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CROP BREEDING
Article

Physical and chemical attributes of beans damaged by the coffee berry borer at different levels of infestation

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ABSTRACT: The physical and chemical attributes of coffee beans have a direct relation with the sensory quality of the beverage and the commodity's market value. The coffee berry borer is a pest that causes a worldwide harm, and its infestation compromises bean health and composition. The objective of this study was to analyze the effect of infestation levels on beans physical and chemical parameters. Coffee samples were collected, and the damaged beans present in the samples were classified as to infestation level. The centesimal composition, color, quantification of bioactive compounds, and organic acids were evaluated. The L* and b* parameters of the CIELab color system were found to be significantly different between infestation levels. For chemical parameters, beans with major levels of infestation were found to differ from the others in the content of sugars, lipids, soluble solids, total titratable acidity, electrical conductivity, potassium leaching, succinic acid, and acetic acid. The presence of pests did not affect the bioactive compounds present in the coffee beans.

Key words: organic acids, sugars, color, brocade beans.

INTRODUCTION

Coffee beans have a variety of chemical compounds, and this composition may vary due to several factors, such as: species, cultivar, place of cultivation, type of post-harvest processing, stage of fruit maturation at harvest, storage conditions, among others (Cid and Peña 2016, Ciaramelli et al. 2019). A factor that can influence the chemical composition of a coffee bean is the health of the beans. When a coffee bean is infested by an external agent, pest or microorganisms, several reactions are triggered from the secondary metabolism to minimize the negative effects of the infecting agent. These reactions can lead to the production and/or degradation of several compounds, which in turn will alter the chemical composition of the bean (Toci and Farah 2008, 2014). In addition, the external agent itself can produce chemical compounds that will alter the chemical composition of the bean, such as microorganisms, which can produce organic acids from fermentation and mycotoxins produced by some species of fungi (Taniwaki et al. 2014, Ha 2015).

The coffee berry borer (*Hypothenemus hampei*) is the pest of greatest importance for coffee culture worldwide, because it is present in the producing localities and its control in the field is difficult. Many studies are currently underway with the objective of developing innovative, efficient, and environmentally safe techniques for the control of the pest. Biological control represents an alternative that is increasingly being explored for use in the field, although it is dependent on climatic factors (Escobar-Ramírez et al. 2019).

A study conducted by Dias et al. (2024) analyzed the risks that arabica coffee-producing regions in Brazil are currently facing and may face in the future because of climate change. This study demonstrated the potential for increased vulnerability to the occurrence of diseases and pests, especially the coffee berry borer, as a consequence of severe climate change, including

increased temperature and water stress. This underscores the crucial importance of implementing strategies to control the pest and minimize its impact on the quality of coffee beans.

The coffee berry borer infests by drilling orifices in the berries, still in the field, causing an orifice from the exocarp that extends to the endosperm. In the endosperm the female oviposits and the larvae feed on the seed until they evolve into the next stage of development (Vega et al. 2009, Infante 2018). The orifice caused by the coffee berry borer can facilitate the contamination of the beans, either by the microbiota present in the environment that will colonize in the fruit through the orifice formed, or by microorganisms present in the insect itself, such as in the cuticle, paws, wings and/or chewing apparatus (Damon 2000, Velmourougane et al. 2010, Plata-Rueda et al. 2019, Silva et al. 2020). However, information on the chemical changes that coffee berry borer and microorganisms associated can trigger in coffee beans are still scarce.

Walker et al. (2019) evaluated the volatile profile of Hawaiian coffee beans with different levels of coffee berry borer infestation and observed that infestation levels generated differences in the volatile profile of the beans and consequently in the quality of the beverage. Compounds such as hexanal and 2-pentilfuran were associated to more severely damaged coffee beans. Santos and Rodrigues (2020) also observed the presence of the hexanal volatile compound when they characterized the volatile carbonic compounds of green/raw coffee bean. This study aimed to analyze color and chemical compounds of coffee beans at different levels of the coffee berry borer infestation.

MATERIAL AND METHODS

Collection and preparation of samples

The total of 120 kg of processed coffee beans were collected, which were manually classified for selection and categorization according to the level of infestation of the coffee berry borer. The categorization was performed according to Silva et al. (2020) taking into account the number of orifices in the bean and the presence or absence of color inside the orifices, resulting in three levels of infestation:

- Clean: beans containing one or two orifices without color change inside the orifices;
- Dirty I: beans containing one or two orifices with color change inside the orifices;
- Dirty II: beans containing three to six orifices with color change inside the orifices.
- Control: perfectly healthy beans were separated and made up the control treatment (Fig. 1).

Figure 1. Coffee beans categories according to coffee berry borer infestation level. Clean: beans containing one or two orifices without color change inside the orifices. Dirty I: beans containing one or two orifices with color change inside the orifices. Dirty II: beans containing three to six orifices with color change inside the orifices. Control: perfectly healthy beans were separated and made up the control treatment.

Color analysis

For color analysis, a sample of coffee beans was spread in a Petri dish of 15 cm in diameter, arranged on a dark surface, so that the whole plate was covered by beans homogeneously. The color was determined using a Minolta R300 colorimeter, and the values of L*, a* and b* were read at five equidistant points of the plate (Oliveira et al. 2016).

Chemical analyses

The chemical analyses were carried out in the food analysis laboratory of Agricultural Research Company of Minas Gerais, Southern Minas Regional Unit. The following analyses were performed: humidity (ISO 2003), ash, ether extract, proteins, total sugars, soluble solids, pH, titratable acidity (AOAC 2000), potassium leaching (Malta et al. 2005), and electrical conductivity (Krzyzanowski et al. 1991).

Quantification of organic acids

The samples were cold ground in an analytical mill model A11 Basic, using liquid nitrogen, for approximately 1 minute. Then, the samples were sieved through 0.42-mm pores, 0.5 g of sample was weighed, with subsequent dilution in 50 mL of perchloric acid 0.23% and transferred to a 50-mL conical tube. The mixture was vortexed for 3 minutes, filtered in quantitative filter paper and then in a 0.45-µm syringe filter with subsequent injection of 20 µL in the chromatograph (Ribeiro et al. 2018).

The equipment used was a Shimadzu, equipped with quaternary high pressure pump model LC-20AT, aerator model DGU-20A5, interface model CBM-20A, automatic injector model SIL-20A-HT and ultraviolet-visible detector (SPD-20A). The column used was supelcogel type C-610H (30 cm × 7.8 MM d. i.) connected to a supelcogel pre-column (5 cm × 4.6 mm d. i.) and the mobile phase composed of 0.23% perchloric acid.

The methodology proposed by Santiago et al. (2020) was used in the establishment of the chromatographic conditions. The elution was performed with a gradient system of an aqueous solution of 0.23% perchloric acid with variation in flow velocity from 0 to 13 minutes (0.5–0.3 mL/min); 13–16 minutes (0.3–05 mL/min); 16–24 minutes (0.5–0.8 mL/min) and 24–28 minutes (0.8–0.5 mL/min).

The standards used were: acetic acid brand J.T. Backer/purity > 99.7%; oxalic acid brand Sigma-Aldrich/purity > 99%; citric acid brand Sigma-Aldrich/purity > 99.7%; malic acid brand Sigma-Aldrich/purity > 98%; quinic acid brand Sigma-Aldrich/purity > 98%; and succinic acid brand Sigma-Aldrich/purity > 99 %.

Quantification of trigonelline, caffeine, and chlorogenic acid

The extraction of bioactive compounds was performed in a water bath at 90 °C for 3 minutes, using 0.5 g of cold ground and sieved sample and 50 mL of distilled water. The mixture was filtered through filter paper no. 4 and in a 0.45-µm PVDF syringe filter. The filtrate was injected into a Shimadzu liquid chromatograph equipped with a quaternary high pressure pump model LC-20AT, degassing model DGU-20A5, interface model CBM-20A, automatic injector model SIL-20A-HT, and ultraviolet-visible detector (SPD-20A). The column used was shim-pack CLC-ODS (6.0 × 150 mm, 5 µm) connected to an Eclipse XDB-C18 pre-column (4.6 × 12.5 mm, 5 µm). The mobile phase for the elution of the analyzed compounds was elaborated with a solution of acetic acid 1% in water (solvent A) and methanol (solvent B), obeying the proportion: water/methanol/acetic acid (85:14:1% v/v). Samples and patterns (caffeine brand Fluka/purity > 99%; trigonelline brand Sigma-Aldrich/purity > 99%; chlorogenic acid 5-CQA brand Sigma-Aldrich/purity > 95%) were isocratic eluted. The wavelength used was 272 nm, flow of 1 mL/min, temperature of 40°C and injection volume of 20 µL (Malta and Chagas 2009).

Statistical analysis

The experiment was completely randomized, with four sample treatments (levels of clean, dirty I, dirty II and control), with three replicas. The data were analyzed by the software SPEED Stat 1.0, in which variance analysis and Student–Newman–Keuls test were performed at 5% probability.

An auxiliary technique to visualize the associations between attributes was the analysis and establishment of the correlation network (Rosado et al. 2017, 2018), constructed from Pearson's correlation matrix, which made it possible to visualize the pattern of relationship between variables. The positive correlations between the variables were represented by blue lines and the negative ones by red lines. The fine lines have lower correlations, and the strong lines have higher correlations.

RESULTS AND DISCUSSION

Color

According to the results of this study, bean staining differed between the levels of the coffee berry borer infestation, and clean and dirty II samples were different from each other and different from control and dirty I samples, for the parameters L* (Fig. 2) and b* (Fig. 3) of the CIELab color system. The CIELab three-dimensional color system determines the color of a given sample or object through the L* values, corresponding to luminosity (0–100), a* color variation from green to red (-120 to +120), and b* color variation from blue to yellow (-120 to +120) (Oliveira et al. 2016). The lowest values of L* and b* were obtained for dirty II samples, which means that the beans of this level of infestation are darker than the other treatments (Fig. 4).

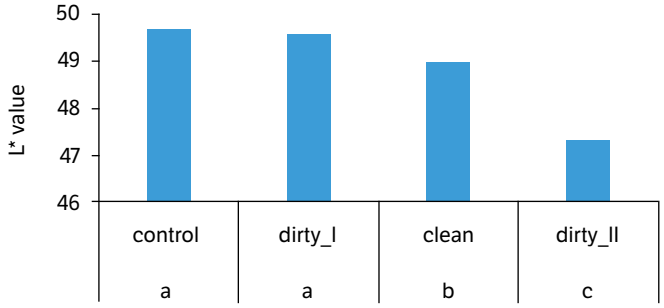

Figure 2. Average of the parameters L* of coffee beans with different levels of coffee berry borer infestation. Means followed by the same letter do not differ significantly from each other according to the Student–Newman–Keuls' test ($p > 0.05$).

Figure 3. Average of the parameters b* of coffee beans with different levels of coffee berry borer infestation. Means followed by the same letter do not differ significantly from each other according to the Student–Newman–Keuls' test ($p > 0.05$).

Figure 4. Color corresponding to the average measurement of L* a* and b* values beans with different levels of coffee berry borer infestation.

One of the hypotheses for color change inside the perforations caused by the coffee berry borer is the presence of microorganisms, especially fungi. Pérez et al. (2003) isolated and characterized fungi associated with the insect's cuticle, intestine, and feces and observed the presence of microorganisms from the genus *Fusarium*, *Penicillium*, and *Aspergillus*. In Silva et al. (2020), these same microorganisms were isolated from beans infested by the coffee berry borer, suggesting that the insect itself can contaminate the beans. The greater the number of perforations in a fruit, the greater the contamination by microorganisms, from the insect's body or the environment, leading to a more pronounced color change in highly infested beans.

The color change is also taken into account when the physical classification of the beans is carried out according to the Brazilian Official Classification (Brasil 2003). Beans with serious defects, such as black and burned, contribute more to the penalty of coffee batches during classification by type–this also applies to dirty II berry borer beans. Therefore, a batch with a higher number of damaged beans of this class will be classified as lower, consequently resulting in lower value for commercialization. This penalty also happens during the classification of specialty coffees, for which the presence of dirty II berry borer berries in a volume of 350 g of raw coffee (SCA 2009) is not allowed.

Chemical analyses

The results of the chemical analyses are summarized in Table 1. Only the protein and humidity parameters showed no significant differences between coffee berry borer infestation levels. The number of soluble solids present in coffee is composed mainly of sugars, caffeine, trigonelline, and chlorogenic acid. For the four levels of coffee berry borer infestation, there was no variation in the concentrations of caffeine, trigonelline, and chlorogenic acid (Table 2). However, for total sugars, the dirty II samples presented lower concentrations, as well as soluble solids.

Table 1. Mean and standard deviation of soluble solids (g·100g MS^{-1}), titratable acidity (mL of NaOH 0.1 N·100g^{-1}), total sugars (g·100g MS^{-1}), ether extract (g·100g MS^{-1}), protein (g·100g MS^{-1}), ash (g·100g MS^{-1}), humidity (%), electrical conductivity (μ S·cm·g^{-1}) and potassium leaching (ppm) in coffee beans with different levels of coffee berry borer infestation*.

Treatment sample	Soluble solids	Titratable acidity	Total sugars	pH	Ether extract	Protein	Ash	Humidity	Humidity Electrical conductivity	Potassium leaching
Clean	35.208^{a} ± 1.671	221.250^{b} ± 3.310	9.344^{b} ± 0.301	6.114^{b} ± 0.020	7.850^{b} ± 0.120	12.883^{a} ± 0.570	4.038^{b} ± 0.131	10.867^{a} ± 0.618	134.383^{b} ± 9.558	35.192^{b} ± 21.310
Dirty I	35.937^{a} ± 1.860	212.500^{c} ± 6.500	9.991^{a} ± 0.299	6.118^{b} ± 0.050	7.975^{ab} ± 0.380	13.287^{a} ± 0.760	4.093^{b} ± 0.211	10.847^{a} ± 0.628	140.978^{b} ± 17.752	45.526^{b} ± 10.810
Dirty II	32.500^{b} ± 0.002	229.166^{a} ± 4.701	7.791^{c} ± 0.311	6.168^{a} ± 0.031	7.191^{c} ± 0.261	13.358^{a} ± 0.641	4.450^{a} ± 0.133	10.809^{a} ± 0.677	244.549^{a} ± 17.897	64.428^{a} ± 16.900
Control	35.625^{a} ± 1.708	214.062^{c} ± 5.200	9.766^{a} ± 0.310	6.103^{b} ± 0.019	8.200^{a} ± 0.419	12.913^{a} ± 0.577	4.152^{b} ± 0.176	11.265^{a} ± 0.776	113.528^{c} ± 14.672	38.809^{b} ± 13.350

*Averages followed by the same letter in the column do not differ significantly from each other according to the Student–Newman–Keuls' test ($p > 0.05$).

Sugars are the main compounds used as substrate for microorganism metabolism, especially fungi (Khosravi et al. 2015). A lower number of sugars in beans with the most severe coffee berry borer infestation levels may be related to microbial contamination, due to the ease of microorganism's entry through the orifice opened by the insect (Velmourougane et al. 2010). The greater the number of orifices in an infested bean, the greater the probability of microbial contamination and the amount of acid compounds from microbial metabolism, contributing to an increase of titratable acidity (Velmourougane et al. 2010). Castro-Moretti et al. (2020) evaluating the differences in the metabolic profile of coffee beans infested and not infested by the coffee berry borer also observed lower levels of sugars such as glucose, fructose, and sorbitol in infested beans.

Regarding sensory attributes, sugars play an important role as precursors of aroma and flavor compounds (Franca et al. 2005) and in the color development of roasted beans (Davídek and Blank 2005). The concentration of sugars present in coffee beans can vary according to the species–*arabica* or *robusta*–, varieties, cultural practices, maturation stage and bean health, as shown in this study. The main sugars related to the sensory quality of coffee are sucrose, glucose, and fructose. These sugars are precursors to an important coffee aroma compound, 2-furaldehyde. Also, studies have shown that high proportions of sucrose are related to better coffee quality (Barbosa et al. 2019, Hall et al. 2022).

Higher acidity values were found for dirty II samples. Naturally, coffee has acidic compounds in its chemical composition. One reason for the increase in acidity of the samples is the presence of microorganisms producing acid compounds, such as fungi, yeast, and bacteria (Chalfoun and Batista 2003, Dezam et al. 2017). The concentration of ether extract in dirty II samples was lower than the observed in other treatments. The damage caused by the coffee berry borer can cause oxidation of coffee's lipid compounds.

According to Pimenta (2003), higher lipid contents is present in better quality coffee and physically arranged in the peripheral regions of the beans. Castro-Moretti et al. (2020) observed reduction in the total content of palmitic acid in robusta coffee infested by the coffee borer. Among the main fatty acids present on coffee, palmitic and linoleic acids stand out, the latter being easily prone to oxidation. The physical damage caused by the pest contributes to the degradation of the lipid fraction within the cells and can compromise the sensory quality of the beverage. Palmitic, stearic, and arachidonic fatty acids were related to better quality coffee in other studies (Pereira et al. 2015, Hurtado-Benavides et al. 2016).

Studies about the influence of coffee berry borer infestation on the sensory quality of the drink are of great relevance since coffee market price can vary according to the quality of the drink. However, this investigation must be done with caution, as sensory analysis is carried out by trained and calibrated tasters and studies have already proven that the insect is a potential vector of toxigenic fungi and high concentrations of ochratoxin A have been found in beans infested by the borer (Velmourougane et al. 2010, Silva et al. 2020).

The dirty II samples also showed higher ash content when compared to other levels of infestation of the berry borer. This increase in mineral content in the sample may be related to the presence of contaminant microorganisms, which have inorganic compounds in their cellular structures, such as calcium (Mouginot et al. 2014). One hypothesis to be considered for increased ash value on the dirty II samples is the presence of the insect itself. During agricultural pest control practices, the insect may die inside the berry, especially when non-dislodging chemical agents are used, thereby increasing the amount of ash present in the sample. This was observed in the study by Silva et al. (2020), who verified in microscopy the presence of whole or parts of insect inside the coffee bean orifice.

The dirty II samples differed from the other treatments in the analysis of electrical conductivity and potassium leaching. It was observed that higher levels of coffee berry borer infestation increase the values of electrical conductivity and potassium leaching due to disruption of cell membranes caused by the physical damage of the insect and larvae that feed on the bean endosperm in the initial stages of its life cycle (Vega et al. 2009, Rodriguez et al. 2013).

Electrical conductivity and potassium leaching analyses are commonly used in seeds in general and especially in coffee beans to evaluate cell structure, in particular regarding cell membrane disruption and cell content extravasation. The greater cellular disruption, the higher values are found for electrical conductivity, potassium leaching and lower the physiological quality of the bean (Costa and Carvalho 2006). The physical damage caused by the pest and the presence of opportunistic microorganisms contribute significantly to cell disruption.

Trigonelline, caffeine, and chlorogenic acid

The results of the quantification of the bioactive compounds of coffee (trigonelline, caffeine, and chlorogenic acid) showed that in this study there was no relationship between the level of infestation of coffee borer and the content of these compounds in the beans (Table 2). Coffee is a food rich in compounds with antioxidant capacity, which can reduce oxidative stress in the human body, performing neuroprotective and hypertensive effects. Chlorogenic acids (3-CQA, 4-CQA and 5-CQA) are the main bioactive compounds of coffee, being found in higher concentrations on robust beans (Jeszka-Skowron et al. 2016b, Wongsa et al. 2019). Bean origin is also a factor that can quantitatively influence the contents of this compound (Dong et al. 2017; Jeszka-Skowron et al. 2016a).

Table 2. Mean and standard deviation of the contents of caffeine, trigonelline, and chlorogenic acids ($g \cdot 100g^{-1}$) in coffee beans with different levels of coffee berry borer infestation-.

Treatment sample	Caffeine	Trigonelline	Chlorogenic acids
Clean	$1.3547^{a} \pm 0.046$	$1.8212^{a} \pm 0.101$	$4.3716^{a} \pm 0.162$
Dirty I	$1.3526^{a} \pm 0.073$	$1.8100^{a} \pm 0.170$	$4.3238^{a} \pm 0.145$
Dirty II	$1.3650^{a} \pm 0.096$	$1.8731^{a} \pm 0.169$	$4.4182^{a} \pm 0.354$
Control	$1.2835^{a} \pm 0.119$	$1.7873^{a} \pm 0.227$	$4.3298^{a} \pm 0.379$

*Averages followed by the same letter do not differ significantly from each other according to the Student–Newman–Keuls' test ($p > 0.05$).

Organic acids

Among the six organic acids quantified in the samples, there was no variation between coffee berry borer infestation levels for citric, malic, and quinic acids (Table 3). The dirty II level contained higher concentrations of acetic and succinic acids when compared to the other levels (Galli and Barbas 2004).

Table 3. Mean and standard deviation of the contents of oxalic acid, citric acid, malic acid, quinic acid, succinic acid, and acetic acid ($g \cdot 100g^{-1}$) in coffee beans with different levels of coffee berry borer infestation*.

Treatment sample	Oxalic acid	Citric acid	Malic acid	Quinic acid	Succinic acid	Acetic acid
Clean	0.0683a ± 0.012	1.3046a ± 0.068	0.4120a ± 0.028	0.3297a ± 0.011	0.3455b ± 0.087	0.4390b ± 0.050
Dirty I	0.0579bc ± 0.013	1.2422a ± 0.143	0.4142a ± 0.030	0.3106a ± 0.015	0.3671b ± 0.041	0.3794b ± 0.079
Dirty II	0.0513c ± 0.006	1.2422a ± 0.077	0.6037a ± 0.081	0.3275a ± 0.015	0.4316a ± 0.031	0.6913a ± 0.149
Control	0.0659ab ± 0.014	1.2276a ± 0.125	0.3688a ± 0.038	0.3059a ± 0.030	0.3541b ± 0.027	0.3933b ± 0.048

*Averages followed by the same letter in the column do not differ significantly from each other according to the Student–Newman–Keuls' test ($p > 0.05$).

The accumulation of organic acids in plants is associated with biotic and abiotic stresses (Lopez-Bucio et al. 2000). Specifically for acetic acid, high concentrations of this compound are associated with the presence of contaminant fungi, which make it as a product of its metabolism. This acid also has close relationship with the sensory quality of the coffee. It is commonly found in beans that have undergone some kind of unwanted fermentation, such as defective black and burned beans, and the concentration of acetic acid is inversely proportional to coffee quality (Casas et al. 2017).

The correlation network (Fig. 5) facilitated the visualizations of associations between groups of attributes evaluated in beans infested by coffee berry borer at different levels. The color parameter (L*) correlated negatively with the attributes electrical conductivity and acetic acid concentration, and positively with the attributes total sugars and ether extract. According to the color data, we observed the lower L* values correspond to darker beans, therefore highly infested beans with color changes inside the orifices showed reduction in the concentration of chemical compounds important for the sensorial quality of the drink, such as sugars and lipids, and increased concentration of compounds that reduced the quality of the drink, such as acetic acid. In Fig. 5, it is also possible to observe the negative correlation between the color parameter b* and electrical conductivity, again, indicating that highly infested beans have cellular disintegration due to the insect and associated microorganisms.

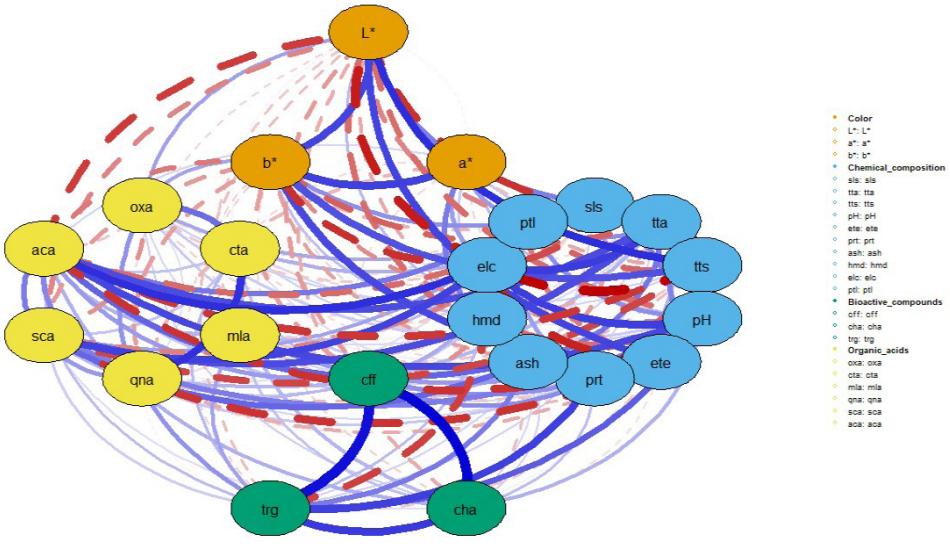

Figure 5. Correlation network between groups of attributes. Color: L*, a* and b*. Chemical composition: soluble solids (sls), titratable acidity (tta), total sugars (tts), pH, ether extract (ete), protein (prt), ash (ash), humidity (hmd), electrical conductivity (elc), potassium leaching (ptl). Bioactive compounds: caffeine (cff), chlorogenic acids (cha), trigonelline (trg). Organic acids: oxalic acid (oxa), citric acid (cta), malic acid (mla), quinic acid (qna), succinic acid (sca), acetic acid (aca).

CONCLUSION

Changes in physical and chemical attributes of coffee beans were observed with an increase on pest infestation. The pest influenced the color of the beans, and the level of infestation dirty II differed the most regarding bean physical-chemical composition, with lower concentrations of sugars, lipids, and soluble solids and higher values of ash, total titratable acidity, electrical conductivity, potassium leaching, succinic acid, and acetic acid. Trigonelline, caffeine, and chlorogenic acids were not quantitatively affected by the damage caused by the coffee berry borer.

This study showed that, even at the lowest level of coffee berry borer infestation, up to two orifices without changing color, some chemical and physical attributes of the beans can be altered. At the highest level of pest infestation, sensorially important chemical compounds are drastically reduced, such as sugars and lipids. The measuring color is an interesting analysis to infer chemical compounds and, consequently, the sensorial potential of the drink in beans infested by the coffee berry borer.

CONFLICT OF INTEREST

Nothing to declare.

AUTHORS' CONTRIBUTION

Conceptualization: Pereira, R. G. F. A.; **Supervision:** Pereira, R. G. F. A. and Chalfoun, S. M.; **Formal Analysis:** Silva, S. A. and Teixeira, A. R.; **Investigation:** Silva, S. A. and Pereira, R. G. F. A.; **Writing – Original Draft:** Silva, S. A.; **Writing – Review and Editing:** Silva, S. A. and Pereira, R. G. F. A.

DATA AVAILABILITY STATEMENT

All dataset were generated and analyzed in the current study.

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