaba, Brazil, 1986.

[4] R. M. Prado, *Nutrição de Plantas*, UNESP, Jaboticabal, Brazil, 2008.

[5] H. Marschner, *Mineral Nutrition of Higher Plants*, Academic Press, New York, NY, USA, 2011.

[6] J. R. Sanders, T. M. Adams, and B. T. Christensen, "Extractability and bioavailability of zinc, nickel, cadmium and copper in three Danish soils sampled 5 years after application of sewage sludge," *Journal of Science Food and Agriculture*, vol. 37, pp. 1155–1164, 1986.

[7] N. K. Fageria, V. C. Baligar, and R. B. Clark, *Micronutrients in Crop Production*, Vol. 77, Advances in Agronomy, New York, NY, USA, 2002.

[8] E. Epstein and A. J. Bloom, *Nutrição Mineral de Plantas: Princípios e Perspectivas*, Planta, Londrina, Brazil, 2nd edition, 2006.

[9] K. Mengel and E. A. Kirkby, *Principles of Plant Nutrition*, Internacional Potash Institute, Horgen, Switzerland, 4th edition, 1987.

[10] R. Hansch and R. R. Mendel, "Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl),"

Current Opinion in Plant Biology, vol. 12, no. 3, pp. 259–266, 2009.

[11] I. Cakmak, H. Marschner, and F. Bangerth, "Effect of zinc nutritional status on growth, protein metabolism and levels of indole-3 acetic acid and other phytohormones in bean (*Phaseolus vulgaris* L.)," *Journal Experimental Botany*, vol. 40, pp. 405–412, 1989.

[12] G. S. Valladares, G. C. G. Santos, C. A. Abreu, O. A. Camargo, and J. P. Ferrero, *Zinco Total e Disponível em Amostras de Perfis de Solos do Estado de São Paulo*, Bragantia, vol. 68, no. 4, pp. 1105–1114, Bragantia, Campinas, Brazil, 2009.

[13] R. H. Santori, A. E. Boaretto, F. C. A. Villanueva, and H. M. G. Fernandes, "Absorção radicular e foliar de ^{65}Zn e sua redistribuição em laranjeiras," *Revista Brasileira de Fruticultura*, vol. 30, no. 2, pp. 523–527, 2008.

[14] N. A. V. D. Pinto, S. M. R. Fernandes, R. G. F. A. Giranda, and V. D. Carvalho, "Avaliação de componentes químicos de padrões de bebida para preparo do café expresso. Ciência e Agrotecnologia," *Lavras*, vol. 26, no. 4, pp. 826–829, 2002.

[15] P. F. P. Goulart, J. D. Alves, E. M. Castro, D. D. Fries, M. M. Magalhães, and H. C. Melo, "Histological and morphological aspects of different grain coffee qualities," *Ciência Rural*, vol. 37, pp. 662–666, 2007.

[16] V. H. Amorim, *Aspectos Bioquímicos do Grão de Café Verde Relacionados Com a Deterioração da Qualidade*, Tese de Livre Docência, p. 85, ESALQ-USP, Piracicaba, Brazil, 1978.

[17] S. J. Chagas, V. D. Carvalho, and L. Costa, "Caracterização química e qualitativa de cafés de alguns municípios de três regiões produtoras de Minas Gerais," *Pesquisa Agropecuária Brasileira*, vol. 31, pp. 555–561, 1996.

[18] L. C. Trugo and R. A. Macrae, "Chlorogenic acid composition of instant coffee," *Analyst*, vol. 109, pp. 263–266, 1984.

[19] M. N. Clifford and K. C. Wilson, *Coffee: Botany, Biochemistry and Production of Beans Beverage*, Croom Helm, New York, NY, USA, 1985.

[20] C. P. Bicchi, O. M. Panero, G. M. Pellegrino, and A. C. Vanni, "Characterization of green and roasted coffees through the chlorogenic acid fraction by HPLC-UV and principal component analysis," *Journal of Agricultural and Food Chemistry*, vol. 43, no. 6, pp. 1549–1555, 1995.

[21] M. Nogueira and L. C. Trugo, "Distribuição de isômeros de ácido clorogênico e teores de cafeína e trigonelina em cafés solúveis brasileiros," *Ciência e Tecnologia de Alimentos*, vol. 23, no. 2, pp. 296–299, 2003.

[22] H. Morais, P. H. Caramori, M. S. Koguishi, J. C. Gomes, and A. M. Ribeiro, "Sombreamento de cafeeiros durante o desenvolvimento das gemas florais e seus efeitos sobre a frutificação e produção," *Ciência Rural*, vol. 39, pp. 400–406, 2009.

[23] A. Farah, M. C. Monteiro, V. Calado, A. S. Franca, and E. L. C. Trugo, "Correlation between cup quality and chemical attributes of Brazilian coffee," *Food Chemistry*, vol. 98, no. 2, pp. 373–380, 2006.

[24] M. C. Monteiro and L. C. Trugo, "Determinação de compostos bioativos em amostras comerciais de café torrado," *Química Nova*, vol. 28, pp. 637–641, 2005.

[25] E. B. Fagan, C. H. E. Souza, N. M. B. Pereira, and V. J. Machado, "Efeito do tempo de formação do grão de café (*Coffea* sp.) na qualidade da bebida," *Bioscience Journal*, vol. 27, pp. 729–738, 2011.

[26] P. T. G. Guimarães, A. W. R. Garcia, V. H. Alvarez et al., "Cafeeiro," in *Recomendações Para o Uso de Corretivos e Fertilizantes em Minas Gerais-5^a Aproximação*, A. C. Ribeiro, P. T. G. Guimarães, and V. H. Alvarez, Eds., pp. 289–302, Viçosa, MG, Brazil, 1999.

[27] E. Malavolta, G. C. Vitti, and S. A. Oliveira, *Avaliação do Estado Nutricional das Plantas: Princípios e Aplicações*, Potafos, Piracicaba, Brazil, 2nd edition, 1997.

[28] Association of Official Analytical Chemists, *Official Methods of Analysis*, AOAC, Washington, DC, USA, 12th edition, 1975.

[29] C. M. Johnson and A. Ulrich, *Analytical Methods for Use in Plants Analyses*, University of California, Los Angeles, CA, USA, 1959.

[30] N. S. Sakyiama, H. E. P. Martinez, M. A. Tomaz, and A. Borém, *Café Arábica: do Plantio à Colheita*, UFV, Viçosa, MG, Brazil, 2015.

[31] Association of Official Analytical Chemists, *Official Methods of Analyses of the Association of Official Analytical Chemists*, AOAC, Washington, DC, USA, 15th edition, 1975.

[32] N. Nelson, "A photometric adaptation of Somogy method for the determination of glucose," *Journal of Biological Chemists*, vol. 153, no. 1, pp. 370–380, 1944.

[33] V. L. Singleton, "The total phenolic content of grape berries during the maturation of several varieties," *American Journal of Enology and Viticulture*, vol. 17, no. 2, pp. 126–134, 1966.

[34] T. M. Loeffler, D. M. Tekrony, and D. B. Egli, "The bulk conductivity test as an indicator of soybean seed quality," *Journal of Seed Technology*, vol. 12, no. 1, pp. 37–53, 1988.

[35] C. E. C. Prete, "Condutividade elétrica do exsudado de grãos de café (*Coffea arabica* L.). Desenvolvimento da metodologia," *Ciências Agrárias*, vol. 21, no. 1, pp. 67–70, 1995.

[36] J. D. Ponting and M. A. Joslyn, "Ascorbic acid oxidation and browning in apple tissue extracts," *Archives of Biochemistry*, vol. 19, pp. 47–63, 1948.

[37] A. Farah, T. De Paulis, L. C. Trugo, and P. R. Martin, "Effect of roasting on the formation of chlorogenic acid lactones in coffee," *Journal of Agricultural and Food Chemistry*, vol. 53, no. 5, pp. 1505–1513, 2005.

[38] P. Mazzafera, A. Crozier, and G. Sandberg, "Studies on the metabolic control of caffeine turnover in developing endosperms and leaves of *Coffea arabica* and *Coffea dewevrei*," *Journal of Agricultural and Food Chemistry*, vol. 42, no. 7, pp. 1423–1428, 1994.

[39] M. D. Vitorino, A. S. França, L. S. Oliveira, and M. L. A. Borges, "Metodologias de obtenção de extrato de café visando a dosagem de compostos não voláteis," *Revista Brasileira de Armazenamento*, vol. 26, no. 3, pp. 17–24, 2001.

[40] A. E. Hagerman, Y. Zhao, S. E. Johnson, and F. Shahadi, "Methods for determination of condensed and hydrolyzable tannins," *ACS Symposium Series*, vol. 662, pp. 209–222, 1997.

[41] A. Sluiter, B. Hames, R. Ruiz et al., *Determination of Structural Carbohydrates and Lignin in Biomass. Laboratory Analytical Procedure*, General Books LLC, Memphis, Tennessee, 2008.

[42] *SAEG-System of Statistical Analysis and Genetics*, Versão 9.1: Fundação Arthur Bernardes, Viçosa, Brazil, 2007.

[43] H. E. P. Martinez, J. F. S. Menezes, R. B. Souza, V. H. Alvarez-Venegas, and P. T. G. Guimarães, "Faixas críticas de concentração de nutrientes e avaliação do estado nutricional de cafeeiros em quatro regiões de Minas Gerais," *Pesquisa Agropecuária Brasileira*, vol. 38, no. 6, pp. 703–713, 2003.

[44] P. H. Brown and B. J. Shelp, "Boron mobility in plants," *Plant soil*, vol. 193, pp. 85–101, 1997.

[45] P. H. Brown and H. Hu, "Phloem boron mobility in diverse plant species," *Botany Acta*, vol. 111, pp. 331–335, 1998.

[46] F. Santinato, F. L. Castanehira, R. M. Prado, and R. Santinato, "Efeito da aplicação de doses elevadas de ácido bórico em cafeeiro jovem, Catuaí Vermelho IAC 144," in *Congresso Brasileiro de Pesquisas Cafeeiras*, vol. 38, pp. 243-244, Procafé, Caxambu, RJ, Brazil, 2012.

[47] O. F. Lima Filho and E. Malavolta, "Calibração de boro e zinco para o cafeeiro (*Coffea arabica* L. cv. Catuaí Amarelo)," in *Reunião Brasileira de Fertilidade do Solo e Nutrição de Plantas*, vol. 20, pp. 54-55, Potafos, Piracicaba, Brazil, 1992.

[48] A. B. Rena and M. Maestri, "Fisiologia do cafeeiro," *Informe Agropecuário*, vol. 11, pp. 26–40, 1985.

[49] R. Santinato, R. P. Camargo, R. B. Batista, and E. M. Pereira, "Quantificação de boro orgânico (ager boro) via foliar para o cafeeiro, seu efeito sobre aplicação na florada e seu comportamento no solo," in Congresso Brasileiro De Pesquisas Cafeeiras, vol. 20, Águas de Lindóia, Brazil, 1994.

[50] U. V. Barros, R. Santinato, and J. B. Matiello, "Fontes, doses e método de aplicação de B no plantio de café na região da Zona da Mata em Minas Gerais," in *Congresso Brasileiro De Pesquisas Cafeeiras*, vol. 22, pp. 117-118, Instituto Brasileiro do Café, Águas de Lindóia, Brazil, 1996.

[51] V. M. M. Andrade, *Influência do Cobre no Crescimento, Morfologia e Anatomia da Folha e na Composição Mineral do Cafeeiro*, Tese (Doutorado), Universidade Estadual de São Paulo, Jaboticabal, Brazil, 1973.

[52] J. R. Loneragan, "The availability and absorption of trace elements in soil-plants systems and their relation to movement and concentrations of trace elements in plant," in *Trace Elements in Soil-Plant-Animal Systems*, D. J. D. Nicholas and A. R. Egan, Eds., pp. 109–134, Academic Press, Cambridge, London, 1975.

[53] W. Reuther and C. K. Labanauskas, "Copper," in *Diagnosis criteria for plants and soils*, H. C. Chapman, Ed., pp. 157–179, University of California, CA, USA, 1966.

[54] P. T. G. Guimarães, J. C. Carvalho, C. C. A. Melles, and E. Malavolta, "Efeitos da aplicação foliar de sulfato de Zn na produção e na composição mineral das folhas de cafeeiro (*Coffea arabica* L.)," *Anais da ESALQ*, vol. 40, pp. 497–507, 1983.

[55] O. F. Lima Filho and E. Malavolta, "Evaluation of extraction procedures on determination of critical soil and foliar levels of boron and zinc in coffee plants," *Commununications in Soil Science and Plant Analysis*, vol. 29, pp. 825–833, 1998.

[56] H. E. P. Martinez, Y. Poltronieri, A. Farah, and D. Perrone, "Zinc supplementation, production and quality of coffee beans," *Revista Ceres*, vol. 60, no. 2, pp. 293–299, 2013.

[57] Y. P. Neves, H. E. P. Martinez, and P. R. Cecon, "Effect of zinc and its form of supply on production and quality of coffee beans," *Journal of Science Food and Agricultural*, vol. 91, pp. 2431–2436, 2011.

[58] P. C. Corrêa, P. C. Afonso Junior, F. A. C. Pinto, and T. T. Oliveira, "Efeito da temperatura de secagem na cor dos grãos de café pré-processados por via seca e via úmida," *Revista Brasileira de Armazenamento*, vol. 5, pp. 22–27, 2002.

[59] M. V. Lima, H. D. Vieira, M. L. L. Martins, and S. M. F. Pereira, "Preparo do café despolpado, cereja descascado e natural na região sudoeste da Bahia," *Revista Ceres*, vol. 55, pp. 124–130, 2008.

[60] W. Heydecker Vigour, in *Viability on Seeds*, E. D. Roberts, Ed., pp. 209–252, University Press, Syracuse, NY, USA, 1972.

[61] J. F. Harrington, "Problems of seed storage,"in *Seed Ecology*, W. Heydecker, Ed., pp. 251–263, The Pennsylvania State University Press, University Park, PA, USA, 1973.

[62] M. R. Malta, M. L. Santos, and F. A. M. Silva, "Qualidade de grãos de diferentes cultivares de cafeeiro (*Coffea arabica* L.)," *Acta Scientiarum*, vol. 24, no. 5, pp. 1385–1390, 2002.

[63] A. D. Lima, T. V. A. Viana, B. M. Azevedo, A. B. Marinho, and J. M. L. Duarte, "Adubação borácica na cultura do girassol," *Revista Agro@mbiente*, vol. 7, no. 3, pp. 269–276, 2013.

[64] I. R. Teixeira, A. Borém, G. A. A. Araújo, and M. J. B. Andrade, *Teores de Nutrientes e Qualidade Fisiológica de Sementes de Feijão em Resposta à Adubação Foliar com Manganês e Zinco*, vol. 64, no. 1, pp. 83–88, Bragantia, Campinas, Brazil, 2005.

[65] H. V. Amorim, M. G. Legendre, V. L. Amorim, A. J. S. Angelo, and R. L. Ory, "Chemistry of Brazilian green coffee and the quality of the beverage. VII. Total carbanyls, activity of polyphenol oxidase, and hydroperoxides," *Turrialba*, vol. 26, no. 2, pp. 193–195, 1976.

[66] S. J. R. Chagas, V. D. Carvalho, L. Costa, and M. M. Romaniello, "Caracterização química e qualitativa de cafés de alguns municípios de três regiões produtoras de Minas Gerais. II-valores de acidez titulável total e teores de açúcares (redutores, não redutores e totais)," *Ciência e Agrotecnologia*, vol. 20, no. 2, pp. 224–231, 1996.

[67] P. Navellier, "Coffee," in *Encyclopedia of Industrial Chemical Analysis*, vol. 19, pp. 73–447, John Wiley & Sons, New York, NY, USA, 1970.

[68] B. B. E. Barrios, *Caracterização Física, Química, Microbiológica e Sensorial de Cafés (Coffea arábica L.) da Região Alto Rio Grande-Sul de Minas Gerais*, Dissertações (Mestrado em Ciência dos Alimentos), p. 72, Universidade Federal de Lavras, Lavras, 2001.

[69] M. Sivetz and N. W. Desrosier, *Coffee Technology*, Avi, Westport, CT, USA, 1979.

[70] P. Mazzafera, "Mineral nutrition and caffeine content in coffee leaves," *Bragantia*, vol. 58, no. 2, pp. 387–391, 1999.

[71] A. Illy and R. Viani, *Espresso Coffee: The Chemistry of Quality*, Academic Press, San Diego, CA, USA, 1995.

[72] H. L. Screenath, *Development of Caffeine in Free Coffee Varieties*, vol. 61, no. 10, Indian Coffee, Bangalore, India, 1997.

[73] M. L. Wolfrom, R. A. Plunkert, and M. L. Laver, "Carbohydrates of the coffee bean," *Journal of Agricultural and Food Chemistry*, vol. 8, no. 1, pp. 58–65, 1960.

[74] J. R. Feldman, W. S. Ryder, and J. T. Kung, "Importance of non-volatile compounds to the flavor of coffee," *Journal of Agriculture and Food Chemistry*, vol. 17, no. 4, pp. 733–739, 1969.

[75] S. Knopp, G. Bytof, and D. Selmar, "Influence of processing on the content of sugars in green Arabica coffee beans," *European Food Research and Technology*, vol. 223, no. 2, pp. 195–201, 2006.

[76] J. J. Camacho-Cristobal, L. Lunar, F. Lafont, A. Baumert, and A. Gonzales-Fontes, "Boron deficiency causes accumulation of chlorogenic acid and caffeoyl polyamine conjugates in tobacco leaves," *Journal of Plant Physiology*, vol. 161, no. 7, pp. 879–881, 2004.

[77] J. C. Brown and R. B. Clark, "Copper as essential to wheat reproduction," *Plant Soil*, vol. 48, no. 2, pp. 509–523, 1977.

[78] M. Fischer, S. Reimann, V. Trovato, and R. J. Redgwell, "Polysaccharides of green Arabica and Robusta coffee beans," *Carbohydrate Research*, vol. 330, no. 1, pp. 93–101, 2001.

[79] J. J. Camacho-Cristobal, D. Anzellotti, and A. Gonzalez-Fontes, "Changes in phenolic metabolism of tobacco plants during short-term boron deficiency," *Plant Physiology Biochemistry*, vol. 40, no. 12, pp. 997–1002, 2002.

[80] D. J. Pilbeam and E. A. Kirkby, "The physiological role of boron in plants," *Journal of Plant Nutrition*, vol. 6, no. 7, pp. 363–382, 1983.

[81] R. Hajiboland and F. Farhanghi, "Remobilization of boron, photosynthesis, phenolic metabolism and anti-oxidant defense capacity in boron-deficient turnip (*Brassica rapa* L.) plants," *Soil Science Plant Nutrition*, vol. 56, no. 3, pp. 427–437, 2010.

[82] E. Karabal, M. Yücel, and H. A. Öktem, "Antioxidant responses of tolerant and sensitive barley cultivars to boron toxicity," *Plant Science*, vol. 164, no. 6, pp. 925–933, 2003.

[83] P. Mazzafera and S. P. Robinson, "Characterization of polyphenol oxidase in coffee," *Phytochemistry*, vol. 55, pp. 285–296, 2000.

[84] H. V. Amorim, A. R. Cruz, S. T. Angelo, A. J. Dias, R. M. Mello, and A. A. Teixeira, "Biochemical, physical and organoleptical changes during raw coffee quality deterioration," in *Proceedings of 8th International Colloq Coffee*, Abidjan, Côte d'Ivoire, December 1977.

[85] E. Malavolta, *Elementos de Nutrição Mineral de Plantas*, Agronômica Ceres, São Paulo, Brazil, 1980.

[86] J. S. Lacerda, *Produção, Composição Química e Qualidade da Bebida de Café Arábica em Razão da Dose de Cobre e Zinco*, Tese de Doutorado, Universidade Federal de Viçosa, Viçosa, Brazil, 2014.

[87] J. M. Clemente, *Boron, Copper and Zinc Effects on Photosynthesis, Enzymatic Activity, Nutritional Status, Production, Chemical Composition and Cup Quality of Coffee*, Tese (Doutorado em Fitotecnia), p. 115, Universidade Federal de Viçosa, Viçosa, MG, Brazil, 2014.

Hindawi
Journal of Food Quality
Volume 2018, Article ID 5908463, 7 pages
https://doi.org/10.1155/2018/5908463

Research Article

Quality of Commercial Coffees: Heavy Metal and Ash Contents

Mariana Teixeira Pigozzi, Flávia Regina Passos, and Fabrícia Queiroz Mendes

Institute of Agricultural Sciences, Federal University of Viçosa, Rio Paranaíba Campus, Road MG 230, Km 07, P.O. Box 22, 38810-000 Rio Paranaíba, MG, Brazil

Correspondence should be addressed to Flávia Regina Passos; flaviapassos1@yahoo.com.br

Received 29 September 2017; Accepted 28 March 2018; Published 6 May 2018

Academic Editor: Gabriel Henrique Horta de Oliveira

This study aimed to quantify the ash content and to determine the concentration of heavy metals in roasted ground coffee and their respective infusions. The ash content was determined by incineration of the samples, and the quantification of heavy metals was performed by flame atomic absorption spectrophotometry for the following metals: cadmium, lead, copper, chromium, nickel, and zinc. According to the ash analysis, 15% of the roasted ground coffee samples were within the standards established by the legislation of the state of São Paulo, which has set an ash content of below 5%. In the roasted ground coffee samples, the Cd, Cu, Ni, and Zn contents did not exceed the limit established by Brazilian legislation. In several samples, both Pb and Cr were found in high levels, exceeding the limits established by Brazilian legislation. In the infusions of roasted ground coffee, the Cd, Cu, Cr, and Ni contents were below the detection limit of the equipment. Zn was found in all infusions and Pb was only detected in seven coffee infusion samples. Overall, the concentrations of heavy metals found in the commercially roasted ground coffee and their respective infusions are lower than the limits recommended by the official inspection agencies and, thus, are suitable for consumption.

1. Introduction

Coffee beans are one of the most widely traded commodities in the world. There are more than 100 different plant species of the *Coffea* genus in the world. Despite this great diversity, only two species have significant economic importance in the world coffee market: *Coffea arabica* L. and *Coffea canephora* Pierre ex A. Froeher, known, respectively, as arabica coffee and robusta coffee or conilon [1].

In the international market arabica coffee represents more than 70% of world production. They are finer coffees recognized for the best aroma and flavor, being more valued than conilon coffee. In addition, it is more commonly used in blends of arabica coffee, which gives more body and reduces the acidity of the beverage [2].

Roasted and ground coffee is classified as Traditional or Extraforte (arabica blended with conilon, without quantity restriction) and Gourmet (arabica only), according to the characteristics of the products identified through the coffee bean species, roasting, grinding, aroma, and flavor [3]. Coffee consumers are becoming increasingly demanding. During the purchasing process, quality factors and purity stamps are frequently sought characteristics. These factors outweigh the price factor, which demonstrates consumer demand for the best coffee [4], free of impurities and adulterants, and is a genuine product.

Normative Instruction No. 16 of the Ministry of Agriculture and Livestock (MAPA), which guarantees the quality of roasted ground coffee, establishes an acceptable level of up to 1% of impurities, sediments, and foreign matter in coffee [5]. However, there are other compounds that, when present in the grains, could cause serious damage to the health of consumers [6]; this is the case for metal contaminants.

Heavy metals are the most frequently evaluated elements in food, because of their ability to accumulate in the food chain [6–11]. Thus, their maximum levels have become quality standards around the world [10]. These elements are stable and, thus, persistent in the environment, accumulating in the soil [8] as a result of factors associated with the weathering of rocks and soil formation, the soil soluble content, pH, plant species, environmental conditions, technological practices, and use of chemical products [6, 7, 12]. Among the chemical products that contribute to increased heavy metal content in the soil are biosolids, which often contain high levels of cadmium, copper, chromium, lead, nickel, and zinc [13]. Additional sources of contaminants include the use

Table 1: Detection limits (DL) and limits quantification (QL) determined for elements quantified by atomic absorption.

	Cu ($mg\,kg^{-1}$)	Cr ($mg\,kg^{-1}$)	Ni ($mg\,kg^{-1}$)	Cd ($mg\,kg^{-1}$)	Pb ($mg\,kg^{-1}$)	Zn ($mg\,kg^{-1}$)
DL	0.0474	0.3105	0.0414	0.1569	0.1104	0.7262
QL	0.1438	0.9408	0.1559	0.4775	0.3347	2.2007

of phosphate fertilizers produced from sedimentary rocks [14] and the application of micronutrients from industrial byproducts [7].

First, these materials are absorbed by plants and accumulate in food, thus becoming sources of contamination for humans [7]. Magna et al. [9] detected the presence of Cd and Pb in many types of food and grasses cultivated in the municipality of Santo Amaro, Bahia. Schmidt et al. [15] observed the presence of many metals in coffee grains cultivated in the state of Paraná, some at concentrations much higher than the values established as safe for daily intake.

Therefore, this study aims to determine, by means of incineration, the ash content and the flame atomic absorption spectrophotometry, the heavy metal contents of commercial samples of roasted ground coffee, and their respective infusions.

2. Material and Methods

The coffee samples were purchased in the local markets of the cities of Rio Paranaíba and Carmo do Paranaíba in the state of Minas Gerais. Fifteen samples of commercially roasted ground coffee were analyzed, three of which were considered "Gourmet" (samples I, N, and O). Two samples of each coffee were purchased to perform the analyses. Two replicates of each sample were taken to the analysis.

For the determination of the ash content in the roasted ground samples, approximately 2 g of sample was weighed in a porcelain capsule and placed in a muffle furnace for incineration at 550°C until the organic matter had been eliminated. At the end of the incineration process, the samples were weighed. The results are expressed in percentages [16].

The coffee infusion consisted of the beverage prepared with 12 g of roasted ground coffee lixiviated by 100 mL of water heated to boiling point (95–100°C), followed by filtration [16]. Then, 25 mL of the beverage was concentrated in an oven at 60°C with air circulation until reaching a final volume of approximately 2.5 mL.

The samples of roasted ground coffee and the infusions were mineralized by the wet route, using 18 mL of the nitric–perchloric digestive mixture in a 3 : 1 ratio (nitric acid : perchloric acid) and heating at 190°C. The solution was maintained under these conditions until a translucent solution had formed [6]. The samples were transferred to 25 mL volumetric flasks, and the volume was made up with water. Then, the contents of the following elements were quantified: cadmium, copper, chromium, lead, nickel, and zinc. The measurements were made in a fast, sequential atomic absorption spectrophotometer (Varian, A240FS, Mulgrave, VIC, Australia) with atomization in an air/acetylene flame at the rate of $13.3\,L\,min^{-1}/2.9\,L\,min^{-1}$ for Cr and $13.5\,L\,min^{-1}/2.0\,L\,min^{-1}$ for the other elements. As a radiation source, a single element hollow cathode lamp (HCL) was used. The intensities of the electric currents were 7 mA (Cr), 5 mA (Mn, Pb, and Zn), and 4 mA (Cd, Cu, and Ni). The absorbances were measured at the following wavelengths (nm): Cd, 228.8; Cu, 324.7; Cr, 357.9; Pb, 217.0; Ni, 232.0; and Zn, 213.9. The procedure used and the appropriate concentrations for the standard curve were performed according to the protocols established in the equipment manual.

Deionized water was used for all analyses. The concentrations were determined by elaborating the calibration analytical curves of each element quantified. The solutions for the construction of the standard curves were obtained through stock solutions of 1000 mg/L for atomic absorption and ICP. The reagent blanks and the samples with the standards of the elements were included in the analysis.

The detection limit (DL) and the quantification limit (QL) were calculated based on the standard deviation of the response and the slope of the calibration curve (mean of 3 curves). They were obtained from the following equations [17]:

$$\mathrm{DL} = 3.3\frac{\mathrm{SD}}{S},$$
$$\mathrm{QL} = 10\frac{\mathrm{SD}}{S}, \tag{1}$$

where

SD is standard deviation of the response or instrumental target (3 curves);

S is slope or angular coefficient of the analytical curve.

The limits of detection and quantification determined experimentally for each of the evaluated elements are described in Table 1.

For optical imaging, the roasted ground coffee samples were placed on slides and humidified. Then, they were analyzed using an optical microscope and digitalized (5-megapixel resolution) using a digital camera (Samsung, WB150F, Manaus, AM, Brazil), CoolSnap Pro image system, and Image-Pro Plus software (Media Cybernetics).

The obtained data were analyzed by descriptive statistics. The heavy metal data in the roasted ground coffee samples were submitted to principal component analysis. Then, the samples were grouped by Euclidean distance, using the values of the first and second main components.

The Pearson correlation analysis was performed between the concentrations of Cd, Cu, Cr, Pb, Ni, and Zn in the samples of roasted ground coffee and their respective infusions.

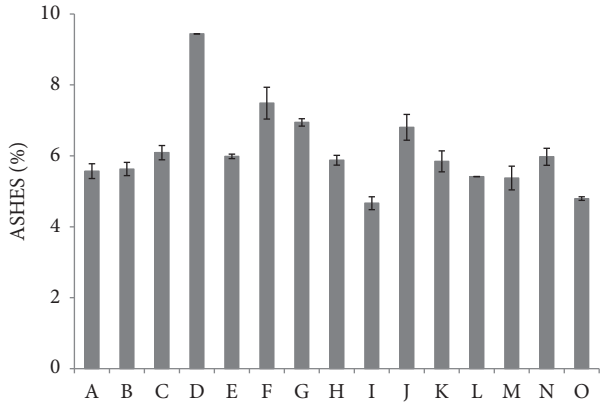


Figure 1: Ash content of the roasted ground coffee samples.

3. Results and Discussion

Figure 1 shows the mean ash content observed in the different roasted ground coffee samples. The ash content ranged from 4.48 to 9.44 g $(100\,g)^{-1}$, where the lowest mean value was obtained in one of the "Gourmet" coffee samples (sample I).

Teixeira et al. [16] analyzed different commercial coffee brands and found ash contents ranging from 3.99 to 6.10 g $(100\,g)^{-1}$, where the lowest value was also found in a "Gourmet" coffee sample. Gourmet coffee is composed only of arabica coffee, which commonly has a lower ash content. The other coffees are composed of blends and the addition of conilon coffee contributes to the increase of ash content [20]. Normative Instruction No. 16 of MAPA [5] does not specify the maximum ash content in roasted ground coffee. However, Resolutions Nos. 19 and 31 of the São Paulo State Board of Agriculture and Supply (Secretaria de Agricultura e Abastecimento do Estado de São Paulo, SAASP) define as standard the maximum fixed content of mineral residue to be 5.00% for both "Traditional" and "Gourmet" roasted ground coffee [21, 22]. Therefore, considering the São Paulo state resolutions, two samples (O and I) meet these standards.

According to a study performed by Durek de Conti et al. [20], this variation could be explained by the difference in the varieties and coffee blends, where the mean ash content in arabica ranged from 2.5 to 4.5%, whereas that of robusta coffee was 4.64%. It is also possible to associate the mineral composition of the coffee with the nutritional state of the coffee plantation and its location [23]. According to Müller et al. [24], when the ash content exceeds 5.00%, there may be a significant quantity of impurities in the sample.

The heavy metal contents found in the roasted ground coffee samples are presented in Figure 2.

The Cu contents ranged from 1.55 to 29.85 mg kg^{-1}, having a mean content of 16.49 mg kg^{-1}. For all roasted ground coffee samples analyzed, the Cu concentrations were below the maximum limit (30 mg kg^{-1}) defined by Brazilian legislation [18]. Morgano et al. [25] obtained mean Cu values higher than the results of the present study (29.86 mg kg^{-1}), considering all the samples analyzed by the authors. However, when analyzing only the samples from the Alto Paranaíba region, MG, the value found (14.17 mg kg^{-1}) is similar to that of the present study. dos Santos et al. [26] found mean Cu contents from 7.15 to 14.9 mg kg^{-1} in coffees produced in two properties in the state of Bahia. These results indicate that the mineral content is associated with the origin of the coffee, species, pesticides, and agricultural treatment, among other factors.

The roasted ground coffee samples had low Ni concentrations. We could only quantify the Ni content in six samples, and these had Ni contents ranging from 0.037 to 0.72 mg kg^{-1}, having a mean of 0.11 mg kg^{-1}. These values do not exceed the maximum limit allowed by Brazilian legislation, which establishes a maximum Ni content of 5 mg kg^{-1} [18]. In the other samples, the Ni content was lower than 0.025 mg kg^{-1}. Morgano et al. [25] determined the mean Ni content of 4.76 mg kg^{-1}, considering all raw coffee samples analyzed, and a mean value of 1.21 mg kg^{-1}, considering only the samples from the Alto Paranaíba region, MG.

The Zn contents varied between 3.31 and 25.97 mg kg^{-1}, having a mean content of 8.07 mg kg^{-1}. The determined values do not exceed the maximum Zn content allowed by Brazilian legislation of 50 mg kg^{-1} [18]. The mean Zn content found in the samples is similar to the values found by Morgano et al. [25], who determined a mean Zn content of 8.33 mg kg^{-1} in raw coffee. Mean contents higher than the results of the present study were found by dos Santos et al. [26], who obtained values from 25 to 45 mg kg^{-1} of Zn when evaluating two coffee plantation properties in the state of Bahia. In addition, Ashu and Chandravanshi [12] found a mean value of 19 mg kg^{-1} of Zn in commercial roasted ground coffee samples.

Cu, Ni, and Zn are essential elements for the development of coffee plants and are applied via the soil or the leaves; therefore, they could also be present in the grains. Copper is an active ingredient of some of the pesticides used in coffee cultures, in the form of copper hydroxide, copper oxychloride, copper sulfate, or copper ethylenediaminetetraacetate (EDTA), which allows the absorption of this element by the plant, explaining its presence in the grain [11]. Furthermore, these elements could also be present in the soil, varying depending on their origin [6].

Cr was only detected in one roasted ground coffee sample, where the content was 3.27 mg kg^{-1}, a value much higher than the maximum limit allowed by Brazilian legislation, which establishes a maximum content of 0.1 mg kg^{-1} [18]. In the other samples, Cr was not detected, and all these contents were below 0.025 mg kg^{-1}. dos Santos et al. [26] did not find Cr in coffee samples from the state of Bahia.

Among the evaluated elements, Cd and Pb are considered the most toxic inorganic contaminants [8]. According to Resolution RDC No. 42 of 2013, the maximum limit of Cd in roasted ground coffee is 0.1 mg kg^{-1} and that for Pb is 0.5 mg kg^{-1} [19].

Cd was not found in the analyzed samples. Hence, the Cd content for all samples was below 0.025 mg kg^{-1}. dos Santos et al. [26] found mean Cd contents between 0.70 and 0.75 mg kg^{-1} in coffee of two different properties in the state of Bahia.

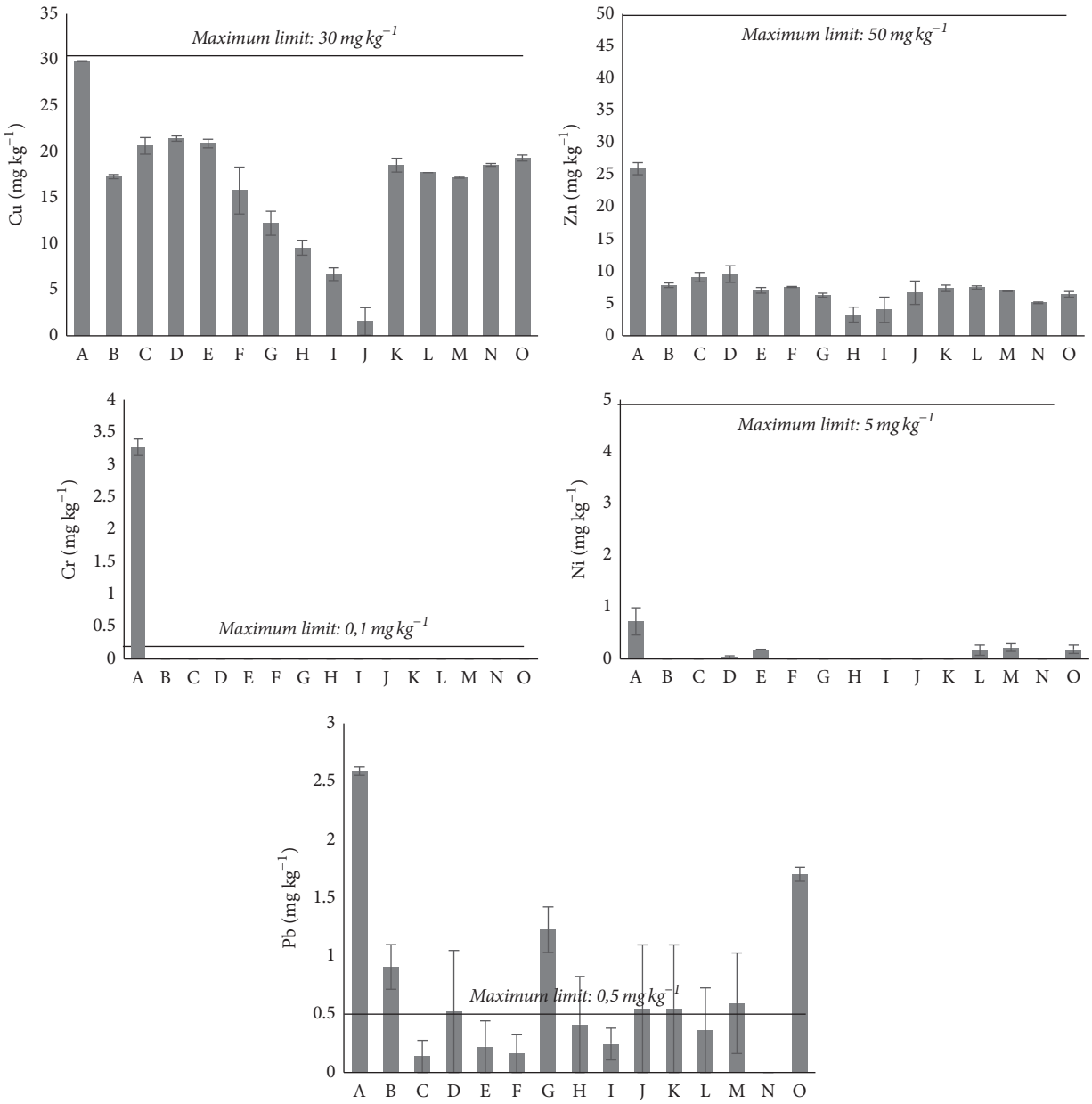


Figure 2: Mean content of heavy metals in roasted ground coffee samples and maximum contents establish by Brazilian legislation [18, 19].

Pb was undetectable only in one sample. The Pb contents ranged from 0.14 to 2.59 mg kg^{-1}, having a mean of 0.69 mg kg^{-1}. Among the analyzed samples, eight had Pb contents above the maximum limit allowed by Brazilian legislation (0.5 mg kg^{-1}) [19]. Most of the samples in the present study originate from local production (Alto Paranaíba region, MG), which indicates that a large part of the coffee produced in this region contains Pb at high levels, indicating that, most likely, this element is found in high concentration in the soils of the Alto Paranaíba region, MG [6]. Pb can act on the neurological system, causing damage to the central and peripheral nervous systems, interfering in the conversion of vitamin D and the homeostasis of calcium, and inhibiting hemoglobin synthesis, in addition to being a potential carcinogen [27].

In the analysis of main components (Figure 3), the first main component explains 76.61% of the variation of the contents of heavy metals of the analyzed samples, while the second main component explains 12.37%.

The distance between the samples suggests the existence of a difference in the heavy metal content of the samples. By calculating the Euclidean distance, it is concluded that the samples differ in three groups.

Sample A showed a positive correlation with the first main component and a great distance from the other samples. It was the sample that presented a greater content of heavy woods. During the acid digestion, precipitate was observed

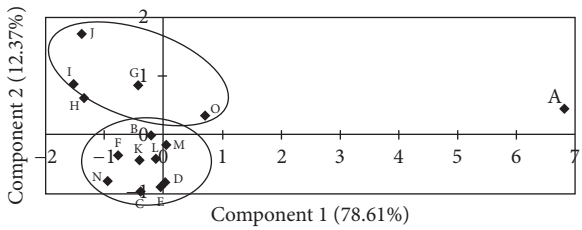


Figure 3: Analysis of major components of heavy metal content in the roasted ground coffee samples. Distinguished groups across Euclidean distance.

in sample A. The presence of precipitate in acidic solution and the high content of heavy woods compared to the other samples suggests the presence of inorganic contamination by material that contains silica, which is an insoluble mineral in acid.

The other samples were divided into two other groups, and the samples of gourmet coffee analyzed (I, N, and O) are distributed in both groups, showing that the fact that they are better classified sensorially is not related to the content of heavy metals. The variations in the contents of heavy metals in the roasted ground coffee can be explained by the chemical composition of the soil and the management and cultivation of the plants and coffee [15]. Arabica and conilon coffee have many distinct characteristics, namely, of botanical, agronomic, and morphological nature. These species can be blended into the commercial coffee industry, which comes from a wide variety of geographical areas. These characteristics may also have contributed to variations in the heavy metal content [28].

In the coffee infusions (50 mL), Cd, Cu, Cr, and Ni were not detected. Zn was detected in all samples, having a maximum content of 15.472 μg 50 mL^{-1} and a minimum of 4.998 μg 50 mL^{-1}, having a mean value of 9.63 μg 50 mL^{-1} (Table 1). Noël et al. [29] found the mean value for Zn for a coffee beverage of 14.25 μg 50 mL^{-1} in the coffee infusions, a value higher than the mean and similar to the maximum value found in the present study. Pb, which was present in almost all roasted ground coffee samples (Figure 2), was only detected in seven coffee infusion samples, having contents ranging from 0.129 to 2.835 μg (50 mL)$^{-1}$ (Table 2).

The maximum acceptable intake of Pb is 25 μg kg^{-1} body weight per week [9], which represents an intake of 250 μg per day based on an adult male weighing 70 kg. Hence, the maximum daily intake of four cups of coffee represents 0.21% to 4.54% of the maximum Pb intake.

The infusion process used to prepare the coffee has a strong influence on the concentration of metals. The lower metal concentration in the infusions is most likely due to the release of all elements in the form of their simple ions with the coffee matrix [30].

The Pearson correlation coefficients were estimated for the Pb and Zn concentrations in the samples of roasted ground coffee and their respective infusions. For Pb, the Pearson correlation coefficient was 0.1204, and for Zn it was 0.0649, indicating that there is no correlation between the content of metals in the roasted ground coffee and their respective infusions. In addition to the differences in the blends and edaphoclimatic differences in the production of the grains, because these are commercial samples, the grain size of the coffee powder could influence the extraction ratio for these metals, making their extraction easier or more difficult (Figure 4).

Table 2: Mean contents (μg 50 mL^{-1}) of heavy metals present in a cup (50 mL) of coffee infusion.

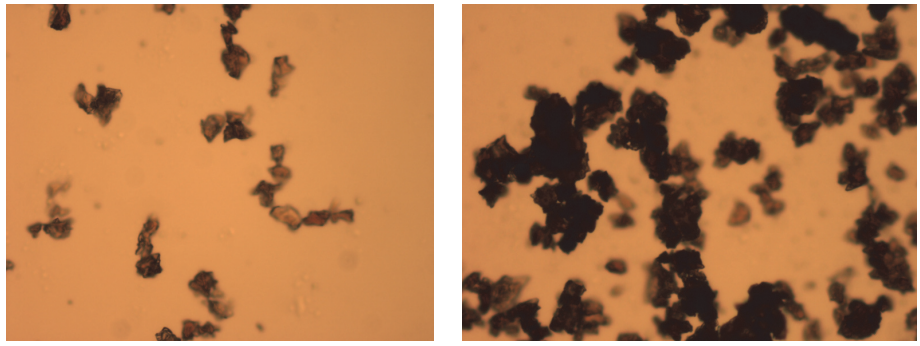
Samples	Zn	Pb
A	10.952 ± 2.963	0.838 ± 0.218
B	12.375 ± 1.401	0.129 ± 0.039
C	9.105 ± 0.1086	nd
D	9.202 ± 1.575	0.322 ± 0.086
E	4.998 ± 3.237	nd
F	7.584 ± 0.239	nd
G	7.116 ± 2.649	nd
H	9.626 ± 0.217	1.289 ± 0.211
I	10.430 ± 2.756	nd
J	9.822 ± 3.322	2.835 ± 0.172
K	9.833 ± 2.901	1.160 ± 0.179
L	9.452 ± 1.043	0.258 ± 0.038
M	7.736 ± 0.043	nd
N	14.635 ± 2.727	nd
O	6.486 ± 0.206	nd
Mean	9.626	0.455

nd: not detected.

The mean extraction of Pb in the "Traditional" and "Extra Strong" coffee infusions, which have smaller grain sizes (Figure 4(a)), was 16.96%. For the coffees of higher quality, named "Gourmet," of larger grain size (Figure 4(b)), Pb was not detected in their infusions, even when high Pb contents were found in the roasted ground coffee.

When comparing the data of the present study with that of the literature, differences are observed in the concentrations found in some elements. Rocks are natural sources of all chemical elements found on Earth [6]. Released by weathering, the elements in the rocks are made available in the soil and then are carried to rivers and groundwater, interacting with the ecosystem where humans live. However, the natural occurrence of these elements is not equally distributed on the surface of the Earth and problems may arise when their concentrations are too low (deficiency) or too high (toxicity) [6].

Metal contamination could also be caused by human activities, such as waste from mining, the steel industry, the cosmetics industry, or agricultural activities. The contamination that affects the agricultural areas currently represents a significant problem, given that many pollutants play an essential role in economic activities, for example, pesticides and fertilizers [6, 31], and many of these products can remain in the soil or water, thus contaminating food [11].



(a) (b)

Figure 4: Microscopy images of grains of the "Traditional" roasted ground coffee sample (a) and a "Gourmet" coffee sample (b).

4. Conclusions

The roasted and milled coffees quantified in the present study present high levels of ash indicating that products available on the market can contain high amounts of impurities.

For most of the quantified samples, the concentrations of heavy metals found in the commercial roasted ground coffees are below the limits recommended by the official inspection institutions and, thus, are suitable for consumption.

Some roasted ground coffee samples have heavy metal contents above the allowed levels. Parameters such as the species and the origin of the coffee beans can contribute to variations in the contents of heavy metals.

There is no correlation between the Pb and Zn contents in the roasted ground coffee and their respective infusions and, consequently, the beverages can be considered uncontaminated by these metals.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

[1] L. R. Cagliani, G. Pellegrino, G. Giugno, and R. Consonni, "Quantification of *Coffea arabica* and *Coffea canephora* var. robusta in roasted and ground coffee blends," *Talanta*, vol. 106, pp. 169–173, 2013.

[2] M. Defernez, E. Wren, A. D. Watson et al., "Low-field 1H NMR spectroscopy for distinguishing between arabica and robusta ground roast coffees," *Food Chemistry*, vol. 216, pp. 106–113, 2017.

[3] ABIC – Brazilian Association of Coffee Industries, Recommended Quality Standard and Good Practices for Manufacturing Roasted Coffee Beans and Roasted and Ground Coffee, Rio de Janeiro, Brazil, 2017.

[4] J. M. Y. Perosa and L. H. F. Abreu, "Economic aspects and opportunities in the market of quality coffees," *Pesquisa Agropecuária Tropical*, vol. 39, pp. 144–150, 2009.

[5] Brazila. Ministry of Agriculture, Livestock and Food Supply. Normative Instruction No 16, May 24, 2010. Technical regulation for roasted coffee beans and roasted and ground coffee. *Federal Official Gazette of Brazil*. May, 2010. Section 1, 11.

[6] S. A. Silva, F. Q. Mendes, M. R. Reis et al., "Determination of heavy metals in the roasted and ground coffee beans and brew," *African Journal of Agricultural Research*, vol. 12, pp. 221–228, 2017.

[7] D. Demírezen and A. Ahmet, "Heavy metal levels in vegetables in turkey are within safe limits for Cu, Zn, Ni and exceeded for Cd and Pb," *Journal of Food Quality*, vol. 29, pp. 252–265, 2006.

[8] Z.-Y. Hseu, S.-W. Su, H.-Y. Lai, H.-Y. Guo, T.-C. Chen, and Z.-S. Chen, "Remediation techniques and heavy metal uptake by different rice varieties in metal-contaminated soils of Taiwan: New aspects for food safety regulation and sustainable agriculture," *Soil Science & Plant Nutrition*, vol. 56, no. 1, pp. 31–52, 2010.

[9] G. A. M. Magna, S. L. Machado, R. B. Portella, and M. R. Carvalho, "Lead and cadmium detected in plant foods and grasses in Santo Amaro, Bahia," *Química Nova*, vol. 36, pp. 989–997, 2013.

[10] J. Malik, J. Szakova, O. Drabek, J. Balik, and L. Kokoska, "Determination of certain micro and macroelements in plant stimulants and their infusions," *Food Chemistry*, vol. 111, no. 2, pp. 520–525, 2008.

[11] V. Souza, R. F. B. Sousa, L. C. Offemann, V. Vaz Alves, and A. C. G. Alves Jr., "Alternatives for remediation and decontamination of soils from Brazil," *African Journal of Agricultural Research*, vol. 9, pp. 3197–3204, 2014.

[12] R. Ashu and B. S. Chandravanshi, "Concentration levels of metals in commercially available ethiopian roasted coffee powders and their infusions," *Bulletin of the Chemical Society of Ethiopia*, vol. 25, no. 1, pp. 11–24, 2011.

[13] R. S. M. P. Nascimento, G. S. Carvalho, L. P. Passos, and J. J. Marques, "Lead and zinc leaching in soil treated with iron smelting residues," *Pesquisa Agropecuária Tropical*, vol. 40, pp. 497–504, 2010.

[14] C. A. Grant and S. C. Sheppard, "Fertilizer impacts on cadmium availability in agricultural soils and crops," *Human and Ecological Risk Assessment*, vol. 14, pp. 210–228, 2008.

[15] C. A. P. Schmidt, É. Miglioranza, G. Nagashima, and F. Grecco, "Heavy metals concentration in coffee grains produced in farming under basalt and Caiuá sandstone soils," *Ciência Rural*, vol. 39, no. 5, pp. 1590–1593, 2009.

[16] O. R. Teixeira, F. R. Passos, and F. Q. Mendes, "Physico-chemical and microscopic quality of 14 trademark roasted and ground coffee," *Coffee Science*, vol. 11, no. 3, pp. 396–403, 2016.

[17] M. E. Scwartz and I. S. Krull, "Validation of chromatographic methods," *Pharmaceutical Technology*, vol. 2, pp. 12–20, 1998.

[18] Brazil. Decree No 55871, March 26, 1965. Modifies the decree No 50.040, January 24, 1961, concerning regulatory standards for the use of food additives, as amended by Decree No 691, March 13, 1962. *Federal Official Gazette of Brazil*. Abril, 1965. Section 1, 3610.

[19] Brazil. Sanitary Surveillance Agency. Resolution No 42, August 29, 2013. MERCOSUL Technical Regulation on Maximum Limits of Inorganic Contaminants in Foods. *Federal Official Gazette of Brazil*. August, 2013. Section 1, 33.

[20] M. C. M. D. Durek de Conti, C. S. Good Kitzberger, M. B. S. Dos Santos Scholz, and S. H. Helena Prudencio, "Physical and chemical characteristics of exotic and conventional roasted and ground coffees," *Boletim Centro de Pesquisa de Processamento de Alimentos*, vol. 31, no. 1, pp. 161–172, 2013.

[21] Brazil. Secretariat of Agriculture and Supply of São Paulo State. Resolution No 31, June 22, 2007. Standard minimum quality standards for roasted and roasted and ground coffee - Characteristic: Gourmet Coffee. *Federal Official Gazette of Brazil*. June, 2007. Section 1, 24.

[22] Brazil. Secretariat of Agriculture and Supply of São Paulo State. Resolution No 19, Abril 5, 2010. Standard minimum quality standards for roasted and roasted and ground coffee - characteristic: Traditional coffee. *Federal Official Gazette of Brazil*. Abril, 2007. Section 1, 26.

[23] P. D. Oliveira, F. M. Borém, E. P. Isquierdo, G. D. S. Giomo, R. R. de Lima, and R. A. Cardoso, "Physiological aspects of coffee beans, processed and dried through different methods, associated with sensory quality," *Coffee Science*, vol. 8, no. 2, pp. 211–220, 2013.

[24] A. J. Müller, L. Huebner, and C. F. V. Souza, "Evaluation of physico-chemical quality of different brands of roasted coffee soluble and in powder marketed in the region of Vale do Taquari/RS," *Revista Brasileira de Tecnologia Agroindustrial*, vol. 7, pp. 1004–1012, 2013.

[25] M. A. Morgano, L. F. Pauluci, D. M. B. Mantovani, and E. E. M. Mory, "Mineral determination in green coffee," *Food Science and Technology*, vol. 22, pp. 19–23, 2002.

[26] J. S. dos Santos, M. L. P. dos Santos, M. M. Conti, S. N. dos Santos, and E. de Oliveira, "Evaluation of some metals in Brazilian coffees cultivated during the process of conversion from conventional to organic agriculture," *Food Chemistry*, vol. 115, no. 4, pp. 1405–1410, 2009.

[27] P. Liu, C.-N. Wang, X.-Y. Song, and Y.-N. Wu, "Dietary intake of lead and cadmium by children and adults - Result calculated from dietary recall and available lead/cadmium level in food in comparison to result from food duplicate diet method," *International Journal of Hygiene and Environmental Health*, vol. 213, no. 6, pp. 450–457, 2010.

[28] M. Grembecka, E. Malinowska, and P. Szefer, "Differentiation of market coffee and its infusions in view of their mineral composition," *Science of the Total Environment*, vol. 383, no. 1–3, pp. 59–69, 2007.

[29] L. Noël, R. Chekri, S. Millour et al., "Li, Cr, Mn, Co, Ni, Cu, Zn, Se and Mo levels in foodstuffs from the Second French TDS," *Food Chemistry*, vol. 132, no. 3, pp. 1502–1513, 2012.

[30] E. Stelmach, P. Pohl, and A. Szymczycha-Madeja, "The suitability of the simplified method of the analysis of coffee infusions on the content of Ca, Cu, Fe, Mg, Mn and Zn and the study of the effect of preparation conditions on the leachability of elements into the coffee brew," *Food Chemistry*, vol. 141, no. 3, pp. 1956–1961, 2013.

[31] M. C. S. De Moura, G. C. Moita, and J. M. Neto, "Analysis and assessment of heavy metals in urban surface soils of Teresina, Piauí State, Brazil: A study based on multivariate analysis," *Comunicata Scientiae*, vol. 1, no. 2, pp. 120–127, 2010.

Hindawi
Journal of Food Quality
Volume 2018, Article ID 5967130, 8 pages
https://doi.org/10.1155/2018/5967130

Research Article

Effect of Yeast Fermentation of Green Coffee Beans on Antioxidant Activity and Consumer Acceptability

Han Sub Kwak,[1] **Yoonhwa Jeong**,[2] **and Misook Kim**[2]

[1]*Division of Functional Food Research, Korea Food Research Institute, 245 Nongsaengmyeong-ro, Iseo-myeon, Wanju-gun, Jeollabuk-do 55365, Republic of Korea*
[2]*Department of Food Science and Nutrition, Dankook University, 19 Dandae-ro, 119 Dongnam-gu, Cheonan-si, Chungnam 31116, Republic of Korea*

Correspondence should be addressed to Misook Kim; mkim5@dankook.ac.kr

Received 16 November 2017; Accepted 23 January 2018; Published 6 March 2018

Academic Editor: Gabriel Henrique Horta de Oliveira

This study assessed the functionality and consumer acceptance of yeast fermented coffee beans. Green coffee beans were fermented for 24 h with three different yeast strains to increase functionality. The yeast fermentation was effective in fortifying the functionality of coffee by significantly increasing antioxidant activity according to the results of ORAC and SOD-like assay ($P < 0.05$). The TPC and TFC contents in the fermented coffee beans were significantly higher than those in the controls ($P < 0.05$). The consumer acceptance for the fermented coffee beans was slightly lower than that of the controls. Fermentation seemed to influence the aroma and flavor of coffee. However, agglomerative hierarchical clustering analysis revealed that approximately 39% of consumers significantly liked one of the fermented coffees (F3) more than the controls ($P < 0.05$). These consumers indicated that the yeast fermentation of green coffee beans did not generate a negative aroma or flavor and can be attractive with high antioxidant activity.

1. Introduction

Coffee is one of the most traded commodities worldwide. It is harvested in tropical regions and mostly exported to developed countries such as the USA, Europe, Russia, Japan, and Korea [1, 2]. Many coffee drinkers consume coffee on a regular basis, similar to tea, and coffee is regarded as a hedonic food. However, coffee contains significant amounts of phenolic compounds such as chlorogenic and hydroxycinnamic acids and antioxidants including caffeine, melanoidins, and other Maillard reaction products and volatile compounds [3–5]. Levels of chlorogenic and hydroxycinnamic acids are also determined on the final aroma and taste of the roasted coffee [6, 7]. Richelle et al. [8] demonstrated that a cup of Robusta and Arabica coffee had two times more antioxidant activity than a cup of green and black tea. A cup of coffee per day was found to reduce the relative risk of diabetes by 7% in meta-analysis [9] and moderate coffee drinking (below 4 cups per day) showed the strongest inverse relation to heart failure [10]. In some European countries, coffee is one of the major sources of antioxidants in the human diet [11–13].

The functionality of food products can be increased by fermentation. Ginseng is one of the best known functional foods. Ginsenoside contents and SOD-like activity after fermentation were significantly increased by solid-microbial fermentation of white ginseng [14, 15]. Increased antioxidant activities and phenolic compounds are also frequently observed in fermented tea products. Jayabalan et al. [16] showed an increase in epicatechin isomers over 18 days of kombucha tea fermentation. DPPH radical scavenging activity and total polyphenol content in Pu-Erh tea were significantly increased by fermentation [17].

The fermentation of coffee is known as coffee cherry fermentation and effectively removes the mucilage layer prior to the drying process for obtaining green coffee beans [18]. Therefore, the primary objective of coffee cherry fermentation is to improve the ease of obtaining green coffee beans rather than to increase the functionality of the coffee beans. Green coffee beans can gain higher functionality with additional processing steps such as soaking in fruit extracts and fermentation. Lim et al. [19] reported higher antioxidant activity, total polyphenol contents, and consumer

acceptability after soaking green coffee beans in mulberry extract. However, the fermentation of green coffee beans has not been widely studied as a second processing step for increasing antioxidant activity and phenolic compounds. As the fermentation of tea products increases their antioxidant activity and the number of phenolic compounds, the antioxidant activity and phenolic compounds in green coffee beans could also be increased by fermentation. The fermented coffee beans can become fortified functional coffee beans. Therefore, the objective of this study was to ferment green coffee beans using commercial yeasts and investigate the resulting physicochemical properties, antioxidant activity, total polyphenol and flavonoid contents, and consumer acceptability with the intention of making fortified functional coffee beans.

2. Materials and Methods

2.1. Chemicals. Sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), monosodium phosphate (NaH_2PO_4), disodium phosphate (Na_2HPO_4), potassium acetate (CH_3COOK), and aluminum chloride ($AlCl_3{\cdot}6H_2O$) were purchased from Duksan Pure Chemicals (Ansan-si, Korea). HCl, NaCl, ethanol, and methanol were supplied by Samchun Pure Chemicals (Pyeongtaek-si, Korea). D-Glucose was bought from Duchefa Biochemie BV (Haarlem, Netherlands). YPD was purchased from Becton Dickinson (Sparks, MD, USA). Folin-Ciocalteu's phenol reagent, fluorescein sodium, 2,2′-azobis(2-amidinopropane)dihydrochloride (AAPH), 2,5-dinitrosalicylic acid (DNS), trolox, ethylenediaminetetraacetic acid (EDTA), pyrogallol, quercetin, and gallic acid were supplied by Sigma-Aldrich LLC (St. Louis, MO, USA).

2.2. Coffee Fermentation, Roasting, and Brewing. Green coffee beans (300 g; Brazil Ipanema Euro Natural, Coiners International Ltd., Bucheon-si, Korea) were put into a water (450 mL) and yeast (3.75 mL, 1.0×10^7 CFU/mL) mixture. Three different commercial yeasts (*Saccharomyces* species) were used for fermentation: F1 (Lalvin 71B, Lallemand Inc., Montreal, Canada), F2 (Lalvin Cy3079, Lallemand Inc.), and F3 (BDX, Lallemand Inc.). Fermentation was conducted for 24 h at 30°C. After fermentation, green coffee beans were washed three times using sterile water. The fermented coffee beans were dried in a dry oven at 45°C until their moisture content reached 10%. Two controls were used in this study; one control (C1) was the original green coffee beans and the other was a control (C2) treated with the same procedure as the yeast fermented coffee beans but without yeasts.

Coffee roasting was conducted with an automatic coffee roaster to ensure a similar roasting condition for all green coffee beans (Behmor 1600, Behmor Inc., Incline Village, NV, USA). The green coffee beans were put into the roaster and roasted at maximum heating power (1,600 W) for 16 min. The roasted coffee beans were cooled for 15 min and kept at room temperature for three days. The roasted coffee beans were ground using the medium option on a coffee grinder (KG79, De'Longhi, Milano, Italy). The ground coffee (36 g) was brewed with 600 mL of filtered water using a coffee maker (HD7564, Philips, Netherlands).

2.3. Fermentation Characteristics. The pH of the fermentation solution was measured using an electronic pH meter (Orion 3 star, Thermo Fisher Scientific Inc., Waltham, MA, USA) at 0 and 24 h.

The reducing sugar content in the green coffee beans was determined by the DNS method [20] with some modifications. A sample (200 uL) was mixed with 600 uL DNS solution and heated at 100°C for 5 min. Water (3 mL) was added to the reaction solution and its absorbance was measured at 550 nm. D-Glucose solutions (0.1–2.5 mg/mL) were used for a standard curve ($R = 0.997$).

Yeast-containing solutions for green coffee bean fermentation were serially diluted with 0.85% NaCl solution and spread onto the surface of YPD agar plates. The spread plates were incubated for 24 h at 30°C to determine the total microbial counts.

2.4. Physicochemical Characteristics of Roasted Coffee. Moisture content determination was performed according to the Association of Official Agricultural Chemists [21] guidelines. Approximately 1 g of ground coffee beans was dried at 105°C after roasting until the weight became consistent.

Crude ash content was also measured with the Association of Official Agricultural Chemists [21] guidelines. The roasted coffee beans (1 g) were incinerated at 550°C until the weight became consistent. Crude ash content was determined gravimetrically.

The color of the ground coffee after roasting was measured using a Hunter colorimeter system (JC-801S, Color Techno system Co., Tokyo, Japan). Ground coffee (2 g) was put into a small Petri dish for measurement. Lightness (L), redness (a), and yellowness (b) were measured. L, a, and b values for the standard white color plate were $L = 98.38$, $a = 0.29$, and $b = -0.41$, respectively.

2.5. Antioxidant Activity. Oxygen radical absorbance capacity (ORAC) was measured using the method described by Ou et al. [22] with some modifications for *in vitro* antioxidant activity. Phosphate (NaH_2PO_4-Na_2HPO_4) buffer (10 mM, pH 7.0) was used to dissolve fluorescein powder. Coffee extract (50 μL) and 25 mM fluorescein solution (150 μL) were mixed and then incubated in a dark room for 10 min. A volume of 25 μL of 120 mM AAPH solution was added to the coffee extract and fluorescein mixture. The control was 10 mM phosphate buffer instead of coffee extract. Fluorescence was measured by the microplate reader (Spectra max M2, Molecular Devices, LLC., Sunnyvale, CA, USA). Measurements were taken every minute for 90 min (excitation wavelength: 485 nm; emission wavelength: 535 nm). The ORAC values were calculated by the following formula and presented as μM trolox equivalent/mL of coffee (μM TE/mL):

$$\mathrm{ORAC}\,(\mu\mathrm{M\ TE/g}) = \frac{C_{\mathrm{Trolox}} \times \left(\mathrm{AUC}_{\mathrm{Sample}} - \mathrm{AUC}_{\mathrm{Blank}}\right) \times k}{\left(\mathrm{AUC}_{\mathrm{Trolox}} - \mathrm{AUC}_{\mathrm{Blank}}\right)}, \quad (1)$$

where C_{Trolox}, k, and AUC were the concentration of trolox (5 μM), the sample dilution factor, and the area under

Table 1: Change of pH, reducing sugar, and viable cells before and after fermentation.

Sample	pH		Reducing sugar (mg/mL)		Viable cell count (log CFU/mL)	
	0 h	24 h	0 h	24 h	0 h	24 h
C2	5.98	5.13	$0.68 \pm 0.01^{c(1)}$	2.57 ± 0.13^{a}	$<1.16 \pm 0.53$	7.78 ± 0.70^{a}
F1	5.79	4.92	1.12 ± 0.02^{b}	2.51 ± 0.12^{a}	6.50 ± 5.83	9.31 ± 0.85^{a}
F2	5.93	4.52	1.28 ± 0.03^{a}	2.47 ± 0.09^{a}	6.41 ± 5.62	9.25 ± 0.86^{a}
F3	5.78	5.04	1.12 ± 0.01^{b}	2.67 ± 0.09^{a}	6.30 ± 5.30	9.03 ± 0.81^{b}

(1)Different superscripts within a column meant significant difference at $P < 0.05$ by Fisher's least significant difference test.

the curve, respectively. AUC was calculated based on the following formula:

$$\text{AUC} = 1 + \sum_{n=1}^{90} \frac{f_n}{f_0}, \tag{2}$$

where f_n is the fluorescence at time n (min).

Superoxide dismutase-like (SOD-like) activity was determined using the method described by S. Marklund and G. Marklund [23] with some modifications. Coffee extract (400 μL), Tris-HCl buffer (600 μL, 50 mM tris(hydroxymethyl)aminomethane and 10 mM ethylenediaminetetraacetic acid (EDTA), pH 8.0), and 7.2 mM pyrogallol (40 μL) were mixed together and kept at 25°C for 10 min. The reaction was stopped by adding 0.1 N HCl (20 μL). The absorbance was measured at 420 nm using a UV/visible spectrophotometer (Ultrospec 2100 Pro, Biochrom Ltd., Cambridge, UK). SOD-like activity was calculated based on the following equation:

$$\text{SOD-like activity (\%)} = \left(1 - \frac{A}{B}\right) \times 100, \tag{3}$$

where A is absorbance of the sample and B is absorbance of the control.

2.6. Total Polyphenol and Flavonoid Contents. The total polyphenol content (TPC) of brewed coffee was measured using Singleton's method [24] with some modifications. Coffee extract (20 μL) was diluted with 1,580 μL of distilled water. Diluted coffee (160 μL) was mixed with Folin-Ciocalteu's phenol reagent (10 μL) and allowed to sit for 8 min. A volume of 30 μL of 20% Na_2CO_3 solution was added and the mixture was incubated in a dark room for 2 h. Distilled water instead of coffee extract was used as a control. The absorbance was measured at 765 nm by a microplate reader (Spectra max M2, Molecular Devices, LLC.). Gallic acid solutions (0-1 mg/mL) were used to generate a standard curve (R^2 = 0.997). The results were presented as mg gallic acid equivalent/mL of coffee extract (mg GAE/mL).

The total flavonoid content (TFC) for each coffee extract was evaluated according to the method described by Dewanto study with some modifications [25]. Coffee extract (250 μL), distilled water (1 mL), and 75 μL of 5% $NaNO_2$ were mixed together. After 5 min, 10% $AlCl_3 \cdot 6H_2O$ solution (150 μL) was added and incubated for 6 min. 1 N NaOH (500 μL) was injected, and the mixture was incubated for 11 min. A blank sample was substituted for the diluted coffee extract with distilled water. The absorbance of the sample was measured at 510 nm against the blank sample using a microplate reader (Spectra max M2, Molecular Devices, LLC). The difference in absorbance between the sample and the blank was compared to the absorbance of the quercetin solution used as the positive control (R^2 = 0.999). The amount of flavonoids in the coffee was presented as mg quercetin equivalent/mL of coffee extract.

2.7. Consumer Acceptance Test. The consumer acceptance test for the yeast fermented coffee was performed with 74 subjects (24 males and 50 females, ages 19–30) who were students and staff at Dankook University (Yongin-si, Korea). Subjects were coffee drinkers that consumed Americano coffee (espresso coffee extract + hot water) at least once a week. Consumer testing was conducted in an open area under incandescent lighting. Subjects were assigned to tables and were prohibited from talking and using cellphones during the test. Coffee (20 mL) was served in 60 mL paper cups using a completed balanced design [26] in order to minimize carry-over effects. The temperature of the coffee was approximately 60°C. Acceptance of overall quality, coffee aroma, bitter taste, astringent taste, and smooth mouthfeel was rated using a nine-point hedonic scale on paper ballots. Each category on the scale was labeled with numbers and descriptors in order to give a clear understanding of the scale. Starting from the left side, the scale was labeled as 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, and 9 = extremely like. A cup of filtered water was served as a palate cleanser. Subjects were monetarily compensated after the test (approximately $8.5).

2.8. Statistical Analyses. Results were analyzed using Excel integrated statistical software (XLSTAT version 2012, Addinsoft, Paris, France) in this study. Analysis of variance (ANOVA) was performed to identify significant differences across samples. Post hoc analysis was carried out using Fisher's least significant test at $P < 0.05$ when the significance was observed by ANOVA. Agglomerative hierarchical clustering (AHC) analysis was conducted to generate consumer segment groups based on their overall quality ratings.

3. Results and Discussion

3.1. Fermentation Characteristics. Measurements of pH values, reducing sugar contents, and viable cell counts were conducted to investigate the effect of yeast fermentation on green coffee beans (Table 1). Reducing sugar contents for

Table 2: Moisture content, crude ash, and color of ground roasted coffee beans after 24 h of yeast fermentation.

Sample	Moisture content (%)	Crude ash (%)	Color			
			L	a	b	ΔE
C1	$1.50 \pm 0.09^{a(1)}$	4.73 ± 0.05^{a}	31.52 ± 1.01^{a}	7.91 ± 0.44^{a}	16.78 ± 1.92^{a}	69.60 ± 1.27^{b}
C2	1.30 ± 0.05^{b}	4.08 ± 0.06^{cd}	25.24 ± 0.62^{c}	4.90 ± 0.25^{b}	8.70 ± 0.89^{c}	74.00 ± 0.62^{a}
F1	1.27 ± 0.17^{b}	4.05 ± 0.06^{d}	30.62 ± 0.81^{ab}	7.11 ± 0.94^{a}	14.74 ± 0.80^{ab}	69.91 ± 0.52^{b}
F2	1.23 ± 0.06^{b}	4.15 ± 0.05^{c}	25.17 ± 0.68^{c}	4.43 ± 0.26^{b}	7.63 ± 0.53^{c}	73.90 ± 0.61^{a}
F3	1.31 ± 0.10^{b}	4.40 ± 0.05^{b}	29.78 ± 0.97^{b}	6.97 ± 0.54^{a}	13.75 ± 1.08^{b}	70.51 ± 0.70^{b}

(1)Different superscripts within a column meant significant difference at $P < 0.05$ by Fisher's least significant difference test.

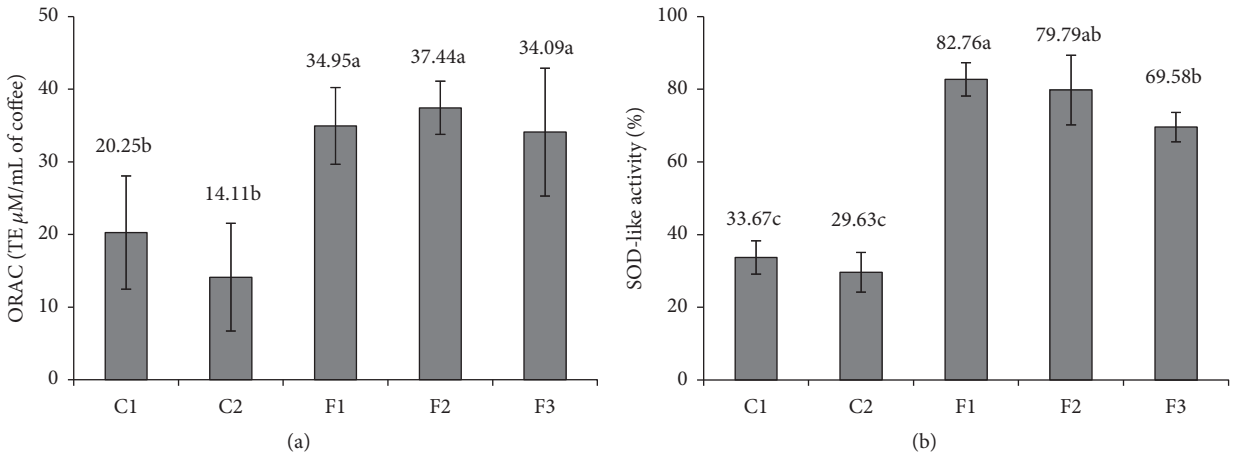


Figure 1: Antioxidant activities of yeast fermented coffee extracts by oxygen radical absorbance capacity (a) and superoxide dismutase-like (SOD-like) activity (b) assays. Different letters meant significant difference at $P < 0.05$ by Fisher's least significant difference test.

C2, F1, F2, and F3 were 2.567, 2.507, 2.469, and 2.673 mg. pH decreased after 24 h of fermentation. The pH for C2 also decreased. This decrease would be due to the soluble organic acids released from green coffee beans [27] and produced by the fermentation of naturally occurring lactic acid bacteria in coffee beans. After 24 h of fermentation, there was no significant difference in reducing sugar content in the fermented solution ($P > 0.05$). Viable cell counts for C2, F1, F2, and F3 showed that fermentation was conducted by yeasts (Table 1). The initial number of viable microbial cells was less than 1.16 log CFU/mL. The number of microbial cells, naturally present in green coffee beans, increased to 7.78 CFU/mL after 24 h fermentation. Solutions from F1, F2, and F3, fermented by starter culture, showed approximately 2.5 higher log CFU/mL of solution than that from C2. This provided supporting evidence for the proliferation of yeasts during fermentation and the role of yeasts in the fermentation. Throughout the 24 h of fermentation, there was no drastic change in pH and reducing sugar content; hence yeast fermentation occurred across all samples.

3.2. Quality Characteristics of Fermented Coffee Beans. The moisture content, crude ash, and color of fermented coffee beans after roasting are presented in Table 2. No significant difference across fermented coffee beans and C2 was observed. This indicates that the drying and roasting of the fermented green coffee beans were performed identically. The moisture content of C1 was 1.50% and was significantly higher than those of C2 and the fermented coffee beans ($P < 0.05$). This was due to slight overdrying prior to roasting. The ash content was also significantly higher in C1 at 4.73%, while the others ranged from 4.05 to 4.40%. Moisture content and crude ash showed a strong negative correlation ($R = 0.894$, $P = 0.041$). The color of the ground roasted coffee beans showed differences across the samples. C1, F1, and F3 had higher L, a, and b values and lower ΔE values than C2 and F2. The color of the ground coffee beans showed a different pattern in comparison with the moisture content and crude ash. Therefore, the different colors of the roasted coffee beans likely partially originated from the fermentation as well as the difference in moisture contents.

3.3. Antioxidant Activity. The antioxidant activities of the coffee extracts in ORAC and SOD-like assays are shown in Figure 1. ORAC for the yeast fermented coffee extracts were 34.95, 37.44, and 34.09 μM TE/mL for F1, F2, and F3, respectively (Figure 1(a)). ORAC for C1 and C2 were 20.25 and 14.11 μM TE/mL, respectively. There was no significant difference in ORAC values after soaking the green coffee beans for 24 h ($P > 0.05$); however, a slight decrease in the ORAC value was observed in C2. It seemed that the soluble antioxidants in green coffee beans might be eluted into water. The ORAC values of the yeast fermented coffee extracts (F1, F2, and F3) were significantly higher than those from the controls (C1 and C2) ($P < 0.05$). There was no significant difference across the fermented coffee beans

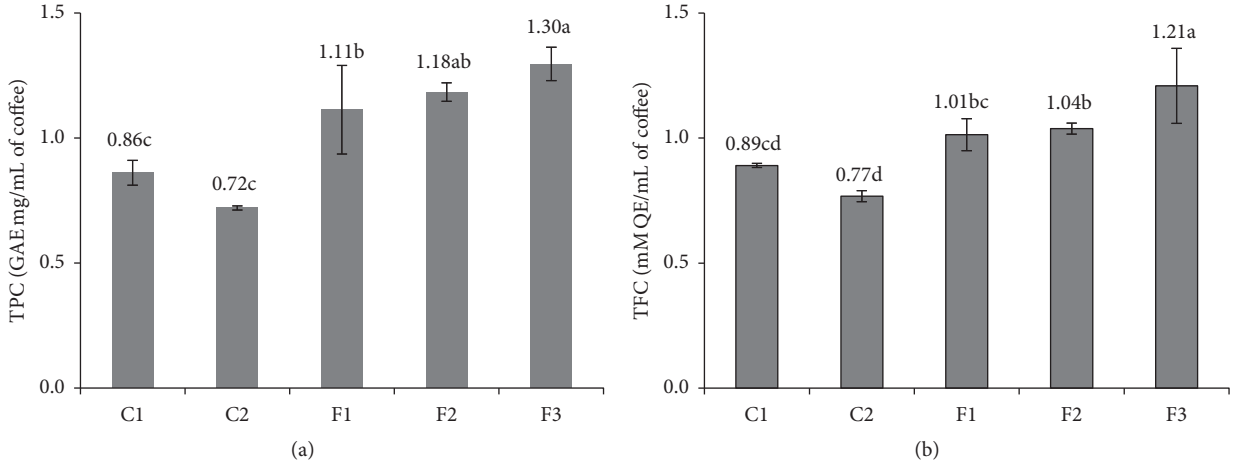


Figure 2: Total polyphenol (a) and flavonoid (b) contents of yeast fermented coffee extracts. Different letters meant significant difference at $P < 0.05$ by Fisher's least significant difference test.

($P < 0.05$). In SOD-like activity, F1, F2, and F3 showed 82.76, 79.79, and 69.58%, respectively (Figure 1(b)). On the other hand, C1 and C2 showed 33.67 and 29.63% SOD-like activity, respectively, and were not significantly different ($P > 0.05$). An increase of more than two times the SOD-like activity was observed after 24 h of yeast fermentation. Among the fermented samples, F1 showed the highest activity and the activity was significantly higher than F3 ($P < 0.05$). Soaking green coffee beans in water (C2) did not significantly influence the antioxidant activity of the coffee extracts ($P > 0.05$) although the ORAC value of C2 was slightly lower than that of C1. Fermentation for 24 h was effective in increasing the antioxidant activity significantly. The increase of antioxidant activity by fermentation was similar to other fermented materials such as ginseng, garlic, tea, and soy [15, 16, 28, 29].

3.4. Total Polyphenol and Flavonoid Contents. The TPC and TFC of the yeast fermented coffee extracts are presented in Figure 2. The yeast fermented coffee extracts (F1, F2, and F3) had 1.11, 1.18, and 1.30 GAE mg/mL of coffee extract, respectively (Figure 2(a)). F3 had significantly higher TPC than F1 ($P < 0.05$). Control samples (C1 and C2) had significantly lower amounts of TPC than the yeast fermented coffee extracts ($P < 0.05$). C2 had the lowest TPC at 0.72 GAE mg/mL. This was due to the elution of soluble phenolic compounds in the green coffee beans during the soaking period (24 h). A similar finding was observed after soaking green coffee beans in mulberry extract [19]. In Lim's study, the TPC of the coffee extracts decreased after 6 h of soaking. Despite the decrease in TPC due to the soaking process in this study, fermentation positively influenced TPC in the coffee extracts. During the fermentation process, strongly bound phenolic compounds in the cell wall may become weakened [30], making them easier to extract after roasting. Phenolic compounds in coffee are mostly in the forms of chlorogenic acids with 5-O-caffeoyl-quinic acid such as caffeic, ferulic, p-coumaric, and caffeoylquinic acid [3]. Feruloylquinic acid, di-caffeoyl-quinic acid, and proanthocyanidins are also detected in coffee [3].

In TFC, the yeast fermented coffee extracts (F1, F2, and F3) had 1.01, 1.04, and 1.21 QE mg/mL of coffee extract, respectively, and F3 had significantly higher ($P < 0.05$) TFC than F1 and F2 (Figure 2(b)). C1 and C2 had lower amounts of TFC than the yeast fermented coffee extracts. Yeast fermentation was effective in increasing the number of flavonoids in the coffee extracts. The increase in TFC might be due to the conversion of insoluble phenolic compounds into soluble flavonoids during fermentation [31]. The ratio of TFC to TPC in C1 and C2 was 1.03 and 1.07, while F1, F2, and F3 had ratios of 0.91, 0.88, and 0.93, respectively. The ratio was lower in the yeast fermented coffee extracts. Fermentation was more effective in producing soluble phenolic compounds than flavonoids. This result contrasted with those for fermented Columbian coffee [32]. However, Columbian coffee fermentation was conducted with broken green beans, which might elute more soluble phenolic compounds easily over seven days of fermentation. This would also influence the roasting process.

3.5. Consumer Acceptance Test. Consumer acceptance for the fermented and control coffee extracts is shown in Table 3. Mean overall quality rating was the highest in C1, followed by F3. F1 and F2 had significantly lower overall quality ratings than C1 at 4.35 and 4.43, respectively ($P < 0.05$). C2 also received slightly lower overall quality ratings than C1. Therefore, the soaking process does appear to slightly influence the coffee quality. Color acceptance was not significantly different ($P < 0.05$), which meant that there was no difference in the appearance of the coffee extract. Aroma acceptance ratings for the fermented coffee samples (F1, F2, and F3) were significantly lower than those for the controls (C1 and C2) ($P < 0.05$). The consumer acceptability was within the range of the previous acceptance study using various roasting conditions [33]. Fermentation might negatively influence the aroma acceptance of coffee. Across the fermented coffee samples (F1, F2, and F3) the differences in consumer acceptability might be related to the presence of different volatile compounds in coffee beans after fermentation. Different yeasts showed different flavor profiles

Table 3: Overall quality and acceptances of color, aroma, sourness, bitterness, astringency, and mouthfeel of yeast fermented coffee extract by 74 consumers.

Sample	Overall quality	Color	Aroma	Sourness	Bitterness	Astringency	Mouthfeel
C1	$5.09 \pm 1.72^{a(1)}$	5.59 ± 1.76^{a}	5.62 ± 1.63^{a}	5.12 ± 1.86^{a}	4.51 ± 1.90^{a}	4.81 ± 1.80a	5.80 ± 1.75^{a}
C2	4.69 ± 1.65^{ab}	5.30 ± 1.75^{a}	5.12 ± 1.70^{a}	5.07 ± 1.49^{a}	4.12 ± 1.94^{ab}	4.57 ± 1.82^{ab}	5.35 ± 1.68^{ab}
F1	4.35 ± 1.70^{b}	5.18 ± 1.48^{a}	4.08 ± 1.85^{b}	4.78 ± 1.50^{a}	3.84 ± 1.76^{b}	4.20 ± 1.76^{b}	4.99 ± 1.72^{b}
F2	4.43 ± 1.46^{b}	5.23 ± 1.44^{a}	4.51 ± 1.51^{b}	4.19 ± 1.58^{b}	4.01 ± 1.85^{ab}	4.03 ± 1.79^{b}	4.85 ± 1.66^{b}
F3	4.84 ± 1.67^{ab}	5.54 ± 1.60^{a}	4.45 ± 1.95^{b}	4.84 ± 1.47^{a}	4.41 ± 1.89^{ab}	4.36 ± 1.58^{ab}	5.12 ± 1.57^{b}

(1)Different superscripts within a column meant significant difference at $P < 0.05$ by Fisher's least significant difference test.

Table 4: Agglomerative hierarchical clustering (AHC) analysis of overall quality.

Class	Subject(1)	C1	C2	F1	F2	F3
1	28	$3.79 \pm 1.20^{b(2)}{}_{B(3)}$	$3.82 \pm 1.61^{b}{}_{B}$	$3.57 \pm 1.48^{b}{}_{B}$	$4.89 \pm 1.66^{a}{}_{A}$	$5.32 \pm 1.61^{a}{}_{A}$
2	43	$5.95 \pm 1.53^{a}{}_{A}$	$5.23 \pm 1.49^{b}{}_{A}$	$4.81 \pm 1.72^{bc}{}_{A}$	$4.09 \pm 1.29^{d}{}_{B}$	$4.51 \pm 1.71^{cd}{}_{B}$

(1)Three subjects were removed from AHC analysis because they marked same ratings for entire samples. (2)Different superscripts within a row meant significant difference at $P < 0.05$ by Fisher's least significant difference test. (3)Different subscripts within a column meant significant difference at $P < 0.05$.

in rice distilled liquor [34] and cachaça [35]. Acceptance ratings for sourness, bitterness, astringency, and mouthfeel were lower in the yeast fermented coffee samples than in C1 and C2.

In order to segment consumers into a small number of groups, AHC analysis was performed using the overall quality ratings for all except three consumers that selected the same acceptance ratings for the entire sample (Table 4). Consumers were divided into two clusters generated by AHC analysis. Cluster 1 was composed of 39.4% of the total consumers. These were the consumers who preferred fermented coffee (F2 and F3), while disliking the controls (C1 and C2) ($P < 0.05$). On the other hand, cluster 2 was composed of 60.6% of consumers. These consumers had higher overall quality ratings for the controls (C1 and C2). They also rated F1 as an average of 4.81 and had the least acceptance for F2 at 4.09. Approximately 40% of consumers preferred the fermented coffee extracts (F2 and F3) although fermented coffee had lower mean overall quality ratings for the entire group of consumers. Therefore, the fermented coffee samples (F2 and F3) did not have lower acceptance ratings for all consumers; they were acceptable and showed higher acceptability for 39.4% of consumers. This result provides supporting evidence that fermentation does not have a negative influence on the consumer acceptance of coffee. Consumers are therefore segmented and another coffee product can be created for approximately 40% of coffee consumers. In addition, the high antioxidant activity and phenolic compounds in fermented coffee can be attractive to those consumers with moderate acceptance for fermented coffee. The consumer acceptance would also increase with awareness of the high antioxidant activity and phenolic compounds content as shown in a previous blind and informed consumer acceptance test for blueberry functional beverages [36].

4. Conclusion

Yeast fermentation of green coffee beans for 24 h was effective in fortifying the functionality of coffee by inducing a significant increase in antioxidant activity, TPC, and TFC. Yeast fermentation of green coffee beans causes bound phenolic compounds to be released after roasting. The consumer acceptance for the fermented coffee beans was slightly lower than for the controls. Fermentation might negatively influence the aroma and flavor of coffee extracts. However, the consumer segmentation revealed that approximately 39.4% of consumers preferred one of the fermented coffees (F3) more than the controls. Therefore, it can be concluded that yeast fermentation did not always generate a negative aroma and flavor for consumers. If fermentation was carried out with properly selected yeasts, fermented coffee can be attractive to coffee consumers, and coffee manufacturers can diversify their products with higher functionality.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

[1] H. D. Vieira, "Coffee: The plant and its cultivation," in *Plant-Parasitic Nematodes of Coffee*, M. Souza, Ed., pp. 3–18, Springer, Dordrecht, Netherlands, 2008.

[2] "International Coffee Organization (ICO)," 2015, http://www.ico.org/trade_statistics.asp.

[3] P. Parras, M. Martínez-Tomé, A. M. Jiménez, and M. A. Murcia, "Antioxidant capacity of coffees of several origins brewed following three different procedures," *Food Chemistry*, vol. 102, no. 3, pp. 582–592, 2007.

[4] M. Pérez-Martínez, B. Caemmerer, M. P. de Peña, C. Concepción, and L. W. Kroh, "Influence of brewing method and acidity regulators on the antioxidant capacity of coffee brews," *Journal of Agricultural and Food Chemistry*, vol. 58, no. 5, pp. 2958–2965, 2010.

[5] I. Sánchez-González, A. Jiménez-Escrig, and F. Saura-Calixto, "In vitro antioxidant activity of coffees brewed using different procedures (Italian, espresso and filter)," *Food Chemistry*, vol. 90, no. 1-2, pp. 133–139, 2005.

[6] J. R. Oliveira-Neto, S. G. Rezende, C. De Fátima Reis, S. R. Benjamin, M. L. Rocha, and E. De Souza Gil, "Electrochemical behavior and determination of major phenolic antioxidants in selected coffee samples," *Food Chemistry*, vol. 190, no. 1, Article ID 17651, pp. 506–512, 2016.

[7] J. A. Vignoli, M. C. Viegas, D. G. Bassoli, and M. D. T. Benassi, "Roasting process affects differently the bioactive compounds and the antioxidant activity of arabica and robusta coffees," *Food Research International*, vol. 61, no. 1, pp. 279–285, 2014.

[8] M. Richelle, I. Tavazzi, and E. Offord, "Comparison of the antioxidant activity of commonly consumed polyphenolic beverages (coffee, cocoa, and tea) prepared per cup serving," *Journal of Agricultural and Food Chemistry*, vol. 49, no. 7, pp. 3438–3442, 2001.

[9] R. Huxley, C. M. Y. Lee, F. Barzi et al., "Coffee, decaffeinated coffee, and tea consumption in relation to incident type 2 diabetes mellitus: a systematic review with meta-analysis," *rchives of Internal Medicine*, vol. 169, no. 22, pp. 2053–2063, 2009.

[10] E. Mostofsky, M. S. Rice, E. B. Levitan, and M. A. Mittleman, "Habitual coffee consumption and risk of heart failure a dose-response meta-analysis," *Circulation: Heart Failure*, vol. 5, no. 4, pp. 401–405, 2012.

[11] R. Pulido, M. Hernñndez-García, and F. Saura-Calixto, "Contribution of beverages to the intake of lipophilic and hydrophilic antioxidants in the Spanish diet," *European Journal of Clinical Nutrition*, vol. 57, no. 10, pp. 1275–1282, 2003.

[12] S. A. Qureshi, A. C. Lund, M. B. Veierød et al., "Food items contributing most to variation in antioxidant intake; a cross-sectional study among Norwegian women," *BMC Public Health*, vol. 14, no. 1, article no. 45, 2014.

[13] A. Svilaas, A. K. Sakhi, L. F. Andersen et al., "Intakes of antioxidants in coffee, wine, and vegetables are correlated with plasma carotenoids in humans," *Journal of Nutrition*, vol. 134, no. 3, pp. 562–567, 2004.

[14] B.-G. Kim, S.-Y. Choi, M.-R. Kim, H. J. Suh, and H. J. Park, "Changes of ginsenosides in Korean red ginseng (Panax ginseng) fermented by Lactobacillus plantarum M1," *Process Biochemistry*, vol. 45, no. 8, pp. 1319–1324, 2010.

[15] S.-I. Lim, C.-W. Cho, U.-K. Choi, and Y.-C. Kim, "Antioxidant activity and ginsenoside pattern of fermented white ginseng," *Journal of Ginseng Research*, vol. 34, no. 3, pp. 168–174, 2010.

[16] R. Jayabalan, S. Marimuthu, and K. Swaminathan, "Changes in content of organic acids and tea polyphenols during kombucha tea fermentation," *Food Chemistry*, vol. 102, no. 1, pp. 392–398, 2007.

[17] K.-C. Jeng, C.-S. Chen, Y.-P. Fang, R. C.-W. Hou, and Y.-S. Chen, "Effect of microbial fermentation on content of statin, GABA, and polyphenols in Pu-erh tea," *Journal of Agricultural and Food Chemistry*, vol. 55, no. 21, pp. 8787–8792, 2007.

[18] C. F. Silva, L. R. Batista, L. M. Abreu, E. S. Dias, and R. F. Schwan, "Succession of bacterial and fungal communities during natural coffee (Coffea arabica) fermentation," *Food Microbiology*, vol. 25, no. 8, pp. 951–957, 2008.

[19] H. H. Lim, S. Ji, H. S. Kwak et al., "Quality characteristics of coffee brewed from green beans soaked in mulberry (Morus bombycis) extract," *Journal of the Korean Society of Food Science and Nutrition*, vol. 44, no. 4, pp. 579–585, 2015.

[20] T. K. Ghose, "Measurement of cellulase activities," *Pure and Applied Chemistry*, vol. 59, no. 2, pp. 257–268, 1987.

[21] AOAC., *Official Methods of Analysis*, Association of Official Analytical Chemists, Gaithersburg, MD, USA, 18th edition edition, 2005.

[22] B. Ou, M. Hampsch-Woodill, and R. L. Prior, "Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe," *Journal of Agricultural and Food Chemistry*, vol. 49, no. 10, pp. 4619–4626, 2001.

[23] S. Marklund and G. Marklund, "Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase," *European Journal of Biochemistry*, vol. 47, no. 3, pp. 469–474, 1974.

[24] V. L. Singleton, R. Orthofer, and R. M. Lamuela-Raventós, "Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent," *Methods in Enzymology*, vol. 299, pp. 152–178, 1999.

[25] V. Dewanto, X. Wu, and R. H. Liu, "Processed sweet corn has higher antioxidant activity," *Journal of Agricultural and Food Chemistry*, vol. 50, no. 17, pp. 4959–4964, 2002.

[26] H. J. MacFie, N. Bratchell, K. Greenhoff, and L. V. Vallis, "Designs to balance the effect of order of presentation and first-order carry-over effects in hall tests," *Journal of Sensory Studies*, vol. 4, no. 2, pp. 129–148, 1989.

[27] C. I. Rodrigues, L. Marta, R. Maia, M. Miranda, M. Ribeirinho, and C. Máguas, "Application of solid-phase extraction to brewed coffee caffeine and organic acid determination by UV/HPLC," *Journal of Food Composition and Analysis*, vol. 20, no. 5, pp. 440–448, 2007.

[28] Y.-H. Pyo, T.-C. Lee, and Y.-C. Lee, "Effect of lactic acid fermentation on enrichment of antioxidant properties and bioactive isoflavones in soybean," *Journal of Food Science*, vol. 70, no. 3, pp. S215–S220, 2005.

[29] E. Sato, M. Kohno, H. Hamano, and Y. Niwano, "Increased anti-oxidative potency of garlic by spontaneous short-term fermentation," *Plant Foods for Human Nutrition*, vol. 61, no. 4, pp. 157–160, 2006.

[30] M. Shi, Y. Yang, Q. Wang, Y. Zhang, Y. Wang, and Z. Zhang, "Production of total polyphenol from fermented soybean curd residue by Lentinus edodes," *International Journal of Food Science & Technology*, vol. 47, no. 6, pp. 1215–1221, 2012.

[31] Y.-K. Lee, S.-I. Lee, J.-S. Kim et al., "Antioxidant activity of green tea fermented with Monascus pilosus," *Journal of Applied Biological Chemistry*, vol. 55, no. 1, pp. 19–25, 2012.

[32] J.-Y. Shin, H. Kim, D.-G. Kim, G.-H. Baek, H.-S. Jeong, and K.-W. Yu, "Pharmacological activities of coffee roasted from fermented green coffee beans with fungal mycelia in solid-state culture," *Journal of the Korean Society of Food Science and Nutrition*, vol. 42, no. 3, pp. 487–496, 2013.

[33] L. C. Mendes, H. C. De Menezes, M. Aparecida, and A. P. Da Silva, "Optimization of the roasting of robusta coffee (C. canephora conillon) using acceptability tests and RSM," *Food Quality and Preference*, vol. 12, no. 2, pp. 153–162, 2001.

[34] H. S. Kwak, J. S. Seo, Y. Hur et al., "Influence of yeast strains on the physicochemical characteristics, methanol and acetaldehyde profiles and volatile compounds for Korean rice distilled spirit," *Journal of the Institute of Brewing*, vol. 121, no. 4, pp. 574–580, 2015.

[35] E. S. Oliveira, H. M. A. B. Cardello, E. M. Jeronimo, E. L. Rocha Souza, and G. E. Serra, "The influence of different yeasts on the fermentation, composition and sensory quality of cachaça," *World Journal of Microbiology and Biotechnology*, vol. 21, no. 5, pp. 707–715, 2005.

[36] M. K. Kim and H. S. Kwak, "Influence of functional information on consumer liking and consumer perception related to health claims for blueberry functional beverages," *International Journal of Food Science & Technology*, vol. 50, no. 1, pp. 70–76, 2015.

Hindawi
Journal of Food Quality
Volume 2018, Article ID 7909467, 8 pages
https://doi.org/10.1155/2018/7909467

Research Article

Shade Trees Spatial Distribution and Its Effect on Grains and Beverage Quality of Shaded Coffee Trees

Francisco José da Silva Neto,[1] **Kátia Priscilla Gomes Morinigo**,[1] **Nathalia de França Guimarães**,[2] **Anderson de Souza Gallo**,[2] **Maicon Douglas Bispo de Souza**,[1] **Rubismar Stolf**,[1] **and Anastácia Fontanetti**[1]

[1]*Department of Rural Development, Universidade Federal de São Carlos (UFSCar), Rodovia Anhanguera, Km 174, Araras, SP, Brazil*
[2]*Department of Soil, Institute of Agronomy, Universidade Federal Rural do Rio de Janeiro (UFRRJ), BR-465, Km 7, Seropédica, RJ, Brazil*

Correspondence should be addressed to Francisco José da Silva Neto; franciscojose331@gmail.com and Anastácia Fontanetti; anastacia@ufscar.br

Received 30 September 2017; Accepted 27 December 2017; Published 25 February 2018

Academic Editor: Fernando M. Botelho

Shading coffee trees has gained importance, especially among smallholders, as an option to improve the products' quality, therefore acquiring place at the specialty coffee market, where consumers are willing to give bonus for quality. This work aims to evaluate the influence of shade trees' spatial distribution among coffee trees' agronomic characteristics, yield, and beans and cup quality of shaded coffee trees. The experimental design consisted of completely randomized blocks with six repetitions and four treatments: coffee trees on shade trees planting rows, distant one meter from the trunk; coffee trees on shade trees planting row, distant six meters from the trunk; and coffee plants between the rows of shade trees, parallel to the previous treatments. The parameters analyzed were plant height, canopy diameter, plagiotropic branches' length, yield, coffee fruits' phenological stage, ripe cherries' Brix degree, percentage of black, unripe, and insect damaged beans, bean size, and beverage quality. Shade trees quickened coffee fruits' phenological stage of coffee trees nearest to them. This point also showed the best beverage quality, except for overripe fruits. The remaining parameters evaluated were not affected by shade trees' spatial distribution.

1. Introduction

Brazilian production processes of specialty coffee have grown in the past decades due to the increase of demand from international markets [1]. The concept of specialty coffee is broad and involves characteristics such as superior beverage quality, rare varieties, and location of cultivation but also can be related to ecological, economic, or social sustainability [2]. Still, coffee quality is the result of complex interactions between the environment, management, and plant genetics [3].

The implantation of shade trees in coffee plantation can bring about many benefits to the agroecosystem, such as temperature reduction of air, soil, and leaf surface [4], as well as the thermal amplitude [5], and softening the effect of biennial bearing [6]. It also protects coffee plants from strong winds, rains, or hail [7] and increases nutrient cycling and soil organic matter [8]. This can significantly increase crop production stability.

Shade trees also impact quality because maturation is more uniform under shade [9]. Coffee beans' size is enlarged as a consequence of fewer flowers and, also, the existence of less fruits per plant once coffee trees are shaded [6]. This benefic effect is possible in sites under optimal [10] and suboptimal [11] conditions for coffee plants. However, Bosselmann et al. [12] found a decrease in coffee quality when shade trees were present in plantations at high altitudes.

However, conditions in shaded coffee plantations are not spatially stable, especially in systems with lower rates of soil

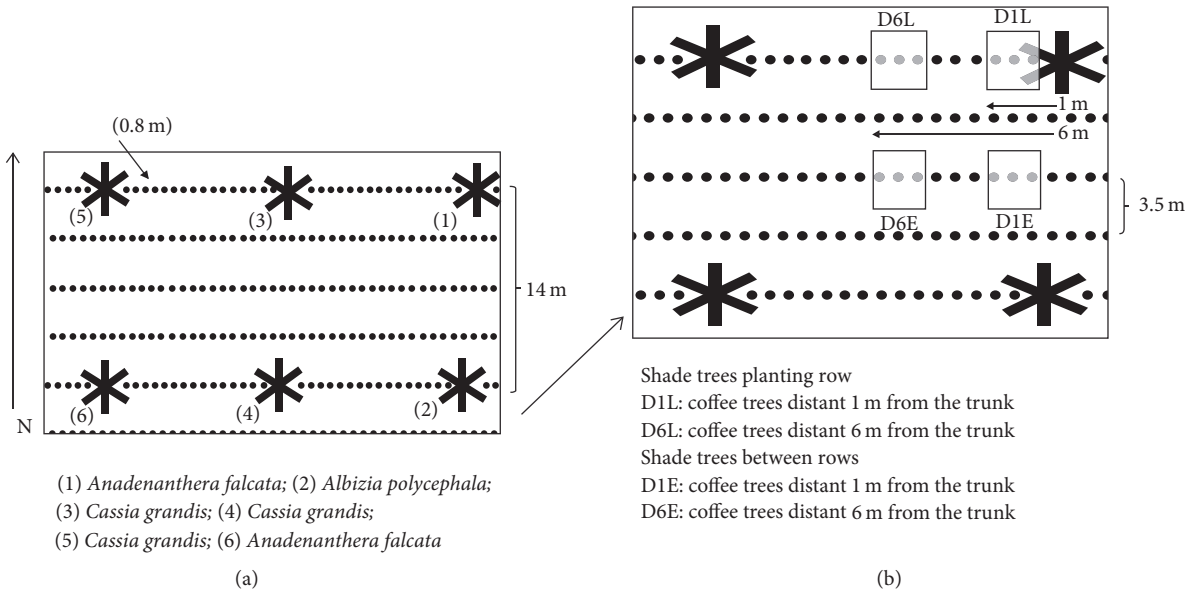


Figure 1: Graphical representation of the shaded coffee plantation (a); detailing of the treatments (b). Santo Antônio do Jardim, SP, 2015.

Table 1: Climate data observed during the months in which the experiment was conducted. Santo Antônio do Jardim, SP, 2015.

Months	RD	PREC (mm)		*T* (°C)			RH (%)		
	Total	Total	Average	Min	Max	Average	Min	Max	Average
Feb	14	348	12.4	19.9	28.3	24.1	80	94	87
Mar	18	475	15.3	18.1	26.6	22.3	87	98	92
Apr	4	165	5.5	17.5	27.2	22.3	70	93	81
May	7	123	4.0	14.6	23.1	18.8	88	98	93
Jun	3	27	0.9	14.7	24.4	19.5	89	95	92
Jul	2	30	1.0	14.5	24.4	19.4	90	95	92
Aug	1	10	0.3	15.0	25.7	20.3	78	92	85

RD: rain days; PREC: precipitation; *T*: temperature; RH: relative air humidity. Source: meteorological station of the Retiro Santo Antônio farm (2015).

cover by trees. In the farthest points from the shade trees, microclimate conditions are rather similar to the ones in full-sun systems [13].

Therefore, this work aims to evaluate the influence of shade trees' spatial distribution on growth and productivity of coffee trees as well as on coffee quality.

2. Materials and Methods

2.1. Research Site. The research took place between April and August 2015 at Retiro Santo Antônio farm, located in Santo Antônio do Jardim, São Paulo state, Brazil (22°06′S and 46°40′W, 850 m above sea level). The climatic conditions were categorized by Köppen [14] as Cwb, temperate with dry winters and warm summers. Maximum and minimum temperature and air relative humidity are shown in Table 1, as well as rainfall volumes. Soil was classified as Red-Yellow Argisol [15]. Soil chemical characteristics are described in Table 2.

A site implanted in 2007 with the coffee variety of Obatã Vermelho and the arrangement of 0.8 × 3.5 meters (3,570 plants ha^{-1}) was used. Shade trees were implanted in 2009. The species used were *Anadenanthera falcata*, *Albizia polycephala,* and *Cassia grandis* with the arrangement of 15.0 × 14.0 meters (44 plants ha^{-1}) (Figure 1).

Fertilization was carried out according to soil chemical characteristics and Raij et al. [16] recommendation. Protected urea was applied three times a year, in the dosage of 50 g per plant, the last applications being done in November and December 2014 and February 2015; potassium chloride was applied twice a year, 10 g per plant, the last one being realized in February 2015; chicken litter and straw of coffee were applied once a year in the dosage of 1 kg per plant, the last applied in October 2014. No fertilization with phosphorus or boron was used during the experiment.

2.2. Treatments and Experimental Design. The experiment design was of completely randomized blocks with six repetitions and four treatments, spatially distributed due to shade trees' localization: coffee trees on shade trees planting rows, distant one meter from the trunk (D1L); coffee trees on shade trees planting rows, distant six meters from the trunk (D6L); coffee plants between the rows of shade trees, parallel to

Table 2: Soil chemical characterization in depths of 0,0–0,20 m and 0,20–0,40 cm of shaded coffee plantations. Santo Antônio do Jardim, SP, 2015.

Depth	Unit	0,0–0,20 m	0,20–0,40 m
pH	$CaCl_2$	5.12	5.20
P	$Mg{\cdot}dm^3$	36.13	30.00
M·O	$g{\cdot}dm^3$	23.96	21.92
K	$mmol_c{\cdot}dm^{-3}$	2.96	3.06
Ca	$mmol_c{\cdot}dm^{-3}$	29.80	24.94
Mg	$mmol_c{\cdot}dm^{-3}$	13.16	11.28
H + Al	$mmol_c{\cdot}dm^{-3}$	27.54	34.63
Al	$mmol_c{\cdot}dm^{-3}$	1.54	2.48
SB	$mmol_c{\cdot}dm^{-3}$	45.94	39.29
CEC	$mmol_c{\cdot}dm^{-3}$	73.48	73.89
V	%	63.60	54.88

SB: sum of bases; CEC: cation-exchange capacity; V: base saturation.

treatments D1L and D6L (D1E and D6E) (Figure 1). The plot consisted of three consecutive coffee trees.

2.3. Evaluations

2.3.1. Phenological Evolution. To determine the ideal time to harvest, the phenological evolution of coffee cherries was tracked once the endosperm expansion has finished. Pezzopane et al.'s [17] methodology was adapted to contemplate only cherry phenological evolution, (1) green, (2) yellowish green, (3) cherry, and (4) overripe. Four productive branches, two in each exposition side to the sun, were marked with a plastic ribbon so the same cherries were always evaluated. The evaluations took place on April 24th, May 1st and 14th, and July 28th.

2.3.2. Coffee Trees' Agronomical Characteristics and Brix Degree. Coffee trees' agronomical characteristics were evaluated previous to harvesting. Plant height, given in meters, was measured from the insertion of the orthotropic branch in the ground to the apical bud. Canopy diameter, in meters, was measured perpendicular to planting rows, measuring the farthest distance between the first pair of leaves from opposites plagiotropic branches. Finally, plagiotropic branch length per plant, given in centimeters, was the mean of ten random branches.

Brix degree evaluation used the juice of three ripe cherries per plot. Cherries were squished in the optical reader of a portable refractometer. The results were given in °Bx; each degree corresponds to one gram of sucrose in 100 grams of solution.

2.3.3. Coffee Yield. Harvest was conducted manually by strip-picking between July 28 and 30, when more than half of the cherries in the hole experiment were ripe. Cherries were washed, to remove impurities, and separated into unripe fruits (UF), ripe fruits (RF), and overripe fruits (OF). Each group of cherries was weighed, in a semianalytic balance machine, separately and together, so the total weight was revealed. Cherries volume was measured with a 2,000 ml graduated cylinder.

Posteriorly, cherries were allocated separately in plastic net bags and naturally dried in the sun over a slab of concrete until 13% of humidity was reached. Then, coffee beans were mechanically peeled (Carmomaq, Tecnologia e Inovação para a Indústria do Café, model: DRC 1, number: 9498, year: 2012); in this operation, the parchment was also removed and once more weighed. Immediately after, beans' humidity was determined with a benchtop grain moisture tester (Moisture and Purity Tester G650, Gehaka Agri).

2.3.4. Coffee Quality. Samples of 300 g of green coffee beans of each plot and each cherry stage (unripe, ripe, and overripe) were analyzed by the Qualicafex Specialty Coffees company. There, the percentage of defect (black, unripe, and insect damaged beans), sieve retention, and cup quality were assessed. Cup quality test follows the SCCA (Specialty Coffee Association of America) guidelines and the notes are given according to Brazilian law (Brazil, 2003). In Brazil, Arabic coffee quality is divided into seven subgroups. In order from the best for wort quality, the subgroups are (i) strictly soft: coffee that has beverage with all the characteristics of aroma and taste of the soft beverage, but strongly accentuated; (ii) soft: coffee beverage with smooth, sweet, and pleasant taste and aroma; (iii) softish: coffee beverage with weakly sweet and smooth, but still with no signs of astringency; (iv) hard: coffee beverage with acrid and astringency taste, but still with no signs of odd flavors; (v) Rioysh: coffee beverage with soft flavor, slightly resembling iodoform; vi) Rio: coffee beverage with typical flavor of iodoform; (vii) Rio Zona: coffee beverage with strongly accentuated aroma and taste, strongly resembling iodoform or phenic acid and repugnant flavor [18].

To determine the percentage of defect, a subsample of 100 g had the defects picked up manually and weighed. The percentages were given dividing the mass of each defect by the initial mass. Sieve retention also used 100 g subsample and, similarly, the percentage was given by dividing the retained mass by the original mass. Samples were passed through sieves 13 (5.15 mm mesh) and 17 (6.75 mm mesh).

2.4. Statistical Analyses. The data expressed in percentage was transformed using the function $y = \arcsin(\sqrt{x}/100)$. The

percentage of defects, sieve retention, plant height, canopy diameter, plagiotropic branch length, and yield were submitted to ANOVA test, and when they were significantly distinct the means were compared with the Tukey test at $P \leq 0.05$. The remaining data was analyzed descriptively.

3. Results and Discussion

Coffee trees' plant height, canopy diameter, and plagiotropic branch length did not differ between the treatments (Table 3). Such a fact, probably, is due to low variation in light intensity among the systems. Ricci et al. [4] related that in systems with low soil cover and little variation in shadows' levels, such as coffee trees shaded with *Erythrina*, which cover only 2 to 6% of the soil, external morphological changes are not observed in coffee trees. Pezzopane et al. [19] evaluating coffee trees shaded with banana trees also did not find differences in plants' heights on points among the systems, closer or farther from shade trees.

However, the same authors found differences in canopy diameters of coffee trees closer to the banana trees. Ricci et al. [20] observed increases in plant height and canopy diameter of coffee trees in shaded systems when compared with full-sun ones. The greater plant height and canopy diameter represent the coffee tree's effort to compensate for the less light availability under shade, as an attempt to reach solar energy [21].

The treatment closest to shade trees (D1L) promoted faster maturation of cherries and the treatment farther from shade trees (D6E) was the latest to ripen (Figure 2). This result differs from the one mentioned by Ricci et al. [22] that reported later maturation in Obatã coffee in agroforest systems with *Erythrina* and banana trees. But this result is similar to the one found by Lunz et al. [9] that showed later and nonuniform maturation when solar exposition of coffee cherries of Obatã was greater due to distancing of coffee trees from shade trees.

Regardless of that, maturation speed is reduced by shadow [10], being an important factor for cup quality increase. This is a result, mostly, of reduction of average temperature, as is the case in high "mountain" coffee, which presents a better cup quality [10]. So, it is possible that maturation ends early not because it was sped up, but due to early flowering. According to DaMatta et al. [23], the flower bud remains dormant until the, so-called, "blossom showers" when flowers finish their growth and blossom. Therefore, in a point close to shade trees, soil moisture may have been conserved as shown by Dhanya et al. [24], and the accumulation of smaller rainfall may have been enough to stimulate blossom at these points. This is corroborated by Lunz et al. [9] that identified latter blossoming in more distant coffee plants of shade trees.

Another hypothesis for the early maturation could be competition for water among coffee trees and shade trees due to shared soil volume by roots of both species in D1L, such as the case observed by Coelho et al. [25]. According to Morais et al. [26], hydric stress associated with high temperature can quicken cherries maturation. However, Morinigo [27], working in the same site, period, and plots, did not find any difference in soil moisture in 0,0–20 and 0,20–0,40 m depths.

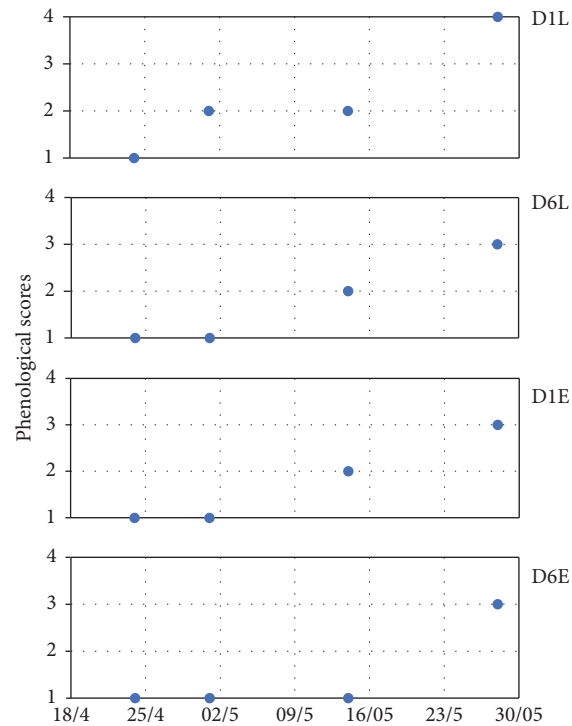


Figure 2: Coffee fruits' phenological stages evolution of shaded coffee plants due to shade trees' spatial distribution. Santo Antônio do Jardim, SP, 2015. Phenological scores: (1) green, (2) yellowish green, (3) cherry, and (4) overripe. D1L: coffee trees on shade trees planting rows, distant one meter from the trunk; D6L: coffee trees on shade trees planting rows, distant six meters from the trunk; D1E: coffee plants between the rows of shade trees, parallel to treatment D1L; D6E: coffee plants between the rows of shade trees, parallel to treatment D6L.

Treatments also did not affect Brix degree of ripe cherries. In the closest treatment to shade trees, D1L, the mean was 19.8°Bx, the intermediate points D6L and D1E showed 21.1°Bx and 19.2°Bx, respectively, and in D6L, the furthest point from shade trees was 18.3°Bx. Similar values were found by Silva et al. [28]. However, those authors consider Brix degree as a poor tool to predict cup quality.

The total yields of cherries and of unripe, ripe, and overripe cherries in mass and volume were not affected by shade trees' spatial distribution (Table 4). There was not any difference in the treatment of green coffee beans in total or in any of the maturation stages of cherries (Table 5).

Those results contradict the ones found by Raij et al. [16] that identified loss of yield in coffee trees near shading banana trees. Yield in this work was greater than the Brazilian average, of 22.49 sacks ha^{-1} [29]. It is worthwhile to highlight that, in highly technified systems of shaded coffee, researches in Brazil showed high yields, such as 141 sacks ha^{-1} in coffee plants shaded by *Swietenia macrophylla* in the Federal District. On the other hand, in native forest coffee yields were a little over 4 sacks ha^{-1} in Minas Gerais [30].

No differences among the treatments were found regarding the percentages of defects (Table 6). Unripe harvested cherries presented a greater percentage of unripe bean defect,

Table 3: Plant height (m), canopy diameter (m), and plagiotropic branch length (cm) of coffee trees due to shade trees spatial distribution. Santo Antônio do Jardim, SP, 2015.

Treatments	Plant height	Canopy diameter	Branch length
	m		cm
D1L	1.49^{ns}	1.45^{ns}	19.56^{ns}
D6L	1.46	1.43	17.12
D1E	1.70	1.50	19.36
D6E	1.57	1.41	18.11
VC%	14.85	9.51	8.58

ns: nonsignificant according to F test at 5% of significance. D1L: coffee trees on shade trees planting rows, distant one meter from the trunk; D6L: coffee trees on shade trees planting rows, distant six meters from the trunk; D1E: coffee plants between the rows of shade trees, parallel to treatment D1L; D6E: coffee plants between the rows of shade trees, parallel to treatment D6L. VC (%): variation coefficient.

Table 4: Total coffee fruits production after harvesting and production of unripe fruits (UF), ripe fruits (RF), and overripe fruits (OF) after washing and fruit separation due to shade trees spatial distribution. Santo Antônio do Jardim, SP, 2015.

	UF	RF	OF	Total (UF + RF + OF)
		Coffee fruits		
		kg·plant^{-1}		
D1L	0.489^{ns}	1.262^{ns}	1.009^{ns}	2.760^{ns}
D6L	0.676	1.267	1.072	3.015
D1E	0.744	1.260	0.680	2.684
D6E	0.845	1.680	0.866	3.391
VC%	46.07	37.04	38.79	34.18
		Volume per plant		
		L·plant^{-1}		
D1L	0,850^{ns}	1,983^{ns}	1,900^{ns}	4,733^{ns}
D6L	1,178	2,044	1,967	5,189
D1E	1,244	2,039	1,183	4,467
D6E	1,400	2,811	1,356	5,567
VC%	41,80	35,95	47,96	35,42

ns: nonsignificant according to F test at 5% of significance. D1L: coffee trees on shade trees planting rows, distant one meter from the trunk; D6L: coffee trees on shade trees planting rows, distant six meters from the trunk; D1E: coffee plants between the rows of shade trees, parallel to treatment D1L; D6E: coffee plants between the rows of shade trees, parallel to treatment D6L. VC (%): variation coefficient.

which was expected. However, that defect was recurrent in all cherries stages of maturation, which is the same as the results found by Carvalho et al. [31]. Black beans were also found in all treatments; still they were more significant in unripe harvested cherries. The fermentation that causes these defects is greater in unripe cherries, once they have higher water content [32].

All treatments presented low percentages, close to zero, of insect damaged beans, 0.3% for D1L, 0.0% for D6L, 1.0% for D1E, and 0.3 for D6E (Table 6). According to Beer et al. [5], trees may be the habitat to natural enemies that control the borer. Besides, the same authors report experiences where the entomopathogenic fungi *Beauveria bassiana*, which were inoculated in the research site, had better persistence due to shade trees.

The variable bean size, represented by the sieve retention, also had no effect on the treatments due to shade trees' spatial distribution (Table 7). There is consensus in the literature that coffee beans enlarge due to shade [9–12, 22, 33]. Still, Muschler [11] observed that, in pollarded systems with *Erythrina* as a shade tree, coffee beans were greater than in systems with heavier shadows. The author then hypothesizes that closer to the shade tree coffee beans were bigger enough to bring the mean up and benefit the system as a whole. However, the same effect was not observed in this work.

Unripe harvested cherries showed a greater percent of small beans (Table 7). This can be explained by the immaturity of cherries. The endosperm is filled until approximately the 17th week after blossoming [23], and during the cherries separation by maturation, all immature fruits are considered unripe, even the ones that have not finished endosperm filling.

Besides, defective beans are smaller than those of high quality [34]. So, beans coming from unripe cherries, the ones that showed the most defects, should be smaller.

To ripe cherries, the worst cup quality was found in D6E, as hard, while the remaining treatments presented beverage softish to D6L and soft to D1L and D1E (Table 8). This result

Table 5: Green coffee production due to shade trees spatial distribution. Santo Antônio do Jardim, SP, 2015.

	UF	RF	OF	Total (UF + RF + OF)
			kg·plant^{-1}	
D1L	0.084^{ns}	0.232^{ns}	0.358^{ns}	0.674^{ns}
D6L	0.122	0.217	0.355	0.694
D1E	0.125	0.228	0.236	0.589
D6E	0.150	0.288	0.253	0.691
VC%	43.61	44.67	37.97	33.53
			sacks·ha^{-1}	
D1L	5.00^{ns}	13.79^{ns}	21.30^{ns}	40.10^{ns}
D6L	7.28	12.91	21.10	41.30
D1E	7.42	13.54	14.02	35.00
D6E	8.94	17.16	14.45	40.60
VC%	43.68	44.68	37.89	33.50
			kg·ha^{-1}	
D1L	299.96^{ns}	827.28^{ns}	1277.82^{ns}	2405.07^{ns}
D6L	436.85	774.74	1265.92	2477.68
D1E	445.18	812.40	840.97	2098.56
D6E	536.25	1029.64	867.16	2433.04
VC%	43.68	44.68	37.89	33.50

ns: nonsignificant according to F test at 5% of significance. D1L: coffee trees on shade trees planting rows, distant one meter from the trunk; D6L: coffee trees on shade trees planting rows, distant six meters from the trunk; D1E: coffee plants between the rows of shade trees, parallel to treatment D1L; D6E: coffee plants between the rows of shade trees, parallel to treatment D6L. VC (%): variation coefficient.

Table 6: Percentage of black, unripe, and insect damaged beans in samples of unripe fruits (UF), ripe fruits (RF), and overripe fruits (OF) of shaded coffee trees due to shade trees spatial distribution. Santo Antônio do Jardim, SP, 2015.

	Black beans			Unripe beans			Insect damaged beans		
	UF	RF	OF	UF	RF	OF	UF	RF	OF
					%				
D1L	14^{ns}	0^{ns}	0^{ns}	29^{ns}	4^{ns}	4^{ns}	0^{ns}	0^{ns}	1^{ns}
D6L	17	1	0	22	5	3	0	0	0
D1E	15	1	1	35	5	5	0	0	3
D6E	14	0	1	26	5	4	1	0	0
VC (%)	14.9	23.7	25.3	7.8	30.0	33.4	13.9	0.0	34.3

ns: nonsignificant according to F test at 5% of significance. D1L: coffee trees on shade trees planting rows, distant one meter from the trunk; D6L: coffee trees on shade trees planting rows, distant six meters from the trunk; D1E: coffee plants between the rows of shade trees, parallel to treatment D1L; D6E: coffee plants between the rows of shade trees, parallel to treatment D6L. VC (%): variation coefficient.

Table 7: Percentage of coffee beans of size smaller than sieve 13 and greater than sieve 17 of shaded coffee trees' unripe, ripe, and overripe fruits due to shade trees spatial distributions. Santo Antônio do Jardim, SP, 2015.

	Beans < 13			Beans > 17		
	UF	RF	OF	UF	RF	OF
			%			
D1L	45^{ns}	11^{ns}	14^{ns}	15^{ns}	19^{ns}	25^{ns}
D6L	52	16	12	11	18	25
D1E	53	16	17	15	9	21
D6E	42	14	13	11	9	21
VC (%)	3.8	26.4	15.0	33.4	30.9	19.6

ns: nonsignificant according to F test at 5% of significance. D1L: coffee trees on shade trees planting rows, distant one meter from the trunk; D6L: coffee trees on shade trees planting rows, distant six meters from the trunk; D1E: coffee plants between the rows of shade trees, parallel to treatment D1L; D6E: coffee plants between the rows of shade trees, parallel to treatment D6L. VC (%): variation coefficient.

Table 8: Beverage scores of samples from unripe fruits (UF), ripe fruits (RF), and overripe (OR) fruits of shaded coffee trees due to shade trees spatial distribution. Santo Antônio do Jardim, SP, 2015.

	Cup quality		
	UF	RF	OF
D1L	Just soft	Soft	Hard
D6L	Hard	Just soft	Soft
D1E	Hard	Soft	Soft
D6E	Hard	Hard	Soft

D1L: coffee trees on shade trees planting rows, distant one meter from the trunk; D6L: coffee trees on shade trees planting rows, distant six meters from the trunk; D1E: coffee plants between the rows of shade trees, parallel to treatment D1L; D6E: coffee plants between the rows of shade trees, parallel to treatment D6L.

was expected once cup quality is well correlated with shadows [10]. Also, in a more distant point from shade trees, microclimate may be similar to full-sun systems [13].

Among overripe cherries, the worst quality was found in D1L, as hard, and the remaining treatments presented beverage as soft. Once again, this was expected, since maturation was over first in these treatments (Figure 2). Those cherries were, probably, exposed to more adverse weather, since they stayed longer after maturation. Increasing the exposure to opportunist microbes and possibility of fermentation while the cherries are still attached to the coffee tree affects quality [35]. Iamanaka et al. [36] found that cherries that are overripe and still attached to the coffee plant presented the worst beverage quality and greater infestation of pathogenic fungi.

But when the cherries were overripe and do not stay long exposed to weather, they did not differ from those harvested on plane maturation [37]. This explains the best quality of overripe cherries on D6L and D1E. According to Silva et al. [28], the harvested unripe cherries present the worst cup quality, as found in this work, as they are just as soft to D1L and hard to the remaining treatments.

4. Conclusion

Shade trees' spatial distribution affects the phenological evolution of cherries and cup quality of coffee trees. Maturation was accelerated in coffee plants closer to shade trees, probably due to anticipated blossom. We recommend monitoring since the blossoming phase to elucidate the results presented here. Also, the best cup quality was obtained in coffee beans coming from coffee trees closer to shade trees, except for overripe cherries.

Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

Acknowledgments

The authors profusely thank Mr. Jefferson Adorno, the owner of Retiro Santo Antônio farm, his family, and staff for the opportunity to learn with their innovative experience at Kaynã Coffee and for all the essential collaborations to the execution of this work.

References

[1] C. G. Z. Teixeira and T. S. Milagres, "Economicidade e certificação da cafeicultura familiar na Zona da Mata de Minas Gerais," *Pesquisa Agropecuária Tropical*, vol. 39, no. 4, pp. 317–329, 2009.

[2] S. P. Pereira, G. F Bartholo, and P. T. G. Guimarães, "Cafés especiais: iniciativas brasileiras e tendências de consumo," *Série Documentos*, vol. 41, 2004.

[3] P. Läderach, T. Oberthür, S. Cook et al., "Systematic agronomic farm management for improved coffee quality," *Field Crops Research*, vol. 120, no. 3, pp. 321–329, 2011.

[4] M. S. F. Ricci, D. G. Cocheto Junior, and F. F. D. Almeida, "Condições microclimáticas, fenologia e morfologia externa de cafeeiros em sistemas arborizados e a pleno sol," *Coffee Science*, vol. 8, no. 3, pp. 379–388, 2013.

[5] J. Beer, R. Muschler, D. Kass, and E. Somarriba, "Shade management in coffee and cacao plantations," *Agroforestry Systems*, vol. 38, no. 1-3, pp. 139–164, 1998.

[6] F. M. DaMatta, "Ecophysiological constraints on the production of shaded and unshaded coffee: A review," *Field Crops Research*, vol. 86, no. 2-3, pp. 99–114, 2004.

[7] A. P. Alvarenga, R. S. Vale, L. Couto, F. A. F. Vale, and A. B. Vale, "Aspectos fisiológicos da cultura do café e seu potencial produtivo em sistemas agroflorestais," *Agrossilvicultura*, vol. 1, no. 2, pp. 195–202, 2004.

[8] M. M. Campanha, R. H. Santos, G. B. Freitas, H. E. Martinez, C. Jaramillo-Botero, and S. L. Garcia, "Análise comparativa das características da serrapilheira e do solo em cafezais (Coffea arabica L.) cultivados em sistema agroflorestal e em monocultura, na Zona da Mata MG," *Revista Árvore*, vol. 31, no. 5, pp. 805–812, 2007.

[9] A. M. P. Lunz, M. S. Bernardes, C. A. Righi, J. D. Costa, J. L. Favarin, and J. G. Cortez, "Qualidade do café arábica em sistema agroflorestal de seringueira (Hevea brasiliensis Müell. Arg.) e em monocultivo in," in *IV Simpósio de Pesquisa dos Cafés do Brasil*, Anais. . . Londrina, Embrapa Café, 2005.

[10] P. Vaast, B. Bertrand, J.-J. Perriot, B. Guyot, and M. Génard, "Fruit thinning and shade improve bean characteristics and beverage quality of coffee (Coffea arabica L.) under optimal conditions," *Journal of the Science of Food and Agriculture*, vol. 86, no. 2, pp. 197–204, 2006.

[11] R. G. Muschler, "Shade improves coffee quality in a sub-optimal coffee-zone of Costa Rica," *Agroforestry Systems*, vol. 51, no. 2, pp. 131–139, 2001.

[12] A. S. Bosselmann, K. Dons, T. Oberthur, C. S. Olsen, A. Raebild, and H. Usma, "The influence of shade trees on coffee quality

in small holder coffee agroforestry systems in Southern Colombia," *Agriculture, Ecosystems & Environment*, vol. 129, no. 1-3, pp. 253–260, 2009.

[13] J. R. M. Pezzopane, M. M. S. Marsetti, W. R. Ferrari, and J. E. M. Pezzopane, "Microclimatic alterations in a conilon coffee crop grown shaded by green dwarf coconut trees," *Revista Ciência Agronômica*, vol. 42, no. 4, pp. 865–871, 2011.

[14] W. K and W. Köppen, *Climatologia: con un estudio de los climas de tierra, Mexico: Fondo de cultura económica*, Fondo de cultura económica, Mexico, 1 edition, 1948.

[15] N. d. Guimarães, A. d. Gallo, A. Fontanetti et al., "Biomassa e atividade microbiana do solo em diferentes sistemas de cultivo do cafeeiro," *Revista de Ciências Agrárias*, vol. 40, no. 1, pp. 34–44, 2017.

[16] B. van Raij, H. Cantarella, J. A. Quaggio, and A. M. C. Furlani, "Recomendação de Adubação e Calagem para o Estado de São Paulo, Instituto Agronômico - Fundação IAC," in *Recomendação de Adubação e Calagem para o Estado de São Paulo*, p. 285, Instituto Agronômico - Fundação IAC, Campinas, 1996.

[17] J. R. Pezzopane, M. J. Pedro Júnior, R. A. Thomaziello, and M. B. Camargo, "Escala para avaliação de estádios fenológicos do cafeeiro arábica," *Bragantia*, vol. 62, no. 3, pp. 499–505, 2003.

[18] Brazil. Ministério da Agricultura, Pecuária e Abastecimento. Intrução Normativa n. 8, de 11 de junho de 2003. Diário oficial da União, poder Executivo, Brasília, 13 jun. 2003.

[19] J. R. Pezzopane, M. J. Pedro Júnior, P. B. Gallo, M. B. Carmargo, and L. C. Fazuoli, "Avaliações fenológicas e agronômicas em café arábica cultivado a pleno sol e consorciado com banana 'Prata Anã'," *Bragantia*, vol. 66, no. 4, pp. 701–709, 2007.

[20] E. Vivier, D. H. Raulet, A. Moretta et al., "Innate or adaptive immunity? The example of natural killer cells," *Science*, vol. 331, no. 6013, pp. 44–49, 2011.

[21] J. I. Fahl, M. L. C. Carelli, J. Vega, and A. C. Magalhaes, "Nitrogen and irradiance levels affecting net photosynthesis and growth of young coffee plants (Coffea arabica L.)," *Journal of Horticultural Science*, vol. 69, no. 1, pp. 161–169, 1994.

[22] M. d. Ricci, J. R. Costa, A. N. Pinto, and V. L. Santos, "Cultivo orgânico de cultivares de café a pleno sol e sombreado," *Pesquisa Agropecuária Brasileira*, vol. 41, no. 4, pp. 569–575, 2006.

[23] F. M. DaMatta, C. P. Ronchi, M. Maestri, and R. S. Barros, "Ecophysiology of coffee growth and production," *Brazilian Journal of Plant Physiology*, vol. 19, no. 4, pp. 485–510, 2007.

[24] B. Dhanya, B. N. Sathish, S. Viswanath, and S. Purushothaman, "Ecosystem services of native trees: Experiences from two traditional agroforestry systems in Karnataka, Southern India," *International Journal of Biodiversity Science, Ecosystem Services & Management*, vol. 10, no. 2, pp. 101–111, 2014.

[25] R. A. Coelho, S. N. Matsumoto, C. L. Lemos, and F. A. Souza, "Nível de sombreamento, umidade do solo e morfologia do cafeeiro em sistemas agroflorestais," *Revista Ceres*, vol. 57, no. 1, pp. 95–102, 2010.

[26] H. Morais, P. H. Caramori, M. S. Koguishi, J. C. Gomes, and A. M. Ribeiro, "Sombreamento de cafeeiros durante o desenvolvimento das gemas florais e seus efeitos sobre a frutificação e produção," *Ciência Rural*, vol. 39, no. 2, pp. 400–406, 2009.

[27] K. P. Morinigo, N. D. Guimarães, R. Stolf et al., "Efeitos da distribuição de árvores sobre atributos do solo em cafeeiro sombreado," *Coffee Science*, vol. 12, no. 4, p. 517, 2017.

[28] S. D. A. Silva, D. M. de Queiroz, F. D. A. C. Pinto, and N. T. Santos, "Coffee quality and its relationship with Brix degree and colorimetric information of coffee cherries," *Precision Agriculture*, vol. 15, no. 5, pp. 543–554, 2014.

[29] *Quarto Levantamento*, vol. 2015, 2016, http://www.conab.gov.br/OlalaCMS/uploads/arquivos/15_12_17_09_02_47_boletim_cafe_d440.

[30] C. Jaramillo-Botero, H. E. P. Martinez, and R. H. S. SANTOS, "Características do café (Coffea arabica L.) sombreado no norte da América Latina e no Brasil: análise comparativa," *Coffee Science*, vol. 1, no. 2, pp. 94–102, 2006.

[31] A. Carvalho, R. S. Garrutti, A. A. Teixeira, L. M. Pupo, and L. C. Monaco, "Ocorrência dos principais defeitos do café em várias fases de maturação dos frutos," *Bragantia*, vol. 29, no. unico, pp. 207–220, 1970.

[32] A. A. Custódio, N. M. Gomes, and L. A. Lima, "Efeito da irrigação sobre a classificação do café," *Engenharia Agrícola*, vol. 27, no. 3, pp. 391–701, 2007.

[33] A. J. J. Souza, S. N. Matsumoto, M. R. Malta, and R. J. Guimarães, "Qualidade do café arborizado e a pleno sol, em manejo pós-colheita no sudoeste da Bahia," *Coffee Science*, vol. 8, no. 2, pp. 109–120, 2013.

[34] J. C. F. Mendonça, A. S. Franca, and L. S. Oliveira, "Physical characterization of non-defective and defective Arabica and Robusta coffees before and after roasting," *Journal of Food Engineering*, vol. 92, no. 4, pp. 474–479, 2009.

[35] G. S. Giomo, "Uma boa pós-colheita é segredo da qualidade," *A Lavoura*, vol. 115, no. 688, pp. 12–21, 2012.

[36] B. T. Iamanaka, A. A. Teixeira, A. R. R. Teixeira, M. V. Copetti, N. Bragagnolo, and M. H. Taniwaki, "Reprint of "The mycobiota of coffee beans and its influence on the coffee beverage"," *Food Research International*, vol. 61, pp. 33–38, 2014.

[37] R. d. Garruti and A. G. Gomes, "Influência do estado de maturação sôbre a qualidade da bebida do café na região do vale do paraíba," *Bragantia*, vol. 20, no. unico, pp. 989–995, 1961.

Hindawi
Journal of Food Quality
Volume 2018, Article ID 3285452, 7 pages
https://doi.org/10.1155/2018/3285452

Research Article

Propositions on the Optimal Number of Q-Graders and R-Graders

Lucas Louzada Pereira,[1] **Rogério Carvalho Guarçoni**,[2] **Gustavo Soares de Souza**,[2] **Dério Brioschi Junior**,[3] **Taís Rizzo Moreira**,[4] **and Carla Schwengber ten Caten**[1]

[1]*Departamento de Engenharia de Produção e Transportes, Universidade Federal do Rio Grande do Sul (UFRGS), Avenida Osvaldo Aranha 99, 5° Andar, 90035-190 Porto Alegre, RS, Brazil*
[2]*Capixaba Institute for Technical Assistance, Research and Rural Extension, Department of Statistics, Rua Afonso Sarlo 160, Bento Ferreira, 29052-010 Vitória, ES, Brazil*
[3]*Federal Institute of Espírito Santo, Food Science and Technology Program, Rua Elizabeth Minete Perim, S/N, Bairro São Rafael, 29375-000 Venda Nova do Imigrante, ES, Brazil*
[4]*Center of Agricultural Sciences and Engineering, Federal University of Espírito Santo, Alto Universitário, S/N, Guararema, 29500-000 Alegre, ES, Brazil*

Correspondence should be addressed to Lucas Louzada Pereira; lucaslozada@hotmail.com

Received 14 September 2017; Accepted 13 November 2017; Published 12 February 2018

Academic Editor: Gabriel Henrique Horta de Oliveira

Sensory analysis or cup testing has been widely used in the coffee production chain for the validation of final quality. The tasters are responsible for defining the patterns and qualitative profiles of the drink based on the sensorial analysis and according to their gustatory sensibilities, which are often acquired by professional experience. However, the literature has not discussed in detail the relationship between the number of tasters and the consistency of sensorial analysis. Thus, using the bootstrap simulation methodology to estimate the optimum plot size, this study quantifies and proposes a specific number of tasters for the process of sensorial analysis of specialty coffees. The results indicate that the use of 6 tasters is sufficient to conduct sensorial analysis following SCA and BSCA protocol for coffees in the Arabica group, as well as 6 tasters for coil and Conilon coffees. From this number, no gains in precision are observed in the process of sensorial analysis of coffee with addition tasters.

1. Introduction

In the coffee industry, the tasting procedure is used to negotiate the price based on the quality of the drink, which is described by the tasters using personal opinion and tasting experience accumulated over the years [1]. Although the tasting process is widely used, for Di Donfrancesco et al. [2], this is not the best method to evaluate coffee quality, due to a range of factors that interfere with the tasting process.

According to Alvarado and Linnemann [3], the "taster" is a judge who performs the sensory evaluation, and this agent is in charge of evaluating the quality of the coffee and, consequently, the evaluation of quality is influenced by his sensorial perceptions.

The taster tends to prefer one sensory profile to the detriment of others or even according to commercial and industrial standards in order to meet the demand of certain clients. This sensorial classification of coffees is based on the taste of the cup. Tasting technique may vary and/or be modified based on the location of the tasting [4]. The field of sensory evaluation of coffees grew rapidly in the second half of the twentieth century, along with the expansion of the processed food and consumer products industries. Sensory evaluation is now an irreplaceable tool in the food industry and is used in key sectors in the production of specialty coffees [5].

However, there is no consensus in the literature about the number of testers to be used during sensory analysis of coffee,

as well as the terminology for the profession. Traditionally, judges should be screened for sensory capabilities that meet the requirements of assessments [6]. In companies that have tasters, it is common that the team consists of a quality control leader and assistants; however, in many producing regions (origins/rural areas) the purchasing offices use only one person in this role.

It is known that tasters trained and experienced in sensorial analysis of coffee are not common because the method is not usually taught in colleges and technical schools in Brazil. For Dzung and Dzuan [5], one of the main problems in using experts in sensory evaluation is that the qualification of the tasters is not well defined. In accordance with ISO 856-2, experience is not the only criterion of a specialist; he must also be trained and have high sensory sensitivity.

Some methodologies, such as the Specialty Coffee Association (SCA), Brazil Specialty Coffee Association (BSCA) protocols for Arabica coffee (*Coffea arabica* L.), and the Uganda Coffee Development Authority (UCDA) for Conilon coffee (*Coffea canephora* Pierre ex Froehner), define procedures and provide protocols for sensory evaluation of specialty coffees. These methodologies are commonly adopted in Brazil and the rest of the world for coffee quality contests and scientific studies with applications of sensorial analysis.

The use of trained judges is an important part of the sensory evaluation tradition [6], but for Ross [7] the cost of sensory tests is very expensive, when a large number of specialists is used due to the difficulty of accessing these professionals.

The amount of tasters used in sensorial analysis of coffee may compromise the quality of the study. On the one hand, the use of few testers can cause loss of accuracy of the analysis. On the other hand, the use of many tasters can be expensive because it uses a greater number of tasters than necessary. In addition, an environment with many people can cause external noise, thus compromising the quality of the study, as already demonstrated by Pereira et al. [8].

Therefore, the definition of the number of trained coffee tasters has not been discussed in studies to define the optimal amount of coffee used in sensory analysis. Aiming for greater consistency in the implementation of SCA, BSCA, and UCDA sensory analysis protocols, this article used the bootstrap method [9] to estimate the variation as a function of the number of tasters, which is a statistical technique of resampling with replacement used in several academic fields [10].

Using the framework presented above, the study estimates the optimal number of Q-Graders and R-Graders (the Q Coffee System identifies quality coffees and brings them to market through a credible and verifiable system; a common standard for both Q Arabica (specialty grade) and Q Robusta (Fine Robusta Grade) coffee has resulted in a universally shared language and standard top scoring lots) to be used in sensory tests that adopt SCA, BSCA, and UCDA methodologies.

2. Materials and Methods

2.1. Preparation of Samples. The studies with Q-Graders and R-Graders were conducted and elaborated in the Laboratory of Analysis and Research in Coffee, LAPC, of the Federal Institute of Espírito Santo in 2016. All the coffees (Arabica and Conilon) come from the 2015/2016 harvest.

The roasting was carried out with a roaster Laboratto TGP2, and all the samples were toasted between 8 and 10 minutes. For the standardization of the roasting process, the set of Agtron-SCA disks, the degree of roast of these samples, was chosen among the colors determined by the disks #65 and #55, for both Arabica coffee and Conilon coffee. The roasting process was performed 24 hours in advance, and after the roasting and cooling the samples remained sealed. The grinding was performed after the time of 24 hours of rest after the roasting process according to the methodology of sensorial analysis established by the SCA. The grinding was carried out in electric mill model BUNN G3, with granulometry in 20 meshes, following the US standard.

For the evaluation of the Arabica coffees, the protocols of the SCA [11] and BSCA [12] were used. For the samples of Conilon coffee, the same procedure of roasting, resting, and grinding of Arabica coffee was established, but the UCDA protocol was used for sensory evaluation.

2.2. Method of Sampling. The quality of the Arabica coffee was evaluated using the SCA protocol, and it is expressed through a centesimal numerical scale. The tasting form provides an opportunity to evaluate eleven important attributes for coffee: fragrance/aroma, uniformity, clean cup, sweetness, flavor, acidity, body, aftertaste, balance, and overall and total note. Highly positive results arise from the perception of a balanced set formed by the evaluated attributes. The attributes of Arabica coffee are the same in the two protocols, SCA and BSCA.

The results of this sensory evaluation are established from a scale of 16 (sixteen) units representing quality levels with intervals of 0.25 (one-quarter of a point) between numerical values between "6" and "10." Coffees were considered good from 6.00 to 6.75, very good from 7.00 to 7.75, excellent from 8.00 to 8.75, and exceptional from 9.00 to 10.00 points. The same interval procedure applies to all three protocols. Theoretically, a scale varies from a minimum value of 0 to maximum of 10 points. There are a few differences between the protocols; BSCA begins with 30 points and UCDA has one different score in sweetness regarding SCA protocol. Nevertheless, the results are the same for all coffees; if >80 points, coffee is considered of specialty grade; if <80 points, coffee is considered below specialty quality.

For Conilon coffee, the quality was evaluated using the UCDA protocol, and the following attributes were obtained: total score, fragrance/aroma, flavor, balance, salinity/acidity, body, bitter/sweetness, and clean cup.

In the case of samples of Arabica coffee and Conilon coffee, which were adopted in the experiments, all presented minimum scores of 80 points, ranging from 80 to 84 points, for both groups.

2.3. Conduction of the Experiment with Q-Graders and R-Graders. The tests were performed in a sensorial laboratory under actinic artificial light, at 23°C and with air circulation. The samples were arranged on two rectangular tables of 110 cm in height, 70 cm in width, and 1.35 meters of length.

For the accomplishment of the study, a blank experiment was conducted, composed of 10 tasters who evaluated 20 samples of Arabica coffee, with a minimum grade ≤ 80 points, considered specialty coffee according to SCA, BSCA, and UCDA sensory protocols.

The table of the 20 samples of Conilon coffee was carried out separately from Arabica coffee using the same 10 tasters who evaluated the Arabica coffee samples. In the case of Conilon coffee, the cut-off score of >80 points was used to select the 20 samples for cupping.

All the tasters used in the studies have certifications, either Q-Grader, R-Grader, or COB, as well as experience in performing sensorial analyses with the following protocols: SCA, BSCA, and UCDA.

2.4. Ideal Number of Tasters. For the grouping of the pair numbers of tasters and their respective coefficients of variation $[X, \mathrm{CV}(X)]$, we used the bootstrap method, where 1000 sample simulations were performed with 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 tasters [13].

In order to determine the optimal size of the tasting room, the linear regression method of plateau response was used [14]. The optimal number of tasters was proposed when the linear model becomes a plateau:

$$Y_i = \begin{cases} \beta_0 + \beta_1 X_i + \varepsilon_i & \text{se } X_i \leq X_0 \\ P + \varepsilon_i & \text{se } X_i > X_0. \end{cases} \quad (1)$$

Here Y_i is the response variable, β_0 is the linear coefficient of the linear model of the segment before the plateau, β_1 is the angular coefficient of this same segment, ε_i is the error associated with the ith observation, P is the plateau, and X_0 is the point of attachment of the two segments. P and X_0 should be estimated.

For the statistical analysis, the free software R was used to perform the bootstrap simulations and to obtain the statistics of the method of reaching the optimal number of tasters [15].

3. Results

Tables 1 and 2 show the results obtained from the 1000 sample simulations of the gustatory characteristics for Arabica and Conilon coffees, respectively, using the bootstrap method, with $1, 2, \ldots, 10$ tasters and their respective coefficients of variation.

According to the results presented in Tables 1 and 2, it is evident that the coefficient of variation as a function of the number of Q-Graders and R-Graders decreases up to a certain point, and from there the increase of the number of tasters for the sensorial analysis does not help to increase accuracy.

Figure 1 shows that 6 tasters are required to evaluate total score (a) and fragrance (b) and 5 for flavor (c) and balance (d) of Arabica coffee using the linear regression method plateau.

In the graphs shown in Figure 2, it is estimated that 5 tasters are needed to evaluate the acidity (a), body (b), sweetness (c), and aftertaste (d) characteristics of Arabica coffee, by the same regression method.

The results related to Figure 3 show that 5 tasters are needed to evaluate fragrance (a), flavor (b), and acidity (c) and 6 tasters for sweetness (d) of Conilon coffee.

Figure 4 shows the results regarding the amount of tasters necessary to evaluate the gustatory quality of Conilon coffee and, specifically, that 5 tasters are required to evaluate the attributes aftertaste (a), balance (c), and total score (d) and 6 tasters to evaluate clean cup (b).

4. Discussion

These results confirm the work done by Bhumiratana et al. [16] and Di Donfrancesco et al. [2] who used six trained testers to perform sensorial analysis of coffee. Further, these results are in parallel with Cook et al. [17], who adopted the use of 15 tasters initially and, after more careful selection based on sensorial sensitivity, used 6 tasters for Arabica coffee.

Other authors used a number of tasters inferior to those found and recommended in this study. The works of Bosselmann et al. [18], who adopted 3 tasters, used the SCA methodology to perform the coffee quality analysis, Alvarado and Linnemann [3] used 1 taster and a judge with 12 consumers trained to conduct their study, and Pereira et al. [19] adopted the use of 3 tasters.

In the study by Ribeiro et al. [20], 11 experienced testers were used to perform the sensorial analysis of coffee.

However, many authors do not indicate if the tasters had any type of international standardization in the materials and methods, indicating once again the lack of standardization of the process of sensorial analysis of coffee. In the work of Pereira et al. [19] and Evangelista et al. [21], the authors do not even identify the number of tasters used in their studies to perform the sensorial analysis of coffee.

Relevant considerations proposed by Chambers et al. [22] emphasize that in addition to the minimum number of tasters it is necessary to study the consistency of who is carrying out the analysis. For the authors, the three-member panel, trained and experienced, had smaller residual error equal to one square of the semitrained panel for a sensory analysis study of chicken, turkey, and other birds, indicating that, in addition to number, consistency should be respected and widely observed. This indicates and reinforces the need to use professionals such as Q-Graders and R-Graders, since these professionals are previously trained to perform such activities. This fact is also verified by Chambers et al. [23], because, for the authors, the training time contributes to the level of accuracy of the evaluator, reinforcing the need for evaluators' training.

Thus it is evident that standardized methodologies can give greater robustness to research and academic works that use this technique. Many studies use Q-Graders, R-Graders, and experts in sensory analysis and often do not provide certification of the specialists. It is necessary to demand more veracity of sensory analysis of coffee, which is impossible without consistency in the number of testers.

Nebesny and Budryn [24] have observed that there are differences between the perceptions of women and men

Table 1: Grouping of the different numbers of Q-Graders and their respective coefficients of variation of the sensorial characteristics of the Arabica coffee samples with the SCA and BSCA protocols.

Number of Q-Graders	Coefficient of variation (%)							
	Total note	Fragrance	Flavor	Balance	Acidity	Body	Sweetness	Aftertaste
1	2.19	9.86	5.04	3.34	4.40	3.03	12.98	4.43
2	1.41	6.51	3.52	2.33	3.20	2.13	9.58	3.15
3	1.09	5.07	2.89	1.93	2.67	1.72	7.70	2.55
4	0.89	4.07	2.47	1.65	2.30	1.52	6.55	2.12
5	0.75	3.31	2.28	1.46	2.06	1.35	5.86	2.01
6	0.60	2.82	2.09	1.36	1.90	1.16	5.29	1.84
7	0.53	2.37	1.81	1.21	1.69	1.14	4.94	1.69
8	0.41	1.92	1.79	1.19	1.52	1.00	4.85	1.48
9	0.32	1.43	1.65	1.10	1.58	0.98	4.36	1.49
10	0.32	0.98	1.57	1.06	1.49	0.93	4.21	1.40

Table 2: Grouping of the different numbers of R-Graders and their respective coefficients of variation of the sensorial characteristics of Conilon coffee samples with the UCDA protocol.

Number of R-Graders	Coefficient of variation (%)							
	Total note	Fragrance	Flavor	Balance	Salt/acid	Body	Bitter/sweet	Clean cup
1	3.18	5.99	7.73	3.32	7.74	6.17	6.18	3.87
2	2.25	4.46	5.59	2.53	5.95	4.39	4.77	3.05
3	1.86	3.30	4.68	1.90	4.52	3.58	3.82	2.40
4	1.57	2.87	4.02	1.74	3.97	3.04	3.36	2.13
5	1.39	2.61	3.47	1.51	3.69	2.79	2.86	1.79
6	1.28	2.44	3.23	1.38	3.29	2.51	2.63	1.62
7	1.16	2.34	2.99	1.26	3.03	2.33	2.48	1.53
8	1.10	2.18	2.78	1.24	2.94	2.15	2.47	1.45
9	1.00	1.97	2.69	1.13	2.78	2.01	2.24	1.36
10	0.98	1.84	2.57	1.10	2.65	2.00	2.12	1.31

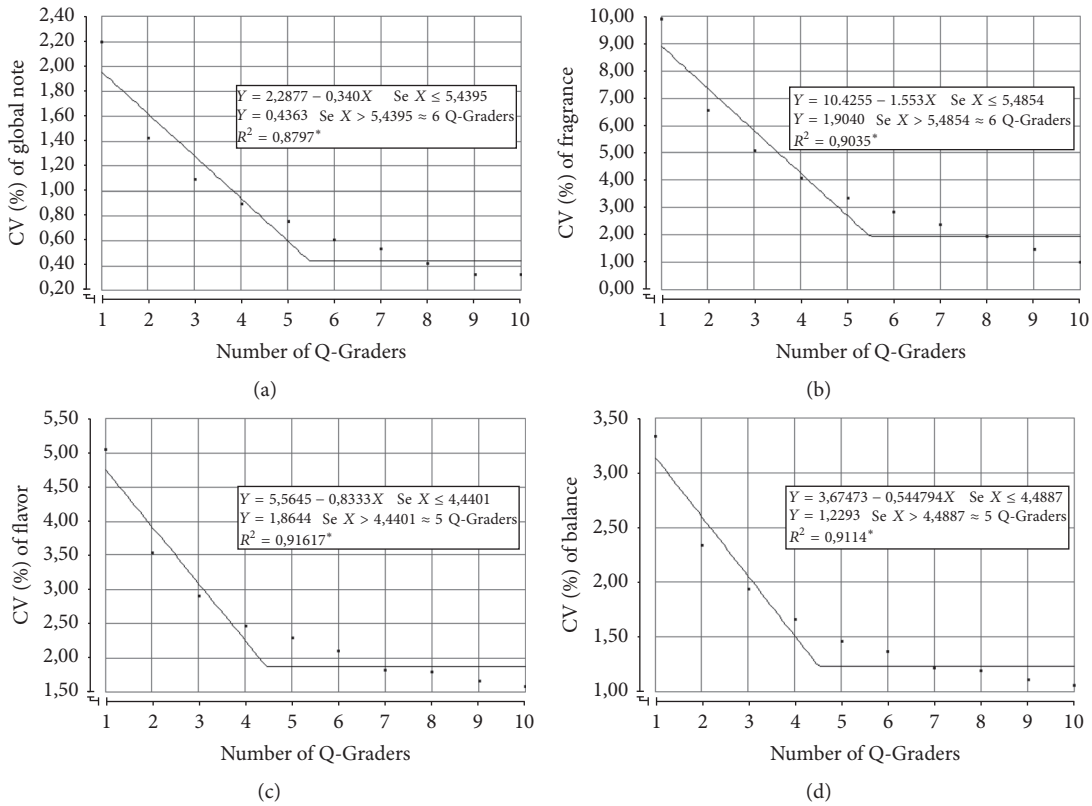


Figure 1: Relation between the coefficient of variation and number of tasters in Arabica coffee for global note (a), fragrance (b), flavor (c), and balance (d). *Significant at 5%, by the F test.

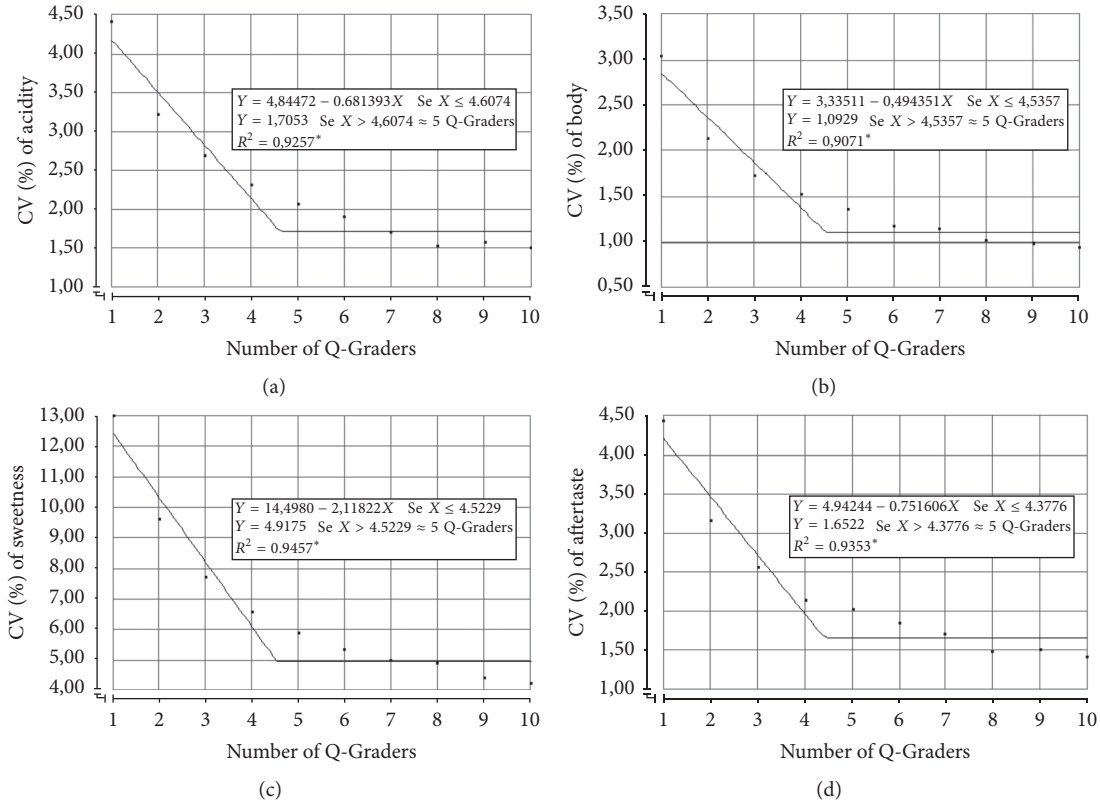


Figure 2: Relationship between the coefficient of variation and number of tasters in Arabica coffee for acidity (a), body (b), sweetness (c), and aftertaste (d). *Significant at 5%, by the F test.

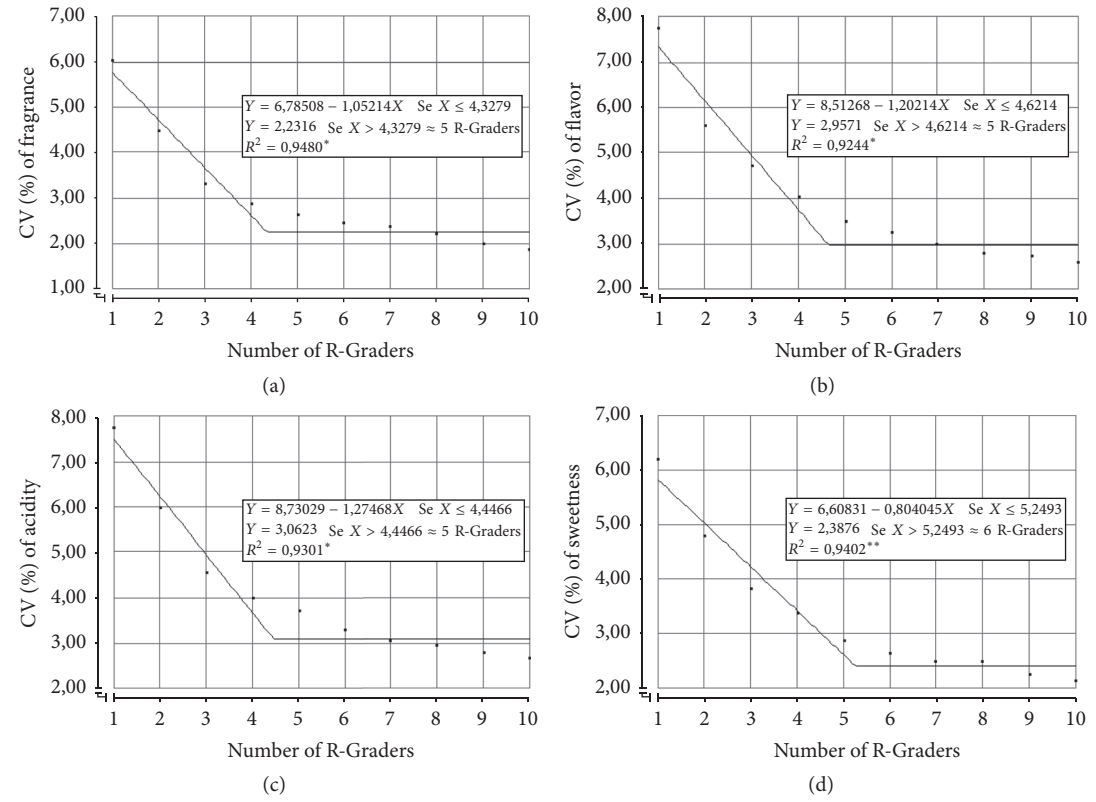


Figure 3: Relation between the coefficient of variation and number of tasters in Robusta/Conilon coffee for fragrance (a), flavor (b), acidity (c), and sweetness (d). *Significant at 5%; **significant at 1%, by the F test.

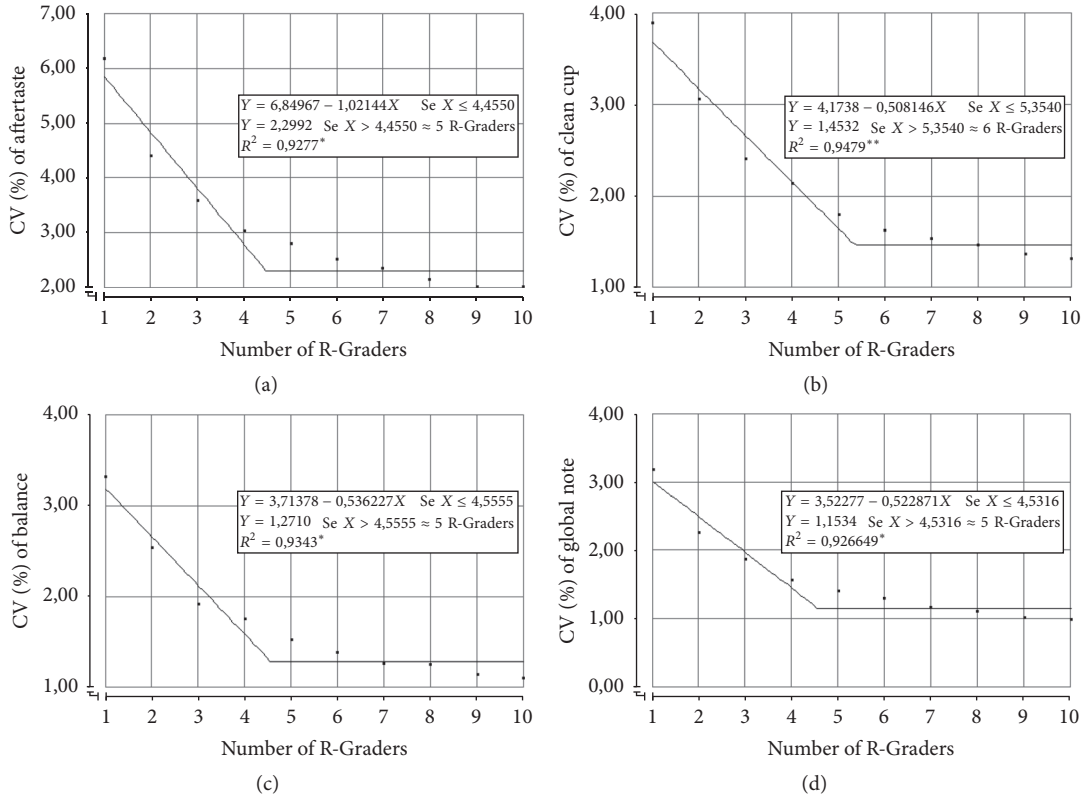


Figure 4: Relation between the coefficient of variation and number of tasters in Robusta/Conilon coffee for aftertaste (a), clean cup (b), balance (c), and global note (d). *Significant at 5%; **significant at 1%, by the F test.

during the sensorial analysis of coffee (in the aroma evaluation question). In the same line, Cook et al. [17] verified that differences in gender and age, as well as psychological factors, have been attributed to the different perceptions of the sensorial analysis. As Pereira et al. [8] have described, the noise factor has been pointed out as one of the greatest villains of the consistency of sensorial analysis with the use of Q-Graders.

It is plausible that judges' perceptions may vary according to these criteria, but this method is more standardized in the process of classification.

In this way, it is possible to express from the results shown that the number of 5 to 6 Q-Graders and/or R-Graders would be sufficient to ensure accuracy of the results of the sensory analysis (cup tests) and that the gains with the same would not be significant with the use of more testers for the decision making. As such, the results presented in Figures 1, 2, 3, and 4 show that the coefficient of variation decreases with more Q-Graders and/or R-Graders in sensory analysis. Moreover, the optimum number of tasters occurred when the linear model becomes a plateau, and the linear regression method of plateau response is a robust tool to quantify and validate the total need of tasters in sensory analysis.

5. Conclusions

The modeling applied in this study allows concluding that according to the data tested, it is possible to recommend the minimum number of evaluators, for these conditions. However, this approach is limited to the data of this study. In the case of limitations on the availability of Q-Graders or R-Graders, the simulation and regression methods with plateau can be a solution model. Based on this the conclusions are as follows.

It is necessary to use 6 or more Q-Graders to perform the sensory analysis with the SCA and BSCA protocol in scientific studies and in routine taste tests for marketing purposes.

For the UCDA protocol, the use of 6 or more R-Graders for the sensory analysis of Conilon coffee is recommended, in research and in sensory analysis for commercialization.

Further studies should be developed about the accuracy and consistency of Q-Graders and R-Graders. In addition, it is necessary to improve and approximate the scientific methods of validation of the level of accuracy of these professionals, so that institutions that offer such courses can raise the level of precision of these professionals through less subjective techniques.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

The authors acknowledge the Federal Institute of Espírito Santo for supporting this research and also the translation

and review of this article, as well as the Q-Graders' and R-Graders' participation, who dedicated themselves to the realization of this study. They also acknowledge National Council for Scientific and Technological Development (469058/2014-5), CNPq, the Secretariat of Professional and Technological Education of the Ministry of Education, SETEC, for the availability of resources for research, and the Credit Unions System in Brazil, SICOOB, for the support and funding of research.

References

[1] A. M. Feria-Morales, "Examining the case of green coffee to illustrate the limitations of grading systems/expert tasters in sensory evaluation for quality control," *Food Quality and Preference*, vol. 13, no. 6, pp. 355–367, 2002.

[2] B. Di Donfrancesco, N. Gutierrez Guzman, and E. Chambers, "Comparison of results from cupping and descriptive sensory analysis of colombian brewed coffee," *Journal of Sensory Studies*, vol. 29, no. 4, pp. 301–311, 2014.

[3] R. A. Alvarado and A. R. Linnemann, "The predictive value of a small consumer panel for coffee-cupper judgment," *British Food Journal*, vol. 112, no. 9, pp. 1023–1032, 2010.

[4] R. N. Dal Molin, M. Andreotti, A. R. Reis, E. Furlani Junior, G. C. Braga, and M. B. Scholz, "Caracterização física e sensorial do café produzido nas condições topoclimáticas de Jesuitas, Paraná," *Acta Scientiarum. Agronomy*, vol. 30, no. 3, pp. 353–358, 2008.

[5] N. H. Dzung and L. Dzuan, "The role of sensory evaluation in food quality control, food research and development: a case of coffee study," 2010, http://www4.hcmut.edu.vn/~dzung/Sensoryrole.pdf.

[6] H. L. Meiselman, "Critical evaluation of sensory techniques," *Food Quality and Preference*, vol. 4, no. 1-2, pp. 33–40, 1993.

[7] C. F. Ross, "Sensory science at the human-machine interface," *Trends in Food Science & Technology*, vol. 20, no. 2, pp. 63–72, 2009.

[8] L. L. Pereira, W. S. Cardoso, R. C. Guarçoni, A. F. A. da Fonseca, T. R. Moreira, and C. S. T. Caten, "The consistency in the sensory analysis of coffees using Q-graders," *European Food Research and Technology*, vol. 243, no. 9, pp. 1545–1554, 2017.

[9] B. Efron and R. J. Tibshirani, *An Introduction to the Bootstrap*, Chapman and Hall-CRC, Washington, DC, USA, 1993.

[10] R. L. Bastos and R. Lemos, *Proposição de testes bootstrap para o índice de qualidade sensorial [Dissertação de Mestrado]*, Universidade Federal de Lavras, UFLA, 2013.

[11] SCA. Specialty Coffee Associationn., "Protocols," 2013, http://www.SCA.org/PDF/resources/cupping-protocols.pdf.

[12] BSCA, Brazil Speciality Coffee Association, Varginha, Brasil, http://bsca.com.br.

[13] F. d. Leonardo, W. E. Pereira, S. d. Silva, R. d. Araújo, and R. M. Mendonça, "Tamanho ótimo da parcela experimental de abacaxizeiro Vitória," *Revista Brasileira de Fruticultura*, vol. 36, no. 4, pp. 909–916, 2014.

[14] P. F. Parnaiba, D. F. Ferreira, and A. R. Morais, "Tamanho ótimo de parcelas experimentais: proposiçãoo de métodos de estimação," *Revista Brasileira de Biometria*, vol. 27, pp. 255–268, 2009.

[15] R Foundation for Statistical Computing, *R Core Team. R: A Language And Environment for Statistical Computing*, R Foundation for Statistical Computing, Vienna, Austria, 2016, https://www.R-project.org.

[16] N. Bhumiratana, K. Adhikari, and E. Chambers, "Evolution of sensory aroma attributes from coffee beans to brewed coffee," *LWT- Food Science and Technology*, vol. 44, no. 10, pp. 2185–2192, 2011.

[17] D. J. Cook, T. A. Hollowood, R. S. T. Linforth, and A. J. Taylor, "Correlating instrumental measurements of texture and flavour release with human perception," *International Journal of Food Science & Technology*, vol. 40, no. 6, pp. 631–641, 2005.

[18] A. S. Bosselmann, K. Dons, T. Oberthur, C. S. Olsen, A. Ræbild, and H. Usma, "The influence of shade trees on coffee quality in small holder coffee agroforestry systems in Southern Colombia," *Agriculture, Ecosystems & Environment*, vol. 129, no. 1-3, pp. 253–260, 2009.

[19] M. C. Pereira, S. M. Chalfoun, G. R. de Carvalho, and T. V. Savian, "Multivariate analysis of sensory characteristics of coffee grains (Coffea arabica L.) in the region of upper Paranaíba," *Acta Scientiarum - Agronomy*, vol. 32, no. 4, pp. 635–641, 2010.

[20] V. S. Ribeiro, A. E. Leitão, J. C. Ramalho, and F. C. Lidon, "Chemical characterization and antioxidant properties of a new coffee blend with cocoa, coffee silverskin and green coffee minimally processed," *Food Research International*, vol. 61, pp. 39–47, 2014.

[21] S. R. Evangelista, C. F. Silva, M. G. P. D. C. Miguel et al., "Improvement of coffee beverage quality by using selected yeasts strains during the fermentation in dry process," *Food Research International*, vol. 61, pp. 183–195, 2014.

[22] E. Chambers, J. A. Bowers, and A. D. Dayton, "Statistical Designs and Panel Training/Experience for Sensory Analysis," *Journal of Food Science*, vol. 46, no. 6, pp. 1902–1906, 1981.

[23] D. H. Chambers, A.-M. A. Allison, and E. Chambers IV, "Training effects on performance of descriptive panelists," *Journal of Sensory Studies*, vol. 19, no. 6, pp. 486–499, 2004.

[24] E. Nebesny and G. Budryn, "Evaluation of sensory attributes of coffee brews from robusta coffee roasted under different conditions," *European Food Research and Technology*, vol. 224, no. 2, pp. 159–165, 2006.

Hindawi
Journal of Food Quality
Volume 2018, Article ID 6408571, 9 pages
https://doi.org/10.1155/2018/6408571

Research Article

Influence of Solar Radiation and Wet Processing on the Final Quality of Arabica Coffee

Lucas Louzada Pereira[1], **Rogério Carvalho Guarçoni**[2], **Wilton Soares Cardoso**[3], **Renato Côrrea Taques**[2], **Taís Rizzo Moreira**[4], **Samuel Ferreira da Silva**[4] and **Carla Schwengber ten Caten**[1]

[1]*Federal University of Rio Grande do Sul, Av. Osvaldo Aranha, n 99/5° Andar, Bom Fim, 90035-190 Porto Alegre, RS, Brazil*
[2]*Capixaba Institute for Technical Assistance, Research and Rural Extension/Department of Statistics, Rua Afonso Sarlo, 160 Bento Ferreira, 29052-010 Vitória, ES, Brazil*
[3]*Federal Institute of Espírito Santo/Food Science and Technology Program, Rua Elizabeth Minete Perim, S/N. Bairro São Rafael. 29375-000 Venda Nova do Imigrante, ES, Brazil*
[4]*Federal University of Espírito Santo/Center of Agricultural Sciences and Engineers, Av. Gov. Lindemberg, 316 Centro, 29550-000 Jerônimo Monteiro, ES, Brazil*

Correspondence should be addressed to Lucas Louzada Pereira; lucaslozada@hotmail.com

Received 17 September 2017; Revised 5 December 2017; Accepted 10 January 2018; Published 11 February 2018

Academic Editor: Silvia C. C. Botelho

The coffee growing in the state of Espírito Santo has some peculiarities that differ from the other regions producing Arabica coffee in Brazil because it has a diversity of edaphoclimatic conditions that influence the final quality of the bean. This study aimed to demonstrate and quantify the effect of solar radiation and of different forms of wet process on the final quality of Arabica coffee in crops located in the altitude range of 950 meters, in order to understand what would be the best wet processing methods for the coffee cultivated to the East (sun-grown) and coffee cultivated to the South-Southeast (shade-grown). The results indicate that shading has a significant effect on the final quality of the Arabica coffee, as well as the type of wet process used to process the beans after harvest. Therefore, there is a need to study in depth the factors related to the processing, edaphoclimatic, and relief conditions inherent to mountain coffee cultivation.

1. Introduction

Arabica coffee in Brazil is usually produced in full sun and in areas that vary from 600 to 1400 meters of altitude, thus creating strata and different sensory perceptions associated with the flavors that each coffee can have due to edaphoclimatic and relief conditions and the amount of solar radiation that it receives.

According to Bosselmann et al. [1] there is a great controversy about the production of shaded and sun-grown coffee. On the other hand, for Somporn et al. [2], little has been discussed about the effect of shading on the final quality of the Arabica coffee. For Pinto Neto et al. [3], the observation of climate change on the planet causes new techniques to be developed, aiming at better adaptation of the crop to the new global climate scenario. Although shading and altitude are empirically known to have beneficial effects on coffee quality, only a few scientific studies have documented these effects [4]. Nevertheless, these kinds of studies need to consider the level of solar radiation as well as the average temperature.

It is known that, normally, coffee of colder regions receives higher grades than samples from warmer regions, regarding taste, aroma, sweetness, and body [5]. This factor is associated with the fact that high temperatures prevent the translocation of chemical compounds to fruits [1, 6, 7].

Another line of research has argued that in addition to the factors mentioned above, it is necessary to study the effects of fermentation in postharvest processing. The works of Silva et

al. [8], Silva et al. [9], and Pereira et al. [10] have discussed the significant effect of yeasts during the fermentation process, and those of Ribeiro et al. [11], Evangelista et al. [12], Masoud and Jespersen [13], and finally De Bruyn et al. [14] discussed the effect of bacteria. These microorganisms were detected in wet fermentation processes of the Arabica coffee, generating significant impacts on coffee quality. Gonzalez-Rios et al. [15] found that the removal of mucilage through degradation in water provided coffees with more fruity, floral, and caramel attributes and characteristics, while the removal of mucilage provided drier, more neutral beverages.

Thus, testing wet processing methods with induced fermentation, associated with the solar radiation index that the crop receives, constitutes an innovative action. Therefore, the hypothesis of this study is as follows: can the incidence of solar radiation, associated with different forms of wet processing with starter cultures, affect the quality of the coffee?

This study had the objective of evaluating the effect of solar radiation, associated with four different forms of wet processing in the sensory quality of Arabic coffee.

2. Materials and Methods

The experiments were conducted on a property located at 950 meters of altitude, as observed in Figure 1, using the variety Catuaí red 44. Table 1 indicates the geographic coordinates, the average duration of annual radiation, the average daily solar radiation for the year, and total solar radiation per year.

The climate of the region of Experiment 1 is characterized as hot and humid, with annual rainfall of 1200 to 1300 mm and average annual temperature of 19°C. The climate of the region of Experiment 2 is also characterized as hot and humid, with an annual rainfall of 1200 to 1300 mm, and average annual temperature of the 19°C.

The soil samples from both experiments which were taken from the depth of 0–20 cm before the implantation of the experiment were analyzed.

The experiments and the management of the fertilization were carried out according to the results of the soil analysis, according to the Liming and Fertilization Manual for the State of Espírito Santo-5th approximation [16]. Fertilization was carried out in three parts from October to March. The soil correction of both experiments was carried out according to the Liming and Fertilization Manual for the State of Espírito Santo-5th approximation [16] according to the results of soil analysis.

Liming was performed in June for both experiments since the base saturation for the coffee crop should be $V = 60\%$ [16]. The phytosanitary control was conducted in October in a preventive way as typical in the region.

For the experimental control of the experiments it was observed that both are located at 950-meter altitude; the soil type of the two experimental areas is dystrophic Red-Dark Podzolic; the soil fertility conditions were corrected through fertilization and liming according to Prezotti et al. [16] in the two experiments; the climatic variations (temperature and rainfall) are similar in both areas, since they are close.

The fresh water used in the processing of the coffees in both experiments is in accordance with CONAMA n° 357/2005 Resolution, which deals with the classification of water bodies [17].

Finally, both experiments were conducted in the same experimental design of randomized blocks, with the same number of replicates (05) and with the same treatments.

Experiment 02, East (sun-grown), received an average of 22.3 more minutes of solar radiation per day than the 01, South-Southeast (shade-grown), representing 255.84 more Wh/m^2 per day, corresponding to 93.381,6 Wh/m^2 more per year.

The South-Southeast and East experiments were conducted in a complete randomized block designed with five replicates and four treatments, one with starter culture for fermentation of Arabic coffee, Yeast Fermentation (*Saccharomyces cerevisiae* sp.), and the others with dry fermentation (Fully Washed) and with water (Washed) and pulped without fermentation (Semidry).

2.1. Raw Materials: Wet Processing. The raw materials used in the formulation of the must were coffee pulp, coffee husk, water, and yeast (*Saccharomyces cerevisiae* sp.).

Ten kilos of coffee were harvested per experimental plot in both experiments, presenting 85% of ripe fruits. After harvest, the fruits were processed according to the treatment.

2.2. Preparation of Musts. Of the four proposed treatments, one was prepared from the must, according to the process of patent BR1020160040531 (African drying beds), with yeast culture (*Saccharomyces cerevisiae* sp.) and coffee husk. The four treatments followed the following methods.

Treatment 01. It is dry fermentation must (Fully Washed, FW), 10 kg of peeled cherry coffee (pulp), and 5 kg of husk, without adding water to the process.

Treatment 02. It is fermentation must with water (Washed, W), 10 kg of peeled cherry coffee (pulp), 5 kg of husk, and 5 liters of water.

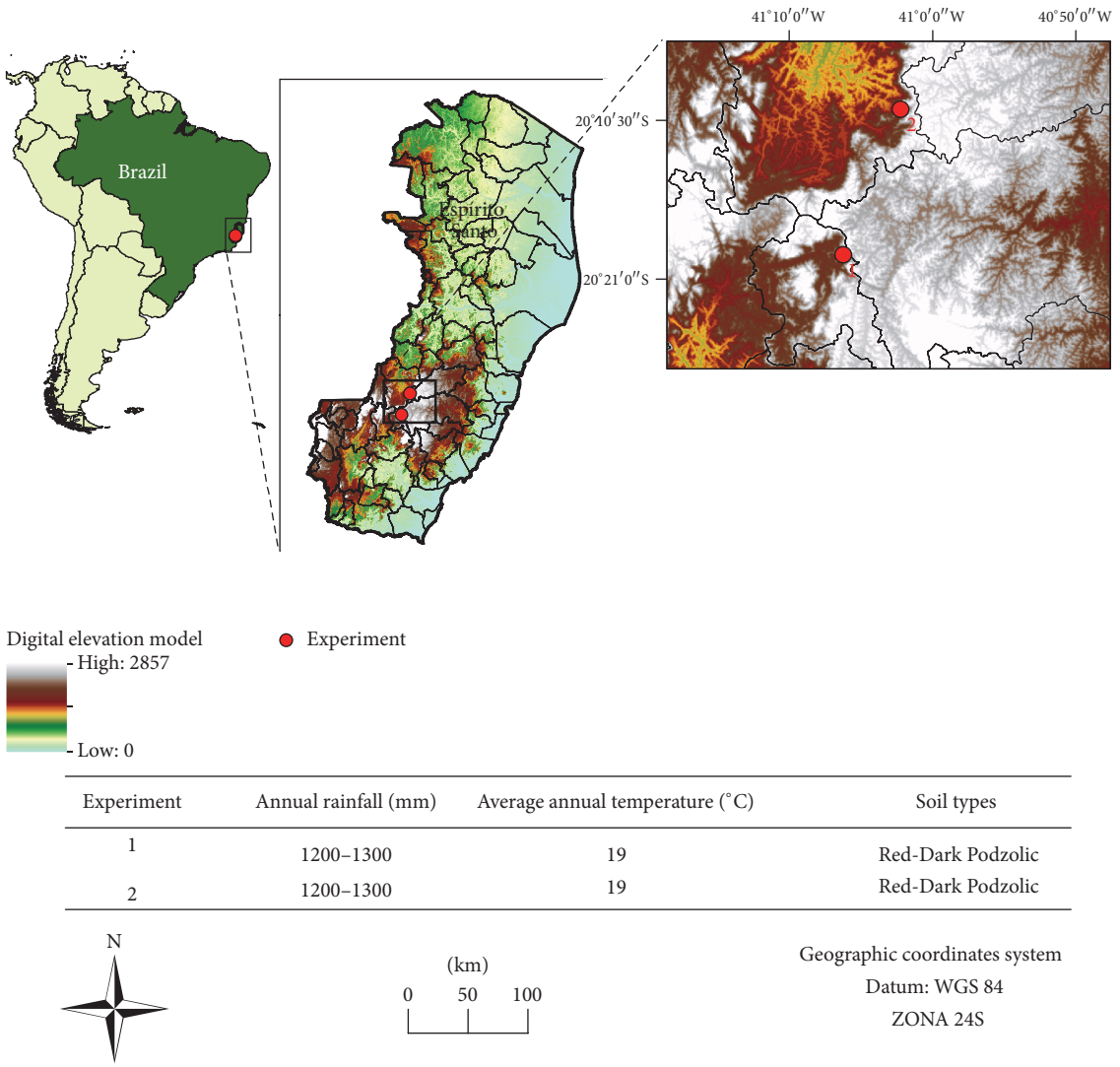
Treatment 03. It is fermentation must with yeast starter culture, *Saccharomyces cerevisiae* sp., (Yeast Fermentation, YF), 1% of the must (p/v), 10 kg of peeled cherry coffee (pulp), 5 kg of husk, 100 grams of yeast, and 5 liters of water.

Patent process (African drying beds) was deposited at the National Institute of Industrial Property.

Treatment 04. It is pulped coffee without mucilage withdrawal (Semidry, SD) and without any addition of microorganisms.

Musts 02 and 03 received water at 38°C and remained immersed in plastic fermentation tanks in the laboratory for 36 hours. The temperature of the fermentation room was stabilized at 21°C.

Treatments 01, 02, and 03 remained in sealed tanks for 36 hours inside the fermentation room and, after this period, they were washed and taken to dry in African drying beds, such as in the farms.



Experiment	Annual rainfall (mm)	Average annual temperature (°C)	Soil types
1	1200–1300	19	Red-Dark Podzolic
2	1200–1300	19	Red-Dark Podzolic

Figure 1: Location of the study area.

Table 1: Geographical coordinates and incidence of solar radiation for the two experiments.

Experiment	Latitude	Longitude	Average annual radiation duration (hours)	Solar radiation, SR, daily average for the year (Wh/m^2)	SR total year (WH/m^2)
01	−20.3263	−41.1069	10.3673	4285.12	1564068.8
02	−20.1667	−41.0375	10.7385	4540.96	1657450.4

Source: [18]; Wh: Watt-hour.

Treatment 04 was pulped and taken to dry African drying beds. The process of fermentation occurred naturally, without any addition of microorganisms.

2.3. Sensory Analyses. The sensory analyses were performed by 06 Q-Graders, according to the method proposed by Pereira et al. [19]. The authors determined the number with simulation of the Bootstrap method.

The evaluations were carried out in the Laboratory of Research and Analysis in Coffee, LAPC, following the evidence protocol of the Specialty Coffee Association of America, SCAA. All tasters tested the 20 plots of each experiment. The SCAA protocol determines the overall quality of the coffee through an additive sum of 10 sensory parameters consisting of fragrance, flavor, aftertaste, acidity, body, uniformity, clean cup, sweetness, balance, and overall.

2.4. Statistical Analysis. A joint analysis of the experiments was performed. The results were compared with the Tukey test at 5% probability, followed by analysis of the main

Table 2: Grades of the means of the sensory attributes for each treatment related to the crop located to the South-Southeast.

Attributes	Pulped without fermentation (Semidry)	Dry fermentation (Fully Washed)	Fermentation with water (Washed)	Yeast Fermentation
Fragrance	7.94	7.99	8.29	8.01
Flavor	7.72	7.80	7.99	7.80
Aftertaste	7.62	7.56	7.78	7.66
Acidity	7.81	7.80	7.98	7.72
Body	7.62	7.61	7.80	7.65
Uniformity	9.78	9.79	9.96	9.88
Clean cup	9.85	9.86	9.92	9.93
Sweetness	9.40	9.44	9.62	9.49
Balance	7.58	7.58	7.74	7.63
Overall	7.61	7.66	7.85	7.64

Table 3: Grades of the means of the sensory attributes for each treatment related to the crop located to the East.

Attributes	Peeled without fermentation (Semidry)	Dry process (Fully Washed)	Fermentation with water (Washed)	Fermentation with yeast (Yeast Fermentation)
Fragrance	7.81	7.85	7.77	7.78
Flavor	7.61	7.66	7.65	7.63
Aftertaste	7.49	7.45	7.41	7.43
Acidity	7.64	7.77	7.65	7.55
Body	7.50	7.51	7.45	7.57
Uniformity	9.85	9.85	9.78	9.81
Clean cup	9.88	9.95	9.57	9.89
Sweetness	9.42	9.42	9.51	9.50
Balance	7.49	7.53	7.39	7.52
Overall	7.45	7.36	7.41	7.52

components to group the treatments, using visual exams in graphical dispersions for the South-Southeast and East experiments, considering an accumulated variability above 70% adequate to perform the analysis. For statistical analysis, the SAEG software was used [11].

3. Results and Discussion

The results of the sensory analyses are presented in Tables 2 and 3, which show the mean values of the sensory attributes by treatment. All average results place the coffees in the range of specialty coffees by the SCAA protocol.

Table 4 presents the average results of the overall quality for the four treatments in the two environments, South-Southeast and East.

The results indicated that for the South-Southeast environment the fermentation must with water was superior in relation to pulped coffee without fermentation. However, it did not differ from the other treatments.

For the experiment located in the South-Southeast, the treatment with water presented higher overall quality than the experiment located in the East. In this way, the results corroborate the propositions of Joët et al. [4] and Muschler [20], indicating that the shading has exerted influence on coffee quality. It is known that usually the coffee in the colder and shaded region (higher altitude) receives higher grades regarding flavor, aroma, sweetness, and body than samples from warmer regions. For DaMatta [5], this factor is associated with the fact that the high temperatures prevent the translocation of chemical compounds to the fruits.

The results expressed in mean values for overall quality between the South-Southeast and East experiments were not statistically different from each other and did not confirm the same results found by Evangelista et al. [21] and Pereira et al. [22]. To these authors, the fermentation induced with yeast culture promoted quality gains to the observed coffees. This indicates that further studies are necessary to understand the modifications that may occur during the fermentation phase, providing greater clarification on the action of the microorganisms, as well as the competition between microorganisms that occurs during the fermentation phase with cultures starters.

It was verified in the experiment located to the East that the treatments did not differ among themselves at 5% of probability.

Table 5 shows the eigenvalues with simple and cumulative percentages of the total variance of the main components of the South-Southeast experiment.

Table 4: Averages of the overall quality evaluated in four treatments and in two environments, Venda Nova do Imigrante, in 2015.

Treatment	Global quality		Average
	South-Southeast	East	
Dry fermentation (Fully Washed)	83.06^{abA}	82.16^{aA}	82.61^{a}
Fermentation with water (Washed)	84.86^{aA}	81.63^{aB}	83.25^{a}
Yeast Fermentation	83.50^{abA}	82.24^{aA}	82.87^{a}
Pulped without fermentation (Semidry)	82.63^{bA}	81.70^{aA}	82.17^{a}
Average	83.51^{A}	81.93^{B}	

Means followed by at least one same horizontal capital letter and at least one same lowercase vertical letter do not differ from one another by the Tukey test at 5% probability.

Table 5: Main components, their respective eigenvalues, and simple and accumulated percentages of the total variance of the South-Southeast experiment.

Main component	Eigenvalues	Simple percentage	Accumulated percentage
CP1	9.998819	99.98819	99.98819
CP2	0.0008436559	0.00844	99.99662

Table 6: Main components, their respective eigenvalues, and simple and accumulated percentages of the total variance of the East experiment.

Main Component	Eigenvalues	Simple percentage	Accumulated percentage
CP1	9.998897	99.98897	99.98897
CP2	0.001005009	0.01005	99.99902

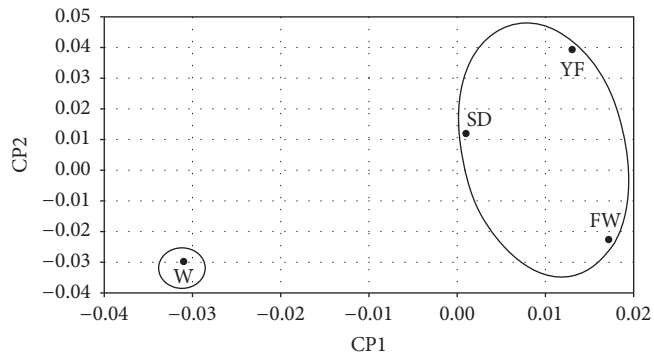


Figure 2: Diagram of dispersion in relation to the first two main components of the treatments: dry fermentation must—Fully Washed (FW), fermentation must with water—Washed (W), fermentation must with yeast—Yeast Fermentation (YF), and pulped without fermentation—Semidry (SD), from the South-Southeast (shade-grown) experiment.

Figure 2 presents the dispersion of the treatments of the South-Southeast experiment, based on the respective coordinates related to the first two main components, CP1 and CP2. The treatments were treatment with dry fermentation must (Fully Washed, FW), fermentation must with starters yeast cultures (Yeast Fermentation, YF), and pulped coffee without fermentation (Semidry, SD) form one group and the treatment fermentation with water (Washed, W) in another group. In addition, the two components absorbed 99.997% of the variation existing in the original features (Table 5).

The results expressed in Figure 2 indicate that for the condition of the experiment located in the South-Southeast, that is, shaded terrains, the fermentation with water, the method commonly adopted in Colombia, is grouped far from the other treatments.

Table 6 shows the eigenvalues and simple and accumulated percentages of the total variance of the East experiment.

Figure 3 shows the dispersion of the treatments of the East experiment based on the respective coordinates relative to the first two main components CP1 and CP2. The treatments with dry fermentation must (Fully Washed, FW), fermentation must with starters yeast cultures (Yeast Fermentation, YF), and pulped coffee without fermentation (Semidry, SD) form a group and the treatment fermentation with water (Washed, W) forms another group, and the two components absorbed 99.999% of the variation existing in the original features (Table 6).

The analyzed data of the eastern crop show results similar to those of the experiment located in the South-Southeast, in relation to the main components. However, in the treatment

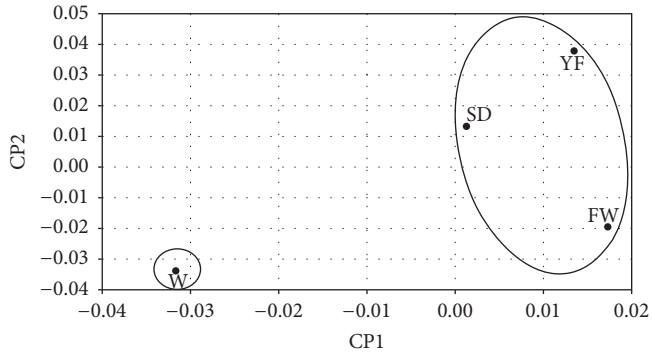


Figure 3: Diagram of dispersion in relation to the first two main components of the treatments: dry fermentation must—Fully Washed (FW), fermentation must with water—Washed (W), fermentation must with starters of yeast—Yeast Fermentation (YF), and pulped without fermentation—Semidry (SD), from the East experiment (sun-grown).

with water, the overall quality was higher for the South-Southeast environment when compared to the East, as shown in Table 4. This result can be explained by the action of the microorganisms to the detriment of the microbiota that is present in the two experiments. Evangelista et al. [21] have argued that the formation of the microbiota, the room temperature, and the pH of the must may undergo changes and modify the intensity and the way the microorganisms act during the fermentation process. The fruits of the coffee when being processed allow the emergence of a spontaneous or wild fermentation. The sugars and pectins present in the mucilage allow the growth of microorganisms, especially bacteria and yeasts.

Yet, the scientific literature contains virtually no studies on microbial interactions at a single cell level occurring on solid and liquid surfaces, involving fermentation microorganisms [23].

This fact may have influenced positively the fermentation with water for the experiment located in the South-Southeast and influenced it negatively in the experiment located in the East, confirming the need for greater monitoring during the fermentation stages of Arabica coffee [14].

Precisely because of the lack of clarification about the medium of fermentation within the coffee fruit, this hypothesis proposes a new perspective for science, the monitoring of the microbiota that is formed inside the coffee fruits and how it develops after harvesting under different fermentation conditions.

According to the perspective of Lee et al. [24], the effects of fermentation during the wet treatment on the aroma profile of coffee are not completely elucidated and are often neglected since the literature has argued that the main function of fermentation is the removal of the mucilage. For De Bruyn et al. [14], further studies should be undertaken to strengthen the understanding of the impact of the microbiota on coffee quality and provide robust data for the development of more controlled fermentation processes.

The coffee fermentation occurs to solubilize polysaccharides, which are present in the coffee pulp, after removal of the mucilage, facilitating the drying of the fruits. During the fermentation, microorganisms act in the degradation of the sugars present in the pulp, thereby creating metabolic routes and differentiated sensory patterns. For Velmourougane [25], it is important to study and understand the fermentation process to develop flavor and a high quality standard of coffee.

It is evident that fermentation is a complex process, involving several factors, with the action of different microorganisms that can act in the improvement, as in the loss, of quality. Consequently, it can contribute or not to the deterioration of the product's final taste, due to the kind of action they can take on processing. Hence the importance of knowing more about the action of the microbiota and about fermentation processes during the production of specialty coffees, in view of the opportunity to create more standardized processes that consider the described factors, aiming to promote quality improvements to the final product, as well as food safety to the consumer.

Developing and controlling processes in the production of specialty coffees have proved to be a complex task and often without a consensus on the best postharvest processing method, which may vary from region to region. Velmourougane [25] reinforces the need to study processing techniques, looking for ways to improve the final quality of Arabica coffee.

Results presented in Figure 4 corroborate with the data presented in Table 4, showing the values of the most significant sensory attributes for the fermentation with water treatment, in relation to the nonfermented (semidry), confirming the data of de Melo Pereira et al. [10] and Lee et al. [24].

Figure 5 shows the dispersion of the sensory attributes of the treatments related to the experiment in the East, corroborating with the results obtained in Table 4 for the overall quality of the Arabica coffee.

It is possible to understand that the washed fermentation can be an engine maker of new routes and nuances for the formation of the sensory features of the wet processed coffee, aiming at the potentialization and optimization of the quality curve of Arabica coffee.

On the difference between the fermentation methods, the results of Somporn et al. [2] indicated that shading exerts an influence on the formation of sugars, chlorogenic acids, and total phenolics. In addition to shading, DaMatta [5]

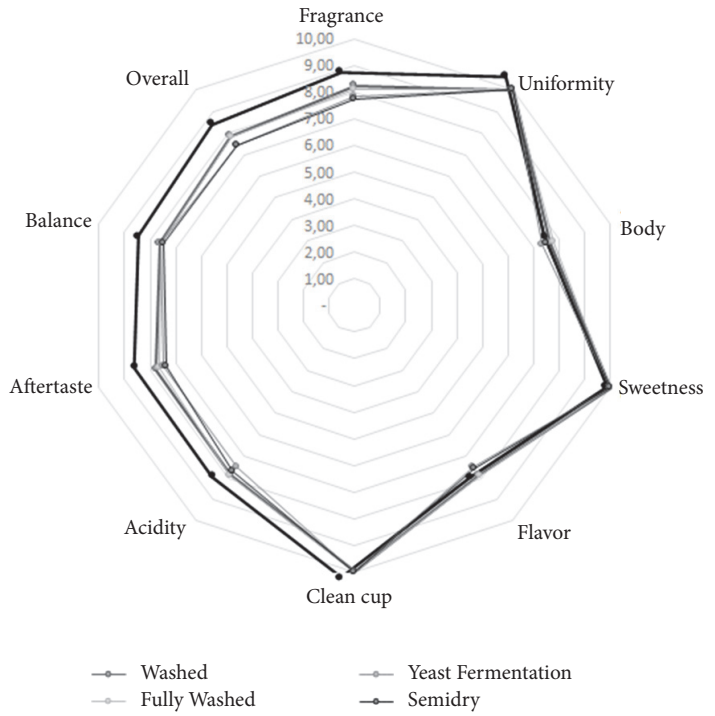


Figure 4: Average dispersion of attributes: fragrance, uniformity, clean cup, sweetness, flavor, acidity, body, aftertaste, balance, and overall, of the coffees located in the South-Southeast (shade-grown).

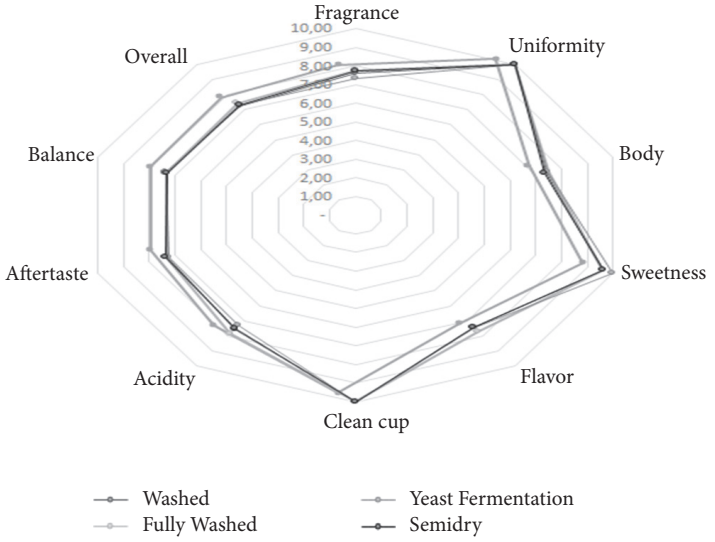


Figure 5: Average dispersion of the attributes: fragrance, uniformity, clean cup, sweetness, flavor, acidity, body, aftertaste, balance, and overall, of the coffees situated to the East *(sun-grown)*.

argues that fruits that are formed under conditions of higher temperatures mature prematurely, preventing the complete translocation of compounds responsible for the typical aroma and flavor of the coffee.

Thus, the microbiota of the experiment located in the South-Southeast associated with the water-induced fermentation method contributed to the modification of the sensory characteristics, in relation to the experiment located in the East, area with higher solar incidence.

4. Conclusion

The overall quality of the coffees presented the most promising results for wet processing through water fermentation in

relation to the nonfermentation method (Semidry) for the experiment located in the South-Southeast region.

The lower incidence of solar radiation in the crop had a significant effect on the overall quality of the Arabica coffee, associated with wet processing and water fermentation (Washed).

These results demonstrate and reinforce the condition of the environment; that is, the incidence of solar radiation can lead to changes in internal metabolites, creating a stress condition, and consequently different conditions for the development of microorganisms. However, this new hypothesis needs to be better clarified.

Variations with respect to the water fermentation method for the experiment located in the South-Southeast and East regions may be related to the action of the microorganisms present in the Arabica coffee and new research and studies are needed to deepen and to quantify the action of these microorganisms in loco.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

The authors thank the Federal Institute of Espírito Santo for supporting this research and also for the translation and review of this article. They also thank National Council for Scientific and Technological Development (469058/2014-5), CNPq, and Secretariat of Professional and Technological Education of the Ministry of Education (SETEC) for the availability of resources for research.

References

[1] A. S. Bosselmann, K. Dons, T. Oberthur, C. S. Olsen, A. Ræbild, and H. Usma, "The influence of shade trees on coffee quality in small holder coffee agroforestry systems in Southern Colombia," *Agriculture, Ecosystems & Environment*, vol. 129, no. 1-3, pp. 253–260, 2009.

[2] C. Somporn, A. Kamtuo, P. Theerakulpisut, and S. Siriamornpun, "Effect of shading on yield, sugar content, phenolic acids and antioxidant property of coffee beans (Coffea Arabica L. cv. Catimor) harvested from north-eastern Thailand," *Journal of the Science of Food and Agriculture*, vol. 92, no. 9, pp. 1956–1963, 2012.

[3] J. N. Pinto Neto, M. I. N. Alvarenga, M. P. Correa, and C. C. Oliveira, "Efeito das variáveis ambientais na produção de café em um sistema agroflorestal," *Coffee Science*, vol. 9, no. 2, pp. 187–195, 2014.

[4] T. Joët, A. Laffargue, F. Descroix et al., "Influence of environmental factors, wet processing and their interactions on the biochemical composition of green Arabica coffee beans," *Food Chemistry*, vol. 118, no. 3, pp. 693–701, 2010.

[5] F. M. DaMatta, "Exploring drought tolerance in coffee: A physiological approach with some insights for plant breeding," *Brazilian Journal of Plant Physiology*, vol. 16, no. 1, pp. 1–6, 2004.

[6] B. Guyot, D. Gueule, J. C. Manez, J. J. Perriot, J. Giron, and L. Villain, "Influence de l'altitude et de l'ombrage sur la qualité des cafés Arabica," *Juillet. Août, Plantations, Recherche, Développement*, vol. 3, no. 4, pp. 272–283, 1996.

[7] B. Bertrand, P. Vaast, E. Alpizar, H. Etienne, F. Davrieux, and P. Charmetant, "Comparison of bean biochemical composition and beverage quality of Arabica hybrids involving Sudanese-Ethiopian origins with traditional varieties at various elevations in Central America," *Tree Physiology*, vol. 26, no. 9, pp. 1239–1248, 2006.

[8] C. F. Silva, L. R. Batista, L. M. Abreu, E. S. Dias, and R. F. Schwan, "Succession of bacterial and fungal communities during natural coffee (Coffea arabica) fermentation," *Food Microbiology*, vol. 25, no. 8, pp. 951–957, 2008.

[9] C. F. Silva, L. R. Batista, and R. F. Schwan, "Incidence and distribution of filamentous fungi during fermentation, drying and storage of coffee (Coffea arabica L.) beans," *Brazilian Journal of Microbiology*, vol. 39, no. 3, pp. 521–526, 2008.

[10] G. V. M. Pereira, V. T. Soccol, A. Pandey et al., "Isolation, selection and evaluation of yeasts for use in fermentation of coffee beans by the wet process," *International Journal of Food Microbiology*, vol. 188, pp. 60–66, 2014.

[11] B. B. Ribeiro, L. M. V. L. Mendonça, G. A. Assis, J. M. A. de Mendonça, M. R. Malta, and F. F. Montanari, "Evaluation of the chemical and sensory characteristics of Coffea canephora Pierre and Coffea Arabica L. blends," *Coffee Science*, vol. 9, no. 2, pp. 178–186, 2014.

[12] S. R. Evangelista, M. G. da Cruz Pedrozo Miguel, C. de Souza Cordeiro, C. F. Silva, A. C. Marques Pinheiro, and R. F. Schwan, "Inoculation of starter cultures in a semi-dry coffee (Coffea arabica) fermentation process," *Food Microbiology*, vol. 44, pp. 87–95, 2014.

[13] W. Masoud and L. Jespersen, "Pectin degrading enzymes in yeasts involved in fermentation of Coffea arabica in East Africa," *International Journal of Food Microbiology*, vol. 110, no. 3, pp. 291–296, 2006.

[14] F. De Bruyn, S. J. Zhang, V. Pothakos et al., "Exploring the impacts of postharvest processing on the microbiota and metabolite profiles during green coffee bean production," *Applied and Environmental Microbiology*, vol. 83, no. 1, Article ID e02398-16, 2017.

[15] O. Gonzalez-Rios, M. L. Suarez-Quiroz, R. Boulanger et al., "Impact of "ecological" post-harvest processing on coffee aroma: II. Roasted coffee," *Journal of Food Composition and Analysis*, vol. 20, no. 3-4, pp. 297–307, 2007.

[16] L. C. Prezotti, J. A. Gomes, G. G. Dadalto, and J. A. Oliveira, "Manual de recomendação de calagem e adubação para o Estado do Espírito Santo – 5a aproximação," *Vitória: SEEEA/INCAPER/CEDAGRO*, article 305, 2007.

[17] CONAMA – CONSELHO NACIONAL DE MEIO AMBIENTE (Brasil), "Resolução n 357, de 17 de março de 2005," *Diário Oficial da União*, pp. 58–63, 2015.

[18] GEOBASES. Sistema Integrado de Bases Geoespaciais do Estado do Espírito Santo. 2016, http://www.geobases.es.gov.br/portal/.

[19] L. L. Pereira, R. C. Guarçoni, I. C. Pulini et al., "Tamanho ótimo do número de provadores de café com uso do protocolo SCAA," *Simposio Internacional De Ingeniería Industrial: Actualidad Y Nuevas Tendencias*, vol. 9, 2016.

[20] R. G. Muschler, "Shade improves coffee quality in a sub-optimal coffee-zone of Costa Rica," *Agroforestry Systems*, vol. 51, no. 2, pp. 131–139, 2001.

[21] S. R. Evangelista, C. F. Silva, M. G. P. D. C. Miguel et al., "Improvement of coffee beverage quality by using selected

yeasts strains during the fermentation in dry process," *Food Research International*, vol. 61, pp. 183–195, 2014.

[22] G. V. M. Pereira, E. Neto, V. T. Soccol, A. B. P. Medeiros, A. L. Woiciechowski, and C. R. Soccol, "Conducting starter culture-controlled fermentations of coffee beans during on-farm wet processing: Growth, metabolic analyses and sensorial effects," *Food Research International*, vol. 75, pp. 348–356, 2015.

[23] R. F. Schwan and G. H. Fleet, *Cocoa and Coffee Fermentation*, Taylor & Francis Group, LLC. CRC Press, 2015.

[24] L. W. Lee, M. W. Cheong, P. Curran, B. Yu, and S. Q. Liu, "Coffee fermentation and flavor - An intricate and delicate relationship," *Food Chemistry*, vol. 185, pp. 182–191, 2015.

[25] K. Velmourougane, "Impact of natural fermentation on physic-ochemical, microbiological and cup quality characteristics of Arabica and Robusta coffee," *Proceedings of the National Academy of Sciences India Section B - Biological Sciences*, vol. 83, no. 2, pp. 233–239, 2013.