Revista Ciência Agronômica, v. 55, e20238738, 2024
Centro de Ciências Agrárias - Universidade Federal do Ceará, Fortaleza, CE
www.ccarevista.ufc.br

Scientific Article
ISSN 1806-6690

Analysis of the chemical composition and glyphosate residue in Conilon coffee beans[1]

Priscilla Moreira Curtis Peixoto[2]*, Vanessa Moreira Osório[3], Tafarel Victor Colodetti[4], Luciana Alves Parreira[3], Leandro Pin Dalvi[5], Marcelo Antonio Tomaz[5]

ABSTRACT - Due to the concern with the contamination of food by pesticides, especially coffee, the aim of this study was to analyze the chemical composition and glyphosate residue in Conilon coffee beans with and without defects and harvested at different times after applying the herbicide. The experiment was in a split-plot design, with the presence or absence of defects in the beans comprising the subplots, and the periods of 15, 30, 45 and 60 days after applying the herbicide corresponding to the split plots. We analyzed the volatile compounds, bioactive compounds, and glyphosate residue in the beans. Regardless of the presence or absence of defects, there was an increase in the concentrations of bioactive compounds in the raw Conilon coffee beans as the interval between applying the herbicide and harvesting the beans was increased. The most abundant volatile compounds per percentage area belonged to the pyrazines, furans and phenols. The amount of glyphosate residue found in the beans exceeded the maximum detectable limit by the ELISA method regardless of the time between application and harvest, corresponding to values that are unacceptable to several purchasing countries, and making the samples unsuitable for export and a risk to food safety.

Key words: *Coffea canephora*. Bioactive compounds. Volatile compounds. Herbicide.

DOI: 10.5935/1806-6690.20240047
Editor-in-Chief: Prof. Bruno França da Trindade Lessa - bruno.ftlessa@univasf.edu.br
*Author for correspondence
Received for publication 06/03/2023; approved on 04/09/2023
[1]This work is extracted from the master's thesis of the lead author, funded by the Fundação de Amparo à Pesquisa e Inovação do Espírito Santo (FAPES)
[2]Postgraduate program in Agrochemistry, Center for Exact, Natural and Health Science (CCENS), Federal University of Espírito Santo (UFES), Alegre-ES, Brazil, priscilla.mcpeixoto@gmail.com (ORCID ID 0000-0002-9060-4187)
[3]Department of Physics and Chemistry, Center for Exact, Natural and Health Science (CCENS), Federal University of Espírito Santo (UFES), Alegre-ES, Brazil, moreirava@yahoo.com.br (ORCID ID 0000-0001-5156-4568), luaparreira@hotmail.com (ORCID ID 0000-0001-7041-9999)
[4]Regional Scientific Development (CNPq/FAPES), Center for Agrarian and Engineering Science (CCAE), Federal University of Espírito Santo (UFES), Alegre-ES, Brazil, tafarelcolodetti@hotmail.com (ORCID ID 0000-0002-9233-5988)
[5]Department of Agronomy, Center for Agrarian and Engineering Science (CCAE), Universidade Federal do Espírito Santo (UFES), Alegre-ES, Brazil, leandropin@yahoo.com.br (ORCID ID 0000-0002-2995-8007), marcelo.tomaz@ufes.br (ORCID ID 0000-0002-5307-0219)

INTRODUCTION

Brazil is the world's largest producer and exporter of coffee and is ranked second among countries in terms of coffee consumption (ASSOCIAÇÃO BRASILEIRA DA INDUSTRIA DE CAFÉ, 2020). The state of Espírito Santo is the second largest coffee producer in the country and the largest producer of Conilon coffee (COMPANHIA NACIONAL DE ABASTECIMENTO, 2021). According to the Centro do Comércio de Café de Vitória (2021), part of the coffee from Espírito Santo is exported. In January 2021, shipments of Conilon coffee reached their third-highest monthly total since 1999, with the export of 240 thousand bags.

The quality of the coffee is directly related to the constituents of the raw beans, which, after roasting and grinding, give the drink its flavor and aroma. It is therefore important to identify the level of compounds and the chemical composition of the beans, as these help to determine the final quality of the product (TAVARES; FERREIRA, 2006).

Weeds are usually found in coffee plantations. By definition, a plant can be considered harmful if it directly or indirectly harms a specific human activity (SILVA; SILVA, 2007). Chemical control using herbicides is the most common method for managing weeds. Among the many different types of herbicides available on the market, the most used are glyphosate-based (AMARANTE JUNIOR *et al*., 2002; VAN BRUGGEN *et al*., 2018).

Glyphosate (N-(phosphonomethyl) glycine)) is an herbicide that is traditionally classified as non-selective, systemic and post-emergent, with rapid leaf absorption and translocation in the plant via both the xylem and phloem (AMARANTE JUNIOR *et al*., 2002; RODRIGUES; ARROBAS, 2017). Many coffee growers adopt this product due to its post-emergent feature that reduces the need for labor (GAZZIERO; ADEGAS; VOLL, 2007).

However, the irrational use of these glyphosate-based agrochemicals has raised concerns about environmental and food contamination and compromised the sustainability of the crops. It is therefore important to analyze the residue in foods such as coffee to assess the degree of toxicity ingested daily by consumers and ensure food safety, and to gain an understanding of the chemical composition of the coffee. In addition, such an investigation is of great interest to Brazilian agribusiness and foreign markets such as the European market, for which the Maximum Residue Limit (MRL) is ten times lower than that authorized in Brazil.

The aim of this study was to analyze the chemical composition and glyphosate residue in Conilon coffee beans with and without defects and harvested at different times after applying the herbicide.

MATERIAL AND METHODS

Characterization of the experimental area

The experimental area is located in the district of Estrela do Norte, part of the rural area of the municipality of Castelo in the southern part of the state of Espirito Santo (ES), in southeastern Brazil, at 20°34'19.6" S and 41°18'51, 7" W, and an altitude of 126 m. The terrain is flat. The historical series for the district shows an average annual precipitation of 1266 mm and an average annual temperature of 22 ºC (CLIMATE-DATA.ORG, 2020). The Conilon coffee plants (*Coffea canephora* Pierre ex A. Froehner) included six genotypes of early, intermediate and late maturation to allow the harvest to be carried out at different times. The plants were two years old and spaced 3.0 x 1.2 m apart, with drip irrigation.

Experimental design

To study the chemical profile of the beans and analyze the glyphosate residue in Conilon coffee, a split-plot scheme was used in a completely randomized design with three replications. The plots corresponded to the two types of bean samples used in the analysis, i.e. beans with and without defects. The split plots corresponded to the four periods during which the Conilon coffee beans were harvested after the application of glyphosate (15, 30, 45 and 60 days).

Applying the glyphosate

The Roundup WG® commercial product was used at the manufacturer-recommended dose of 3.0 kg. ha^{-1}, corresponding to 300 g per 20 L back sprayer tank (Jactor®). The applications were carried out during the morning, no later than 11 am, using a uniform flat fan nozzle. The herbicide was applied in a single dose throughout the experimental area to allow for harvesting 15, 30, 45 and 60 days after application.

Collecting the bean samples

The beans were harvested from April to May 2020, beginning when 85% of the fruit reached the cherry stage of ripeness following application of the herbicide. For each period, 10-L samples of cherry coffee were collected and immediately taken to be sun-dried on a suspended terrace until reaching a moisture level of around 12% wb. After drying and processing, the bean samples from each treatment were split into three subsamples (replications), which were then separated into samples with and without defects. The beans were passed through nos. 9 and 10 (mocha) oblong (4 × 19 mm) sieves (Pinhalense Produtos SA), to remove any impurities. Defective beans were manually separated based on the classification table for raw processed coffee beans, as per

Normative Instruction 8, of June 11, 2003 (Ministério da Agricultura, Pecuária e Abastecimento – MAPA).

Determining the levels of chlorogenic acid, trigonelline and caffeine (bioactive compounds)

The levels of chlorogenic acid (5-CQA), trigonelline and caffeine were analyzed in samples of raw Conilon coffee beans by high performance liquid chromatography (HPLC), employing the external standard method. This was done using 40 g of ground coffee and 150 mL of Mili-Q water at 90 °C. The mixture was stirred in a magnetic stirrer for 15 minutes and filtered. The filtrate was collected in a 50 mL volumetric flask. After cooling to room temperature, the filtrate was again filtered using a syringe containing a 0.45 µm membrane filter. The aqueous coffee extracts were then transferred to 1mL vials.

The extracts were analyzed by HPLC using a Shimadzu model Prominence-i chromatograph with a Shimadzu C-18 Slim-pack VP-ODS reverse phase column (250 mm long × 4.6 mm internal diameter). The system was coupled to a Shimadzu model SPD-20A UV/VIS spectrophotometric detector connected by a CBM-20A interface to a microcomputer to process the data. The analysis was carried out under the following conditions: flow of 1 mL. min^{-1}; mobile phase: HPLC grade methanol, Mili-Q water and HPLC grade acetic acid at a ratio of 20:80:1; column temperature of 40 °C and wavelength of 272 nm (ABRAHÃO *et al*., 2008).

The levels of chlorogenic acid, trigonelline and caffeine in the Conilon bean samples were quantified simultaneously using the external standard method. Solutions of known concentrations of the standards of these substances, acquired from Sigma Aldrich, were prepared, and analyzed under the same conditions as above.

Determining the volatile compounds

Headspace solid phase microextraction (HS-SPME) was used on the roasted coffee beans to analyze the volatile compounds, followed by gas chromatography coupled with mass spectrometry (GC-MS). The beans were subjected to a medium roast at a temperature range of 185 °C to 200 °C using a Probat Leogap roaster.

Three grams of ground and roasted coffee were weighed and placed in a headspace vial with a magnetic screw cap and silicone septum. The vial was then heated to 70 °C for 30 minutes.

The volatiles were collected by HS-SPME using 50 µm thick DVB/CAR/PDMS (Divinylbenzene/ Carboxene/Poldimethylsiloxane) fibre, and injected into the Shimadzu GC-MS QP-PLUS-2010. An Rtx-5MS fused silica capillary column (30 m long with an internal diameter of 0.25 mm) was used, with helium as the carrier gas at a flow rate of 1.67 $mL.min^{-1}$. The temperature of the injector and detector was 250 °C and 300 °C, respectively. The oven temperature followed a linear program that began at 40 °C and increased by 3 °C per minute until reaching 125 °C, at which it remained for one minute; the temperature was then increased by 10 °C per minute to 245 °C, which was maintained for a further three minutes (PEREIRA *et al*., 2020). To determine the chemical constituents, the resulting mass spectra were compared with those from the device library, including data from other studies and the Kovats indices (ADAMS, 2007).

Determining the glyphosate in the bean samples

Glyphosate was extracted from the samples of raw Conilon coffee beans, which were ground in a knife mill, using the ELISA (enzyme-linked immunosorbent assay) method acquired from Eurofins Abraxis.

We transferred 0.5 g of the ground sample to a 20 mL bottle. We then added 10 mL of deionized water and vortexed the mixture vigorously for 10 to 15 seconds. The sample was then placed on a shaker at 40 rpm for 10 minutes. After mixing, the sample was left to rest for two minutes, when 1.5 mL of the extracted sample were aspirated into a clean and suitably labeled 2.0 mL microcentrifuge tube and centrifuged for five minutes.

Eight hundred µL of glyphosate sample diluent was added to a suitably labeled 4 mL glass vial. Two hundred µL of supernatant (after mixing and standing for two minutes) was added to the glyphosate diluent (1:5) and mixed for 15 seconds on a vortex mixer. The standard and control samples were derivatized as per the 'Test Preparation' section of the Glyphosate ELISA Plate Kit User's Guide. The ELISA test for analyzing glyphosate residue in raw coffee beans has been patented by the company without discriminating the solutions.

The analysis consists of two steps: the derivatization procedure and the ELISA procedure using the glyphosate plate, followed by analysis using the ELISA plate. The derivatization step involves preparing the derivatization reagent, adding the samples to the test tubes, and then adding the buffer and the reagent. This is followed by analysis using the ELISA plate.

During the second step of the ELISA procedure, the standards and samples were added to the ELISA plate, followed by the addition of the antibody solution. The plate was left to react for 30 minutes before adding the enzyme conjugate, which was then left to rest for a further 60 minutes. The plate was then washed using buffer wash solution, and any remaining buffer in the wells was removed by drying the plate on a stack of paper towels. The substrate/ color solution was added and left to act for 30 minutes. The stop solution was then introduced. To measure the color, an

absorbance reading was taken at 450 nm using an ELISA microplate reader, and the results calculated.

A correction factor (x 100) was used to adjust the ELISA results for the required dilution. Samples with concentrations less than the standard of 7.5 ppb glyphosate are considered below the quantifiable value, while samples with concentrations > 400 ppb are considered above that value.

Statistical analysis

Homogeneity of variance and error normality were checked as per Storck, Ribeiro and Cargnelutti Filho (2011). Data on the trigonelline, chlorogenic acid and caffeine concentrations were submitted to analysis of variance (ANOVA) by F-test ($p \leq 0.05$). When there was a significant effect for the source of variation, Tukey's test ($p \leq 0.05$) was used to compare the mean values between the samples with and without defects. Regression analysis was used to determine the mean values as a function of the periods between applying the glyphosate and the harvest. The regression model (first or second degree) was chosen based on the significance of the angular coefficients and the values of the coefficients of determination (R^2). The SISVAR statistical software was used (FERREIRA, 2011).

Principal Component Analysis (PCA), a technique of multivariate analysis, was carried out on volatile compounds from the samples with and without defects (harvested 15, 30, 45, and 60 days after applying the glyphosate) based on the mean peak areas of the compounds, using the GENES statistical software (CRUZ, 2013). To standardize the data, the expression: $x_i = X_i/\sigma x$ was used, where x_i is the standardized mean of the variable, X_i is the original mean of the variable, and σx is the standard deviation of variable X_i.

RESULTS AND DISCUSSION

Bioactive compounds

The retention time identified for each of the compounds was 15 minutes for chlorogenic acid (5-ACQ), 13.5 minutes for caffeine and 2.76 minutes for trigonelline. Significant effects ($p = 0.05$) were seen for the concentrations of the three compounds in the raw Conilon coffee beans based on the interactions between sample type (with and without defects) and the interval between applying the glyphosate and harvesting the fruit (15, 30, 45, and 60 days). Figure 1 shows a breakdown of the factors.

The concentrations of chlorogenic acid in the bean samples with no defects did not adjust to the regression models under study (first and second degree). As such, the value shown corresponds to the mean value (60.4 μg. mL^{-1}). For the bean samples with defects, the data adjusted to the first-degree linear regression model, with increases in the concentration of the compound as the interval between applying the glyphosate and the harvest increased (Figure 1A). Figure 1B shows significant differences between the beans with and without defects harvested at 15, 45, and 60 days after applying the glyphosate. Samples with no defects had a higher concentration of chlorogenic acid at 15 and 45 days, while samples with defects had a higher concentration at 60 days. There was no significant difference in the concentration of chlorogenic acid for the different types of bean samples 30 days after application.

Organic acids, such as chlorogenic acid, are important compounds for the quality of the coffee in certain concentrations and are recommended as selection criteria for improving the quality (GUERRERO; SUÁREZ; MORENO, 2001).

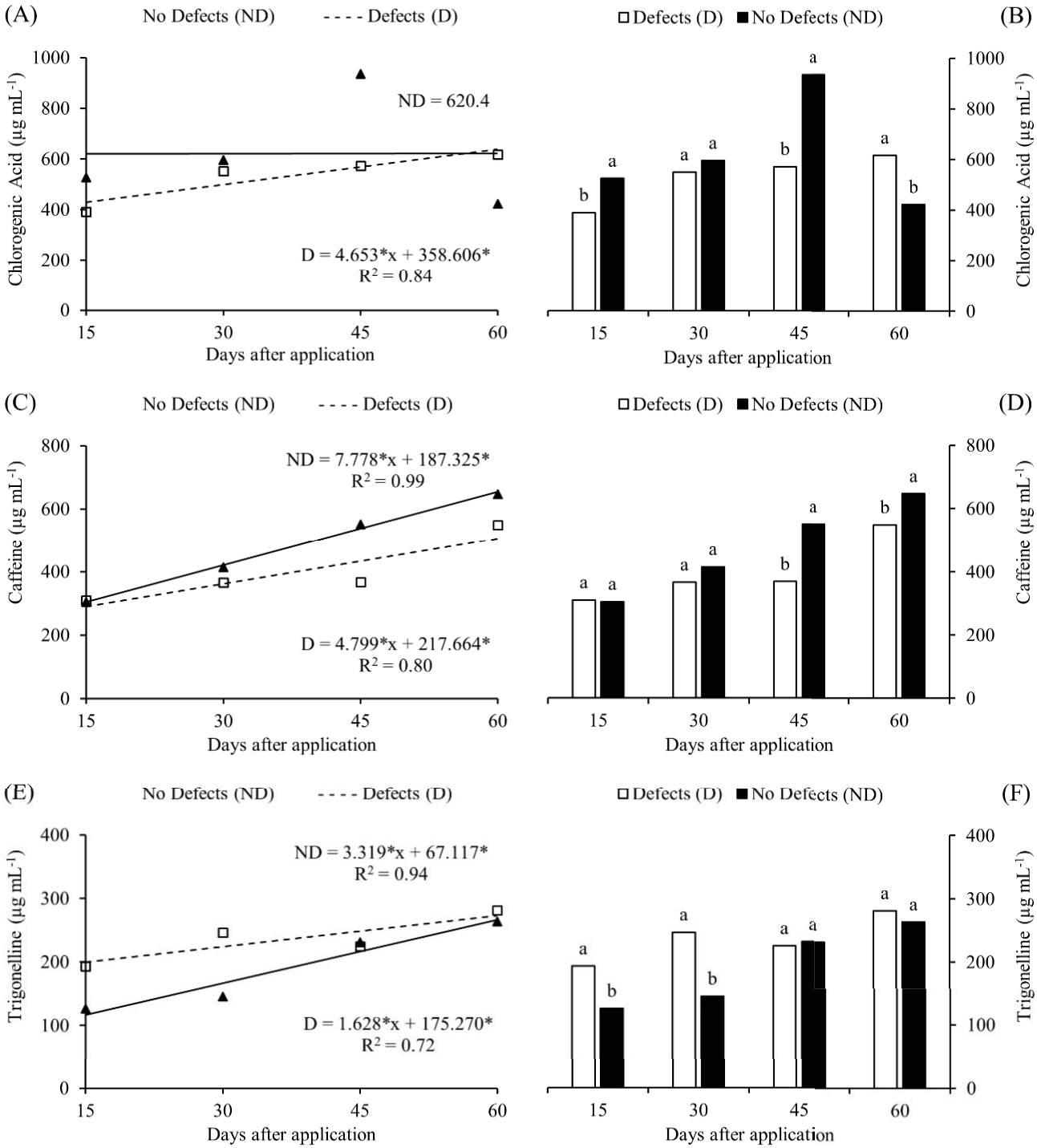
The caffeine concentration in the bean samples with and without defects adjusted to the first-degree regression models, where an increase in the concentration of the compound was seen as the interval between applying the glyphosate and the harvest increased (Figure 1C). Figure 1D, shows significant differences between coffee with and without defects harvested 45 and 60 days after applying the glyphosate, with higher concentrations of caffeine found in the samples with no defects. According to Monteiro and Trugo (2005), caffeine remains stable while roasting the beans, and despite being odorless, is bitter, which may favor this sensory attribute.

Trigoneline concentrations in samples with and without defects adjusted to the first-degree linear regression model, with an increase in the concentration of the compound as the interval between applying the glyphosate and the harvest increased (Figure 1E). In Figure 1F, there were significant differences between samples with and without defects harvested 15 and 30 days after application, where higher concentrations of trigonelline were seen in the samples with defects.

Trigonelline is a nitrogenous compound found in unripe fruit and is important for the flavor and aroma of the coffee. It is responsible for the formation of degradation products during the roasting process, which are crucial for the aroma. (MONTEIRO; TRUGO, 2005; MORAIS *et al*., 2008; VIGNOLI *et al*., 2014).

The results show that the concentration of caffeine and trigonelline in raw Conilon coffee beans tends to increase when the interval between applying the glyphosate and harvesting the beans is increased, regardless of the presence or absence of defects.

Figure 1 - Chlorogenic acid, caffeine and trigonelline concentrations in samples of Conilon coffee with and without defects, as a function of the interval between applying the glyphosate in the experimental area and harvesting the fruit (Castelo, ES; altitude 126 m; 2020 harvest). Regression analysis for the concentrations (µg. mL^{-1}) of chlorogenic acid (A), caffeine (C) and trigonelline (E) in samples of raw Conilon coffee beans with and without defects, as a function of the interval between applying the glyphosate and the harvest (15, 30, 45 and 60 days). Comparison test of the mean values for the concentrations (µg. mL^{-1}) of chlorogenic acid (B), caffeine (D) and trigonelline (F) between samples of raw Conilon coffee beans with and without defects, for each day after the applying the glyphosate (15, 30, 45 and 60 days)



Angular regression coefficient significant at 5% probability. Mean values followed by the same lowercase letter when comparing the bars do not differ by Tukey's test at 5% probability

Coffee quality is normally evaluated using criteria that include determining the number of defects and the size of the beans (MATIELLO, 1998). However, the quality is also associated with different physical and chemical components, which are responsible for the flavor, texture, bitterness and characteristic aroma of the coffee. Important among these components are the organic acids profile, and the levels of soluble solids, 5-caffeoylquinic chlorogenic acid, caffeine (PIMENTA, 2003) and trigonelline (FIGUEIREDO *et al.*, 2013).

Volatile compounds

Thirty-three volatile compounds were identified in the roasted coffee using the Kovats index and by analyzing their respective mass spectra obtained from the GC-MS library (Table 1). These compounds belong to different classes, including carboxylic acids, alcohols, ketones, phenols, furans, pyrazines and others that remain unidentified. Nascimento *et al*. (2007) also identified some of these compounds belonging to the pyrazine, furan and phenol classes in Conilon coffee.

Roasting the coffee beans causes physical, chemical and sensory changes in the matrix. The intensity and type of these changes depend primarily on the chemical composition of the beans, as well as the time taken, and the temperature applied during the process. This procedure leads to the degradation, formation, and volatilization of various compounds (RODARTE *et al*., 2009).

Pyrazines are compounds found in food that has undergone thermal processing. They are responsible for the aroma of the food, but degrade during prolonged roasting, and are characteristic of light and medium roasts (CLARKE; MACRAE, 1985; FLAMENT; ENGELHARDT, 2002). In this study, the class of pyrazines represented between 19.2% and 28.6% of the total areas of the analyzed chromatograms. The compounds methypyrazine, 2.5-dimethylpyrazine, and 3-ethyl-2.5-dimethylpyrazine were found with a greater percentage area in each of the samples under analysis, methypyrazine being the most abundant in the bean samples with no defects.

From 12.1% to 5.8% of the total area of the chromatogram corresponded to furan compounds. According to Czerny, Mayer e Grosch (1999) and Akiyama *et al*. (2005), in roasted coffee, the furan and pyrazine compounds are not only the principal compounds, but also the main contributors to the aroma.

Ketone compounds identified in the samples represented only 1.0% – 4.1% of the area of the chromatogram, making it the least abundant class in terms of chromatogram area. Bandeira *et al*. (2009) found an abundance of ketone compounds together with aldehydes and pyrazines.

The number of phenols varies depending on the degree of roasting and the type of coffee. In the case of C. canephora and darker roasts, there was a greater number of phenolic compounds (CLARKE; MACRAE, 1985; MOREIRA; TRUGO; MARIA, 2000).

Only one phenol compound was identified: 4-ethylguaiacol. Toci and Farah (2008) identified the following potential PVA defect markers in roasted coffee: pyrazine, 2,3-butanediol meso, 2-methyl-5-(1-propenyl) pyrazine, hexanoic acid, 4-ethylguaiacol and p-cresol isopropyl sulfite. In this study, there was a greater concentration of 4-ethyguaiacol in defective beans, which is consistent with the literature. Bandeira *et al*. (2009) found that the classes of volatile compounds seen in the defects represented their formation process and, therefore, confirmed the hypothesis that it is possible to identify compounds as markers of specific defects. According to the same authors, it is possible to differentiate the main defects of raw and roasted beans using headspace analysis.

Alcohols were also represented by only one compound: 1.2.3-Propanetriol diacetate. According to Flament (2002), the compounds in raw beans that stood out in order of abundance were alcohols, carboxylic acids, esters and ketones (primary compounds), while in roasted beans the classes were pyrazines, furans, ketones, pyrroles, pyridines and esters (secondary compounds). Bandeira *et al*. (2009) also identified more alcohol compounds in raw beans than in roasted beans, corroborating the present study.

Figure 2 displays the results of the dispersion of 33 volatile compounds in the samples with and without defects. The biplots were obtained through Principal Component Analysis using standardized data and the mean percentages of the peak areas of each compound.

The first two principal components (PC) of the bean samples with defects were responsible for 83.96% of the total variation in volatile compounds as a function of the interval between applying the glyphosate and the harvest (15, 30, 45, 60 days), where PC1 was responsible for 51.42% and PC2, for 32.54% of the variation in the data. For the samples with no defects, the first two principal components were responsible for 81.06% of the total variation in volatile compounds as a function of the interval between applying the herbicide and the harvest, where PC1 corresponded to 51.80% and PC2 to 29.26%.

When analyzing the biplot of the samples with defects, it was found that certain compounds showed a high correlation with each other, resulting in simultaneous increases or decreases, especially Trimethylpyrazine [16], 2-ethyl-3-methylpyrazine [17] with 2,3-dimethyl-5-ethylpyrazine [20]; Ethylpyrazine [9] with 1,2-Benzenedicarboxylic Acid [32]; 2-ethyl-5-methylpyrazine [15] with hexadecanoic acid [33]; 2,3-dimethylpyrazine [10] with 1-(2-furanylmethyl) [24];

Table 1 - Volatile compounds and their classes identified in samples of Conilon coffee with and without defects, at different intervals between applying the glyphosate in the experimental area and harvesting the fruit (Castelo, ES; altitude 126 m; 2020 harvest)

		Area (%)							
		15 days		30 days		45 days		60 days	
Class	Compound	With defects	No defects	With defects	No defects	With defects	No defects	With defects	No defects
Pyrazine	Methylpyrazine	4.983	5.089	4.138	5.050	6.145	7.349	5.270	6.227
	2.5-dimethylpyrazine	3.469	3.308	3.259	3.493	3.538	4.168	2.466	3.984
	2.6-dimethylpyrazine	1.565	1.711	1.322	1.113	1.824	1.520	0.934	1.500
	Ethylpyrazine	1.275	1.245	1.253	1.030	0.756	1.356	0.789	1.204
	2.3-dimethylpyrazine	0.597	0.500	0.286	0.398	0.661	0.421	0.249	0.383
	2-ethyl-6-Methylpyrazine	4.088	-	4.660	4.463	-	3.542	3.130	5.019
	2-ethyl-5-Methylpyrazine	1.778	1.615	1.583	1.947	1.110	1.430	1.330	2.431
	Trimethylpyrazine	1.158	1.077	1.150	0.739	0.911	0.817	0.518	1.030
	2-ethyl-3-methylpyrazine	1.444	1.210	1.364	1.209	1.077	1.091	0.748	1.017
	3-ethyl-2.5-dimethylpyrazine	5.204	4.369	6.034	5.042	4.489	6.700	5.002	5.581
	2.3-dimethyl-5-ethylpyrazine	-	0.490	0.817	0.566	0.602	0.661	-	0.709
	2.3-diethyl-5-methylpyrazine	-	-	0.741	0.496	-	0.699	0.393	0.528
	3.5-diethyl-2-methylpyrazine	0.524	0.642	0.955	0.797	-	0.821	0.741	0.875
	2-Isoamyl-6-methylpyrazine	1.000	0.943	1.447	0.939	1.024	1.429	1.282	1.123
	Total pyrazines	25.964	19.624	26.210	23.347	19.207	28.585	18.480	27.993
Furan	3-(2H)-Furanone. dihydro-2-methyl	0.492	0.467	0.327	0.318	0.632	0.554	0.459	0.429
	Furfural	3.155	3.119	3.674	2.757	2.406	3.859	4.091	3.752
	2-furanmethanol	5.521	5.787	3.538	5.004	5.615	5.074	5.383	5.543
	2-Furancarboxaldehyde. 5-methyl	2.655	2.846	3.075	2.667	4.039	4.541	3.988	3.281
	1-(2-furanylmethyl)	1.455	1.234	1.347	1.372	1.533	1.744	1.313	1.021
	Total furans	13.278	13.453	11.961	12.118	14.225	15.772	15.234	14.026
Carboxylic acid	Butanoic acid. 3-methyl	-	-	0.130	-	0.061	0.091	0.149	0.137
	Benzoic acid. 2-hydroxy	4.074	4.298	5.023	4.161	2.433	3.657	3.155	3.095
	1,2-Benzenedicarboxylic acid	0.664	0.656	0.980	0.307	0.168	0.038	-	0.108
	Hexadecanoic acid	0.186	0.150	0.594	0.576	0.306	0.027	0.144	-
	Total carboxilic acids	4.924	5.104	6.727	5.044	2.968	3.813	3.448	3.340
Ketone	2-Propanone	1.332	1.255	0.761	0.914	2.333	1.411	1.155	0.950
	2-butanone	0.305	0.378	0.273	0.189	0.606	0.490	0.275	0.265
	Ethanone	1.380	1.217	-	0.928	1.153	-	-	0.622
	Total ketones	3.017	2.850	1.034	2,031	4,092	1.901	1.430	1.837
Phenol	Phenol. 4-ethenyl-2-methoxy (4 – ethylguaiacol)	13.256	11.746	15.446	14.109	14.286	13.834	16.098	14.367
Alcohol	1.2.3-Propanetriol. diacetate	6.325	5.277	4.665	6.119	7.585	3.132	-	-
Others	N-benzylidene-dimethylammonium chloride	0.138	0.106	0.160	0.082	-	0.083	0.122	0.057
	Maltol	-	1.050	-	0.877	1.025	-	0.577	-
	Triacetin	5.755	5.323	5.970	6.816	7.581	3.119	7.196	5.159
	Delta-Cadinene	0.301	0.266	0.131	-	-	-	0.745	0.645
	Caffeine	5.586	4.358	4.343	7.263	4.155	1.472	5.933	3.388
	Total others	11.780	11.103	10.604	15.038	12.761	4.674	14.573	9.249
Unidentified		21.456	30.843	23.353	22.194	24.876	28.289	30.737	29.188

3-(2-H)-Furanone, dihydro-2-methyl [1], 2-Propanone [6] with 2-Butanone [13]; Methylpyrazine [2] with 2-furanmethanol [5]; Delta-Cadinene [30], Caffeine [31], Phenol, 4-ethenyl-2-methoxy [27] with Butanoic acid, 3-methyl [4]. On the other hand, various compounds showed an inverse correlation, with one increasing as the other decreased, namely: Ethylpyrazine [9] with 1,2-benzenedicarboxylic acid [32] in detriment to 2-furancarboxaldehyde, 5-methyl [12] and Triacetin [29]; Delta-Cadinene [30] with 2,5-dimethylpyrazine [7]; Caffeine [31] and Phenol,

4-ethenyl-2-methoxy [27] with 1,2,3-Propanetriol diacetate [28]; Maltol [21] with Benzoic acid, 2-hydroxy [26]; Methylpyrazine [2] and 2-furanmethanol [5] with 3-ethyl-2,5-dimethylpyrazine [19]; 2-Propanone [6] and 2-Butanone [13] with 3,5-diethyl-2-methylpyrazine [23]; 2,3-dimethylpyrazine [10] and 1-(2-furanylmethyl) [24] with Furfural [3].

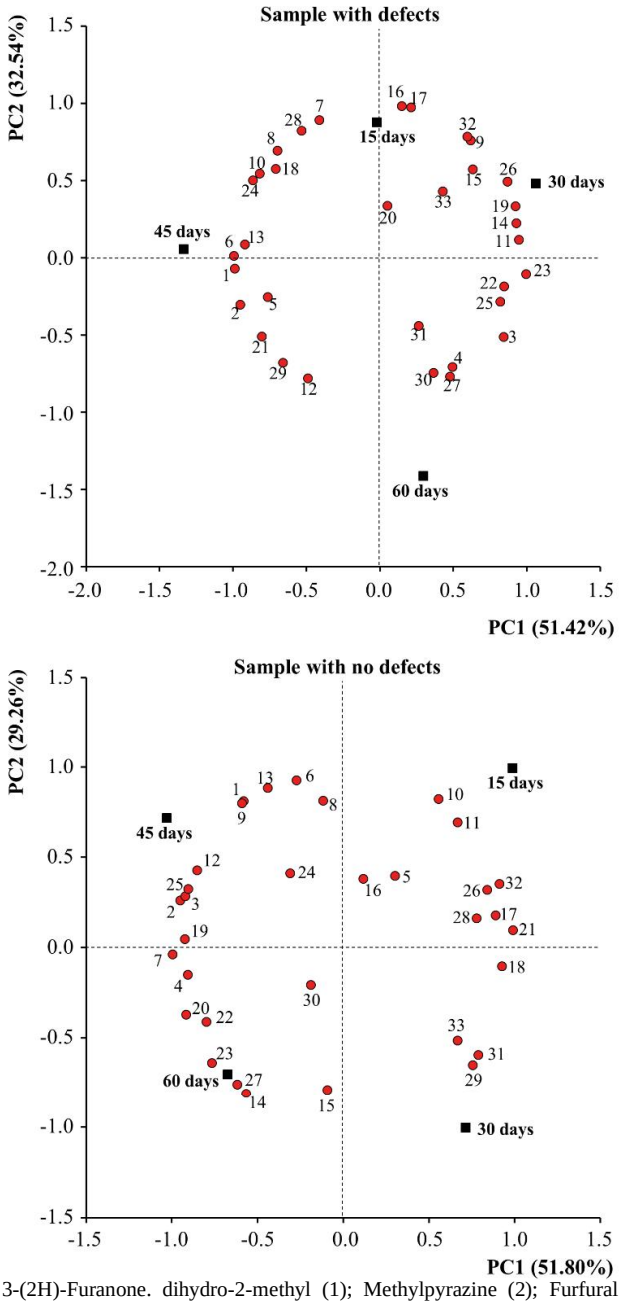
Compounds with a high positive correlation were also seen in the samples with no defects: Ethylpyrazine [9] with 1-(2-furanylmethyl) [24]; Methylpyrazine [2], Furfural [3] and 2-Isoamyl-6-methylpyrazine [25]; 2,5-dimethylpyrazine [7] with 3-ethyl-2,5-dimethylpyrazine [19]; 2,3-dimethyl-5-ethylpyrazine [20] with 2,3-diethyl-5-methylpyrazine [22]; Phenol, 4-ethenyl-2-methoxy [27] with 2-ethyl-6-methylpyrazine [14]; Triacetin [29], Hexadecanoic Acid [33] and Caffeine [31]; Benzoic acid, 2-hydroxy [26] with 1,2-benzenedicarboxylic acid [32]; 2-furanmethanol [5] with 2,3-dimethylpyrazine [10]; 2-ethyl-3-methylpyrazine [17] with 1,2,3-Propanetriol diacetate [28]; 2,3-dimethylpyrazine [10] compounds with 1-(2-furanylmethyl) [24]; 2-Furanmethanol [5] with Phenol, 4-ethenyl-2-methoxy [27]; Benzoic acid, 2-hydroxy [26] and 1,2-benzenedicarboxylic acid [32] with 2,3-dimethyl-5-ethylpyrazine [20]; 1,2,3-Propanetriol diacetate [28] and 2-ethyl-3-methylpyrazine [17] with butanoic acid, 3-methyl [4]; Maltol [21] with 2,5-dimethylpyrazine [7]; Ethanone [18] with 3-ethyl-2,5-dimethylpyrazine [19] while 3,5-diethyl-2-methylpyrazine [23] showed a negative correlation with N-benzylidene-dimethylammonium chloride [11].

The 33 compounds showed different dispersions depending on the type of sample (with and without defects) and the number of days after applying the glyphosate (15, 30, 45 and 60 days). In other words, there was no trend between the values for peak area in compounds of a given class as a function of the type of sample. However, it was noted that, in addition to most compounds belonging to the class of pyrazines, they showed a strong correlation with each other. This correlation was seen in samples with and without defects, meaning they displayed areas of higher or lower peaks simultaneously.

Glyphosate residue

Table 2 shows the absorbance values, coefficients of variation and results after applying the ELISA method for measuring glyphosate. Glyphosate residue was detected in all samples where the herbicide was applied, exceeding the maximum detectable limit for the method used, of 400 ppb. Based on these results, it is important to pay attention to the limits for glyphosate residue in coffee beans. The maximum limit was exceeded even for the longest interval between applying the herbicide and the harvest (60 days), raising concerns about food safety.

Figure 2 - Principal component analysis (PCA) of the volatile composition of Conilon bean samples with and without defects, and the interval between applying the glyphosate and harvesting the fruit (Castelo, ES; altitude 126 m; 2020 harvest)



3-(2H)-Furanone. dihydro-2-methyl (1); Methylpyrazine (2); Furfural (3); Butanoic acid. 3-methyl (4); 2-furanmethanol (5); 2-Propanone (6); 2,5-dimethylpyrazine (7); 2,6-dimethylpyrazine (8); Ethylpyrazine (9); 2,3-dimethylpyrazine (10); N-benzylidene-dimethylammonium chloride (11); 2-Furancarboxaldehyde. 5-methyl (12); 2-Butanone (13); 2-ethyl-6-methylpyrazine (14); 2-ethyl-5-methylpyrazine (15); Trimethylpyrazine (16); 2-ethyl-3-methylpyrazine (17); Ethanone (18); 3-ethyl-2,5-dimethylpyrazine (19); 2,3-dimethyl-5-ethylpyrazine (20); Maltol (21); 2,3-diethyl-5-methylpyrazine (22); 3,5-diethyl-2-methylpyrazine (23); 1-(2-furanylmethyl) (24); 2-Isoamyl-6-methylpyrazine (25); Benzoic acid. 2-hydroxy (26); Phenol. 4-ethenyl-2-methoxy (27); 1.2.3-Propanetriol. diacetate (28); Triacetin (29); Delta-Cadinene (30); Caffeine (31); 1,2-Benzenedicarboxylic acid (32); Hexadecanoic acid (33)

Table 2 - Results of the analysis of glyphosate residue in samples of raw Conilon coffee beans

Sample/Control	Absorbance	CV (%)	Result (ppb)
Control	0.729	6.402	-
60 days - no defect	0.194	11.664	> 400
60 days – with defects	0.0925	5.351	> 400
45 days - no defect	0.181	16.408	> 400
45 days - with defects	0.1485	8.095	> 400
30 days - no defect	0.275	12.342	> 400
30 days - with defects	0.1375	1.543	> 400
15 days - no defect	0.193	0.000	> 400
15 days - with defects	0.1205	0.587	> 400

CV - coefficient of variation

Researchers used the ELISA method in a published study to measure glyphosate levels in samples of water, food (including coffee) and human urine. The results showed that the water samples contained undetectable or very low levels of glyphosate (≤ 0.08 ppb). On the other hand, in addition to glyphosate being detected in human urine, the food samples showed varying levels of glyphosate contamination. Especially coffee powder, that presented 11 and 26 ppb in two samples and was among the foods with the highest concentrations of glyphosate (JOHN; LIU, 2018). In the present study, the bean samples had levels greater than 400 ppb, showing them to be contaminated by this residue.

For each registered agrochemical there is a maximum residue limit (MRL). In Brazil, the MRL of each active ingredient is defined by the Brazilian Health Regulatory Agency (Anvisa) and is based on scientific studies. However, the limit for glyphosate varies from country to country due to the different conditions of production. It is important to consider the MRL accepted by each of the purchasing countries, as well as correct agricultural practices and the rational use of agrochemicals. This is particularly important for Brazil, a major coffee exporter, to ensure that Brazilian coffee does not exceed the authorized MRL.

In 2019, Anvisa completed a reassessment of glyphosate, defining it as safe for use in the country and maintaining the MRL in coffee at 1 $mg.kg^{-1}$ (AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA, 2019). Since 2013, the MRL for glyphosate in coffee in the European Union (EU) has been 0.1 $mg.kg^{-1}$ (BOMBARDI, 2017), a limit 10 times lower than in Brazil.

Based on the analysis of glyphosate residue using the ELISA technique, it was found that all Conilon bean samples treated with the herbicide exceeded quantifiable limits. As a result, these bean samples are not suitable for export to the EU as they present residual levels at least four times higher than the maximum acceptable limit for EU countries.

CONCLUSIONS

1. There was an increasing trend in the concentration of caffeine and trigonelline in raw Conilon coffee beans regardless of the presence or absence of defects as the interval between applying the glyphosate and harvesting the fruit increased;
2. Thirty-three volatile compounds were identified in the beans, especially pyrazines, furans, and phenols, confirming their abundance in Conilon coffee. Pyrazine compounds showed a high positive correlation with each other in beans with and without defects. However, there was no relationship between the composition of the volatile compounds and the interval between applying the glyphosate and harvesting the beans.
3. Residual levels of glyphosate exceeding the maximum limits quantifiable by the ELISA method were found in the raw Conilon coffee beans. This highlights concerns about coffee safety, as even in samples with a longer interval between applying the herbicide and the harvest (60 days), the residual limits could not be quantified. In addition, such high residual levels make it impossible to export these coffees to consumer markets such as the EU, as they present high levels of glyphosate contamination.

ACKNOWLEDGEMENTS

The authors would like to thank the Fundação de Amparo à Pesquisa do Estado do Espírito

Santo - FAPES and the Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq for their financial support of this research and for granting the scholarships. The authors would also like to thank the Centro de Ciências Exatas, Naturais e da Saúde da Universidade Federal do Espírito Santo (CCENS/UFES) for providing access to the necessary facilities and laboratories.

REFERENCES

ABRAHÃO, S. A. *et al.* Compostos bioativos em café integral e descafeinado e qualidade sensorial da bebida. **Pesquisa Agropecuária Brasileira**, v. 43, n. 12, p. 1799-1804, 2008.

ADAMS, R. P. **Identification of essential oil components by gas chromatography/mass spectrometry**. 4. ed. Illinois: Allured Publishing Corporation, 2007. 804 p.

AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA. **Publicada reclassificação toxicológica de agrotóxicos**. 2019. Available at: https://www.gov.br/anvisa/pt-br/assuntos/noticias-anvisa/2019/publicada-reclassificacao-toxicologica-de-agrotoxicos. Accessed on: Jan. 18, 2021.

AKIYAMA, M. *et al*. Characterization of flavor compounds released during grinding of roasted robusta coffee beans. **Food Science and Technology Research**, v. 11, n. 3, p. 298-307, 2005.

AMARANTE JUNIOR, O. P. de *et al.* Glifosato: propriedades, toxicidade, usos e legislação. **Química Nova**, v. 25, n. 4, p. 589-593, 2002.

ASSOCIAÇÃO BRASILEIRA DA INDUSTRIA DE CAFÉ. **O café brasileiro na atualidade**. 2020. Available at: https://www.abic.com.br/o-cafe/historia/o-cafe-brasileiro-na-atualidade-2/. Accessed on: Dec. 3, 2020.

BANDEIRA, R. D. C. C. *et al*. Volatile composition of intrinsic defective coffee beans by GC/MS-headspace. **Química Nova**, v. 32, n. 2, p. 309-314, 2009.

BOMBARDI, L. M. **Geografia do uso de agrotóxicos no Brasil e conexões com a União Europeia**. 2017. Available at: https://ecotoxbrasil.org.br/upload/587ed92192e9dbe77bddffd31cbe25a7-e-book_atlas_agrot_axico_2017_larissa_bombardi.pdf. Accessed on: Apr. 19, 2020.

BRASIL. Conselho Nacional do Meio Ambiente. Resolution number 420 of December 28, 2009. Soil quality guiding criteria and values regarding the presence of chemical substances and establishes guidelines for the environmental management of areas contaminated by these substances as a result of human activities. **Diário Oficial da União**: section 1, Brasília, DF, Dec. 30, 2009. 81 p.

CENTRO DO COMÉRCIO DE CAFÉ DE VITÓRIA. **Exportação**: estatística mensal. 2021. Available at: http://www.cccv.org.br/imagens/gerais/divulg_012021_634.pdf. Accessed on: Jan. 22, 2021.

CLARKE, R. J.; MACRAE, R. **Coffee**. v. 1: Chemistry. Amsterdã: Elsevier Science, 1985. 306 p.

CLIMATE-DATA.ORG. **Clima**: Castelo-ES. 2020. Available at: https://pt.climate-data.org/america-do-sul/brasil/espirito-santo/castelo-43566/?amp=true. Accessed on: Apr. 14, 2020.

COMPANHIA NACIONAL DE ABASTECIMENTO. **Acompanhamento da safra brasileira**: café. 2021. Available at: https://www.conab.gov.br/info-agro/safras/cafe. Accessed on: Mar. 9, 2021.

CRUZ, C. D. Genes: a software package for analysis in experimental statistics and quantitative genetics. **Acta Scientiarum. Agronomy**, v. 35, n. 3, p. 271-276, 2013.

CZERNY, M.; MAYER, F.; GROSCH, W. Sensory study on the character impact odorants of roasted Arabica coffee. **Journal of Agricultural and Food Chemistry**, v. 47, n. 2, p. 695-699, 1999.

FERREIRA, D. F. Sisvar: a computer statistical analysis system. **Ciência e Agrotecnologia**, v. 35, n. 6, p. 1039-1042, 2011.

FIGUEIREDO, L. P. *et al*. The potential for high quality bourbon coffees from different environments. **Journal of Agricultural Science**, v. 5, n. 10, p. 87, 2013.

FLAMENT, I. **Coffee flavor chemistry**. New York: John Wiley and Sons, 2002. 157 p.

FLAMENT, I.; ENGELHARDT, U. H. BOOKS-Coffee Flavor Chemistry. **Angewandte**

Chemie-International Edition, v. 41, n. 13, p. 2413, 2002.

GAZZIERO, D. L. P.; ADEGAS, F. S.; VOLL, E. **Indicações para o uso de glyphosate em soja transgênica**. Londrina: Embrapa Soja, 2007. 4 p.

GUERRERO, G.; SUÁREZ, M.; MORENO, G. Chlorogenic acids as a potential criterion in coffee genotype selections. **Journal of Agricultural and Food Chemistry**, v. 49, n. 5, p. 2454-2458, 2001.

JOHN, J.; LIU, H. O monitoramento do glifosato na água, alimentos e urina revela uma associação entre o glifosato urinário e o consumo de chá: um estudo piloto. **Revista**

Internacional de Engenharia de Saúde Ambiental, v. 7, n. 1, 2018.

MATIELLO, J. B. **Café conillon**. Rio de Janeiro: MAPA, 1998. 162 p.

MONTEIRO, M. C.; TRUGO, L. C. Determinação de compostos bioativos em amostras comerciais de café torrado. **Química Nova**, v. 28, p. 637-641, 2005.

MORAIS, S. A. L. de *et al*. Bioactive compounds, acids groups and antioxidant activity analysis of arabic coffee (Coffea arabica) and its defective beans from the Brazilian savannah submitted to different roasting degrees. **Food Science and Technology**, v. 28, p. 198-207, 2008.

MOREIRA, R. F. A.; TRUGO, L. C.; MARIA, C. A. B. de. Componentes voláteis do café torrado. Parte II: Compostos alifáticos, alicíclicos e aromáticos. **Química Nova**, v. 23, n. 2, p. 195-203, 2000.

NASCIMENTO, E. A. do. *et al*. Composição química do café Conilon em diferentes graus de torração. **Ciência & Engenharia**, v. 16, n. 1/2, p. 17-21, 2007.

PEREIRA, L. L. *et al*. New propositions about coffee wet processing: chemical and sensory perspectives. **Food Chemistry**, v. 310, p. 125943, 2020.

PIMENTA, C. J. **Qualidade de café**. Lavras: UFLA, 2003. 297 p.

RODARTE, M. P. *et al*. Compostos não voláteis em cafés da região sul de Minas submetidos a diferentes pontos de torração. **Ciência e Agrotecnologia**, v. 33, n. 5, p. 1366-1371, 2009.

RODRIGUES, M. A.; ARROBAS, M. Manutenção do solo. *In*: RODRIGUES, M. A. (ed.). **Amendoeira**: estado da produção. Bragança: CNCFS, 2017. p. 85-231.

SILVA, A. A.; SILVA, J. A. **Tópicos em manejo de plantas daninhas**. Viçosa, MG: UFV, 2007. 367 p.

STORCK, L.; RIBEIRO, N. D.; CARGNELUTTI FILHO, A. Experimental precision in common bean trials using the Papadakis method. **Pesquisa Agropecuária Brasileira**, v. 46, n. 8, p. 798-804, 2011.

TAVARES, L. A.; FERREIRA, A. G. Análises quali-e quantitativa de cafés comerciais via ressonância magnética nuclear. **Química Nova**, v. 29, n. 5, p. 911-915, 2006.

TOCI, A. T.; FARAH, A. Volatile compounds as potential defective coffee beans' markers. **Food chemistry**, v. 108, n. 3, p. 1133-1141, 2008.

VAN BRUGGEN, A. H. C. *et al*. Environmental and health effects of the herbicide glyphosate. **Science of The Total Environment**, v. 616/617, p. 255-268, 2018.

VIGNOLI, J. A. *et al*. Roasting process affects differently the bioactive compounds and the antioxidant activity of arabica and robusta coffees. **Food Research International**, v. 61, p. 279-285, 2014.