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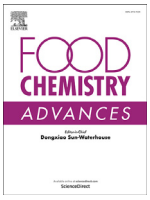

Impact of the coffee berry borer on the volatile and semi-volatile compounds; qualitative profile of Coffea arabica berries

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ABSTRACT

One of the main attributes that highlight the final quality of a gourmet cup of coffee is its aroma. Aromas vary according to a variety of plant and environmental variables, among others. This study aimed to characterize volatile and semivolatile compounds according to the Coffee arabica "Limani" berries ripening stages (healthy and brocaded). The study used different extraction methodologies to capture the broad spectrum of volatile, semivolatile organic compounds in coffee berries and berry borer (CBB). The methodologies used in the study included: enfleurage, headspace SPME (solid-phase microextraction), absorbent trap, and direct immersion SPME. Our study generated a Profile for coffee berries and CBB w with 228 compounds. Esters, cyclic, and benzyl compounds represent 65.6% of the total. The first three types of compounds that most attract our sense of smell constitute 40.5% of the compounds found; 1.3% aldehydes, 2.6% alcohols, and 36.6% benzyl. Overripe berries have high volatile emissions and show a composition dominated mainly by esters followed by alcohols, ketones, and aldehydes. The lowest-level compounds were monoterpenes. The number of compounds found in CBB varied according to sex. In summary, the CBB damage harms coffee berries' quality and aroma. The complete profile compounds generated will help better understand insect-plant relationships and potentially develop effective bait traps.

1. Introduction

One of the main attributes that highlight the quality of a good cup of coffee is its aroma. The aromas vary according to plant variety, altitude, geographical origins, soil, environmental variables, application of integrated pest management, harvesting, drying, and roasting (Bastian et al., 2021; Colzi et al., 2017; Sunarharum et al., 2014; Toledo et al., 2016). Coffee crops are among the most important agricultural export products worldwide. The two most commercial species of the more than 100 knowns are *Coffea canephora* Pierre ex A. Froehner and *Coffea arabica* L. (Melese et al., 2021). Arabica coffee accounts for ~60% of the world's annual commercial production (International Coffee Organization, 2022), with a higher price than robusta. This achievement of arabica coffee is due to its low caffeine content and excellent aroma (Guerreiro Filho & Mazzafera, 2003; Philippe et al., 2009).

Plants emit volatile compounds that olfactory receptor neurons allow insects to locate their food and place of origin Bruce et al. (2005). The emission of coffee aromas is one of the main attractants for some pests. Coffee berry borer (CBB, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae), is the most devasting coffee pest and directly affects their quality and production (Mathieu et al., 1997). The world has only two countries without CBB; Nepal and Australia (Johnson et al., 2020; Sun et al., 2020). Therefore, for the CBB, a monophagous insect, it is vital to locate its host (i.e., coffee berry). Chemical signaling helps the CBB find its coffee berry host (De la Rosa-Cancino et al., 2021; Jaramillo et al., 2013) while it still greens and changes to ripening. In this way, the coffee berry's maturation stage could influence the bean's rapid and effective localization by the CBB and other organisms, such as natural enemies (Cheng et al., 2018).

In the literature, we found studies on the characterization of volatile coffee compounds, from green berries to roasted berries (De la Rosa-Cancino et al., 2021; Franca et al., 2009; Procida et al., 2020; Toledo et al., 2016). Procida et al. (2020) compared green and roasted berries of arabica and robusta varieties of different geographical origins and concluded that geographical origin influences the profile of both varieties. Samples of *Coffee arabica* from Africa, the Caribbean, Central America, and South America and robusta from Asia and Africa. The African robusta samples were greater than 14 μg/kg of 4-methyl-2.3-

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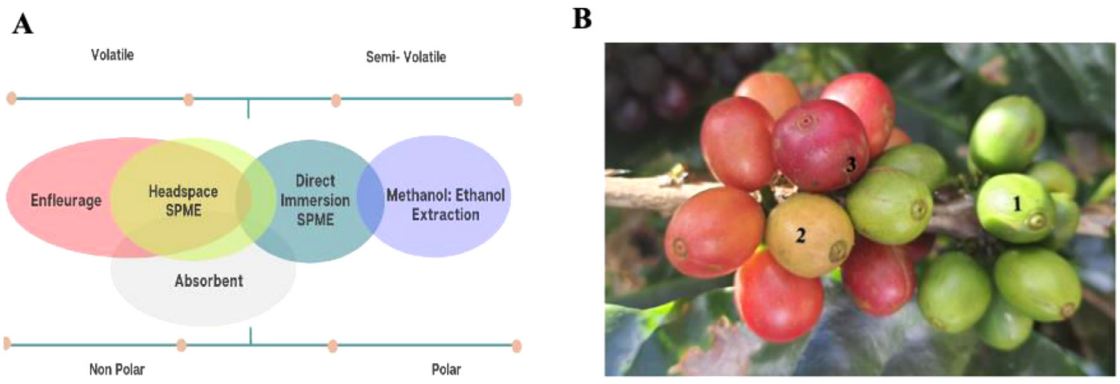

Fig. 1. A) Methods used to capture/extract volatile and semi volatile compounds in coffee berries and coffee berry borer according to their polarity: Enfleurage, Headspace SPME, Absorbent trap, Direct Immersion SPME and Methanol: Ethanol extraction. B) Different stages of coffee ripening. 1) Healthy green berry, 2) yellow berry and 3) Red coffee berry. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

dihydrofuran, Asia lower than 4.3 μg/kg of n-hexanol (Procida et al., 2020). Samples arabica Africa greater than 9.6 μg/kg, Caribbean samples lower than 8 μg/kg, South America between 8 and 22 μg/kg, and Central America are greater than 22 μg/kg (Procida et al., 2020). De la Rosa-Cancino et al. (2021) evaluate volatile compound emissions in healthy berries and berries with different levels of CBB infestation and conclude that volatile compounds decrease as the level of infestation increases.

Other research reported using volatile compounds from coffee berries as a management strategy for CBB control (Castro et al., 2018; Ortiz et al., 2004; Ramli et al., 2019; Vega et al., 2017; Gongora et al., 2020). These volatile compounds of the berries could be used as bait to obtain better results with traps or as a repellent for CBB control (Castro et al., 2018; FAO, 2014; Filho & Mazzafera, 2000; Gongora et al., 2020; Vega et al., 2017). However, to the best of our knowledge, there is no complete profile of volatile and semivolatile compounds of the arabica coffee berry species' different stages of maturation.

The coffee berry matrix comprises the analytes bearing different hydrophobicities, molecular weights, chemical functionalities, polarities, and volatility. Obtaining a natural fragrance is challenging, as the scent could change during extraction. The study aims to generate a comprehensive profile of volatile and semivolatile compounds from coffee berries at different ripening stages. Such as pea-sized, green, yellow, red, healthy, brocaded berries (Ballesteros et al., 2014; Pohl et al., 2013; Steingass et al., 2014). Additionally, we characterized the volatile and semivolatile chemical compounds of CBB females and males (Takata et al., 2019). Since various factors influence the uptake of the fragrant compound, such as coating chemistry, extraction mode, the physicochemical properties of analytes, and matrix complexity, different extraction techniques were used to obtain a broader profile of the compounds present in the coffee berries' fragrance.

2. Materials and methods

2.1. Coffee berries samples

The samples were collected in the Agricultural Experiment Station of Adjuntas of the University of Puerto Rico, PR (18 10′ 28.89″ N 66 47′ 52.27″ W) at 585 m above sea level in *Coffea arabica* "Limani."The berries collected were coffee plants with an average 2.5 m height in monoculture conditions at a full-sun cropping system between May to November 2020. The personnel who collected the samples wore nitrile-free gloves and were free of volatile or semivolatile compounds to avoid contamination of the samples. Four different ripening stages were collected. Our first ripening stages are pea-size healthy (PSH), green healthy (GH), yellow healthy (YH), and red healthy (RH), Fig. 1B.

In addition, berries-brocaded fruits were also collected; green brocaded (GB), yellow brocaded (YB), and red brocaded (RB). The berries were collected in labeled glass gas tie containers (PSH, GH, YH, RH, GB, YB, and RB) and stored in a cooler for transport to the laboratory for analysis.

2.2. Coffee berry borer samples

A total of 300 female and 30 male CBB followed the rate of male: female (Vega et al., 2010) were used to analyze volatile and semivolatile compounds. All CBBs were collected from an established colony at the Center of Excellence in Quarantine and Invasive Species of the Agricultural Experiment Station of the University of Puerto Rico at Río Piedras. The BBC were rinsed with distilled water on three occasions after being extracted from rearing tubes with an artificial diet of ground coffee (Mariño et al., 2017) to remove dietary debris from their bodies. After drying on a paper filter (Lab Nerd, 202 mediums, 9 cm), live specimens were kept in a laboratory beaker (250 mL) for 4 h. They were then transported for chemical analysis in labeled glass containers (50 mL) to the University of Puerto Rico Environmental Chemistry laboratory at the Río Piedras campus, Fig. 1SC.

2.3. Infestation and phenology

At the Agricultural Experiment Station of Adjuntas (EEA Adjuntas), PR (18 10′ 28.89″ N 66 47′ 52.27″ W) at 585 m above sea level (masl) was collected phenology and infestation data from May to December 2020 (harvest end in December), the period when berries are present. Fifteen branches from different trees were randomly selected at the study site. Once the branch was selected, the total number of berries and brocaded berries were counted. The total number of brocade coffee berries over the total number of berries per branch represented the level of infestation. The infestation level of the site per monitoring date was the average infestation level of the fifteen branches. In addition, the total number of green, yellow, and red in each branch selected was counted per sampling site. The total number of coffee berries (green, yellow, and red) per branch over the total number of berries per branch represented the percentage by different ripening stages. The three ripening stages per monitoring data were calculated with the average of each ripening stage of the 15 branches per site.

2.4. Extraction method

Eight different extraction and capture procedures were used and compared with five methods (Fig. 1A): Head space-solid phase micro extraction-gas chromatography-mass spectrometry (HS-SPME-GC–MS), coffee beans solid-liquid extraction in a MeOH: EtOH (3:1) mixture, coffee extracts analysis via injection to gas chromatography-mass

spectrometry (GC–MS). Coffee extracts analysis via direct immersion solid phase microextraction (DI-SPME-GC–MS), CBB volatile analysis via solid-phase microextraction-gas chromatographic-mass spectrometric (SPME-GC–MS), coffee extracts (without maceration) analysis via GC–MS, adsorbent analysis via GC–MS and enfleurage extraction and analysis via GC–MS.

The Enfleurage technique was used for trapping low molecular weight non-polar compounds. Solid-phase microextraction in two modalities of headspace and direct immersion trap non-polar to intermediate polarity compounds (Pensuk et al., 2007). Due to the differences in the mass transfer mechanisms in these two SPME modalities, it was expected that headspace mode would trap non-polar volatile compounds, and direct immersion extraction would allow enhanced extraction of more polar compounds (Gionfriddo et al., 2015). Combining these two modalities will detect compounds in trace amounts within a broad range of molecular masses. Solvent extraction using methanol and alcohol mixtures was included to capture semivolatile compounds with higher polarities and a molecular weight that cannot be detected through SPME techniques Fig. 1A.

2.4.1. Whole coffee beans characterization via HS-SPME-GC-MS

Three whole coffee beans were weighed and transferred into a 20 mL vial with a septa cap for each replication. The vials were incubated for 30 min at 60 °C. Meanwhile, Divinylbenzene/ Carboxen/ Polydimethylsiloxane (DVB/CAR/PDMS) SPME fiber was for 15 min at 270 °C in the injector. The fiber was then exposed for 30 min to the coffee sample and desorbed for 15 min at 260 °C in the injector of the GC and analyzed using the conditions described in the GC–MS method section.

2.4.2. Coffee beans solid-liquid extraction in a MeOH: EtOH (3:1) mixture

Solid-Liquid extractions were performed by mixing 20 g of macerated beans, or 5 g of Coffee skin, with a MeOH: EtOH (3:1) mixture at 10 mL/g. The mixtures were done in 500 mL Erlenmeyer flasks, which were heated for 30 min in a water bath with magnetic agitation at 60 °C. Afterward, the extracts were filtered and stored at −20 °C until further analysis with the described GC–MS method.

2.4.3. Coffee extracts analysis via DI-SPME-GC-MS

A 20 mL vial with a septa cap was filled with coffee extract for each sample replica. The vials were incubated for 5 min at 60 °C. The DVB/CAR/PDMS SPME fibers were conditioned for 15 min at 270 °C in the GC injector. The fiber was then immersed for 5 min into the extracts and desorbed for 15 min at 260 °C in the injector and analyzed using the described GC–MS method.

2.4.4. CBB volatile analysis via SPME-GC-MS

A sample of female CBB, and another of male CBB, were transferred to 20 mL vials with a septa cap, Fig. 1SC. DVB/CAR/PDMS SPME fibers were conditioned for 15 min at 270 °C in the injector. The fiber was then exposed for 30 min to the sample and desorbed for 15 min at 260 °C in the injector. This sampling was carried out at room temperature and heating at 37 °C. After the fiber exposition, it was analyzed with the GC–MS methodology described previously.

2.4.5. Coffee extracts (without maceration) analysis via GC-MS

Another set of Coffee extracts was prepared by collecting whole coffee beans and storing them directly in a MeOH: EtOH (3:1) mixture, Fig. 1SD. A solid-liquid extraction was carried out afterward by heating for 30 min in a water bath with magnetic agitation at 60 °C. For the analysis, a 1 μL aliquot of each extract was injected into the GC using an autosampler and analyzed using the GC–MS methodology described previously in Fig. 1SD.

2.4.6. Adsorbent analysis via GC-MS

One gram of different adsorbents (Florisil and C_{18}) was transferred to a glass Petri dish at duplicate, Fig. 1SE. This was exposed for 30 min; these were stored at −80 °C until further analysis. For this analysis, the Florisil samples were extracted with 10 mL of acetone, and the C_{18} samples with 10 mL of acetonitrile, for 10 min with magnetic agitation. For the analysis of these samples, a 1 μL aliquot was injected into the GC using an autosampler and analyzed using the GC–MS methodology described previously.

2.4.7. Enfleurage extraction and analysis via GC-MS

Whole coffee beans samples were transferred into glass containers, Fig. 1SB. These were covered with commercial-grade vegetable shortening and stored at −20 °C for 16 h. After this period, the coffee beans were removed, and the vegetable shortening was heated to its melting point and transferred to a clean bottle (250 mL). Then, 75 mL of EtOH was added, left to sit, and stored at −20 °C overnight. The next day, the supernatant was filtered and analyzed. This procedure was repeated with another set of samples, with an incubation period of 49 h. For the analysis of these samples, a 1 μL aliquot was injected into the GC using an autosampler.

2.5. GC–MS analysis

All the samples were analyzed with an Agilent Technologies 6890 N Network GC system 5973 Network Mass Selective Detector using high purity Helium as carrier gas at a 1.5 mL/min constant flow a 5% Phenyl-Methylpolysiloxane phase column (HP-5MS, 30 m × 0.32 mm, $df = 0.25$ μm, Agilent J&W Columns). The GC oven temperature program was as follows: an initial temperature at 40 °C for 2 min, then raised by 8 °C/min until 300 °C, where it was held for 2 min. The total run time was 36.5 min. The MS was in positive ion and total ion scan mode with a detection of 50 m/z to 500 m/z. The compounds were identified using the NIST17 mass spectral database with retention index (RI) matching and a factor of correlation of 800 (80% spectra match) or higher. Identification of target analytes was accomplished by comparing their mass spectra with the electron impact spectra of authentic standards. To analyze the coffee berries liquid extracts, 1 μL was injected into the GC using an autosampler. The analysis of SPME samples was performed manually using a desorption time of 15 min and a temperature of 260 °C.

2.6. Data analysis

A Kruskal Wallis was used to analyze the total number of compounds and the healthy stage of the coffee berry. Moreover, we used an analysis of variance to analyze coffee berry phenology and infestation. All data were transformed according to (Warton, 2011) to fit a normal distribution. The figures and statistical analysis were performed using RStudio Team V2022.02.3, and we used the package ggplot2 (R Core Team, 2022).

3. Results and discussion

3.1. Classification of compounds by categories

As a result of the eight methods used to capture the aromatic profile of coffee berries stages and berry borer, there were 228 different compounds divided into 20 functional categories, Fig. 2, Table 1S. The detected compounds may be incorporated in the coffee berries by a different mechanism, including the interactions of the plant with the soil, water, or air, anthropogenic sources, or be the product of the metabolic pathway of coffee macromolecules or compounds used to develop berries. However, it is essential to note that all the reported compounds were consistently detected in all the sample replicas. Some were just detected in a specific berry stage, as reported in Table 1S.

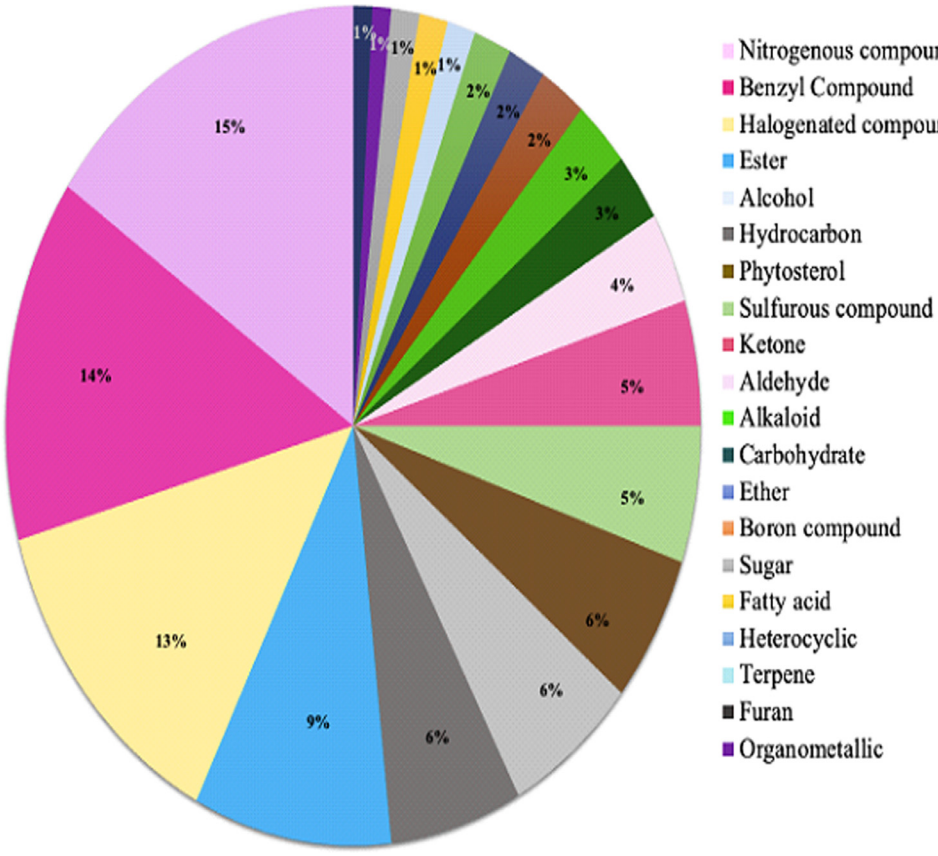

Fig. 2. Pie diagram showing the 20 categories that embody the 228 compounds identified from different ripening stages healthy and brocaded berries, also male and female coffee berry borer.

Table 1
The total compounds from the different ripening stages of coffee berries, berry skin, females, and males of the coffee berry borer.

Coffee Berries	# Compounds
Skin healthy berries	
Pea size healthy skin	22
Green Healthy skin	17
Yellow Healthy	27
Red Healthy	26
Healthy berries	
Pea size healthy	23
Green Healthy	61
Yellow Healthy skin	75
Red Healthy skin	55
Brocaded Berries	
Green Brocaded	71
Yellow Brocaded	53
Red Brocaded	28
Coffee Berry Borer	
Female	11
Female Hot	4
Male	9
Male Hot	6

*Only compounds detected in all replicas were considered in the tabulation. Identifications were done using commercial NIST library and standards when available.

The identification of compounds was made starting at a mass-to-charge rate of 50 UMA/z, and a solvent delay was used to start the detection after its chromatographic detection. For this reason, although it is well known that ethanol is one of the most abundant compounds in coffee aroma, it was not detected or reported in our study. The ten categories with the most components were (in descending order of the number of compounds): Nitrogen compounds, benzyl compounds, halogenated compounds, esters, hydrocarbons, alcohol, phytosterol, sulfurous compound, ketone, and aldehyde representing 83.3% of the total number of compounds identified, Fig. 2.

Overall, healthy berries accounted for 70% of the compounds found. There was also a decrease in the total number of compounds found in CBB-infested berries by 30% concerning healthy berries.

For CBBs, the compounds found were distributed in seven categories. The two categories with the most compounds were halogenated compounds and benzyl compounds, these categories representing 53% of the compounds identified.

We find the three most attractive categories for our sense of smell within the first ten categories of classification of the total number of compounds, representing 23.6%; aldehydes with 3.5%, alcohols with 6.1%; and benzyl aromatic compounds with 14.0%. Fig. 3 shows the change in compounds per these categories as the coffee berry ripens through time. A drastic decrease in the number of compounds is observed when the berries have been brocaded at different stages of berry ripening. The healthy yellow berries showed the highest number of compounds of the other three ripening stages (pea size, green and red). Brocaded berries had the highest number of healthy yellow berries and reduced the total number of compounds in these three categories by 45%. These results suggest that brocaded berries would be less attractive to our senses.

The total compounds found in PSH and PSHS berries were classified only into 14 categories. With nitrogenous compound (20.6%), halogenated (14.7%), benzyl (14.7%), and alcohol (8.8%). In the next stage of GH-GHS ripening, the total number of compounds doubled, and the classification categories of the compounds increased to 18. The 56.5% of total compounds were nitrogen (14.5%), halogenated compounds (13.0%), benzyl compounds (21.7%), and phytosterol (7.2%).

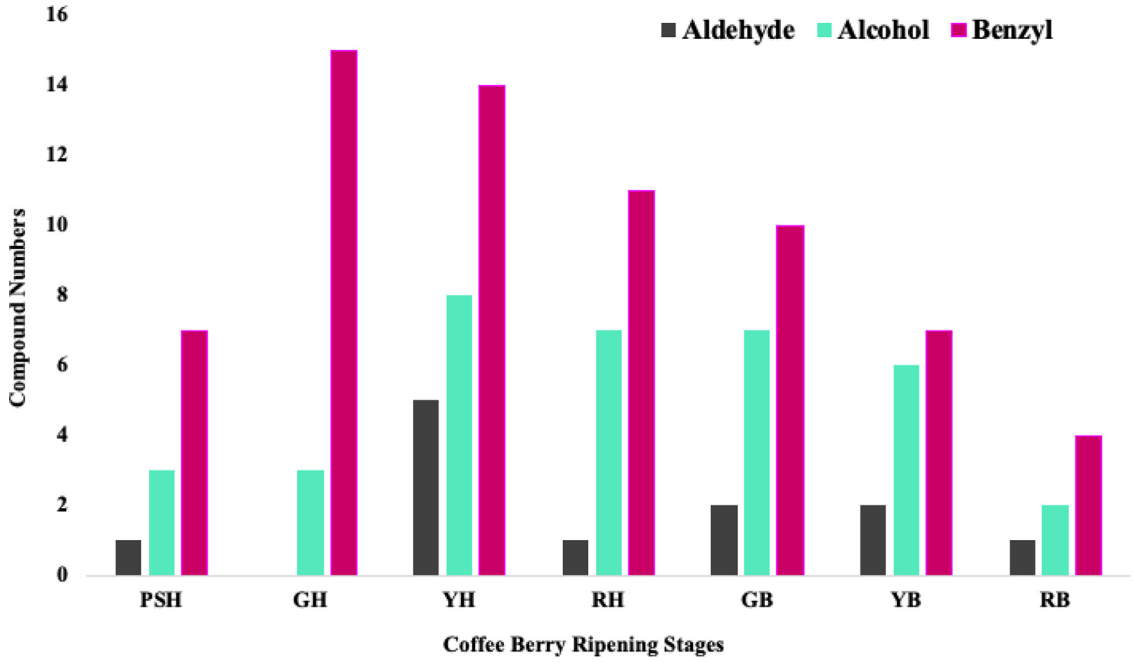

Fig. 3. The number of compounds found associated with the coffee berry ripening stages of the three categories (aldehyde, alcohol, benzyl) that most attract our sense of smell; PSH = pea size healthy, GH = green healthy, YH = yellow healthy, and RH = red healthy. The berries brocaded GB = green brocaded, YB = yellow brocaded, and RB = red brocaded. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The number of compounds increases as the coffee berry matures. However, for the next maturity stage YH-YHS, the same number of compound classification categories was maintained. Compounds in YH-YHS increased by 20% concerning the previous stage (GH-GHS), with the same first three categories having the highest number of compounds benzyl compounds (16.3%), halogenated compounds (12.8%), nitrogen compounds (10.5%). The following categories were alcohol, with 9.3%, and phytosterol, which increased to 8.1%. These five categories represent 57.0% of the total compound of the third ripening stage of the coffee berries selected for our study. At the last stage of berry ripening (RH-RHS), the number of categories was reduced to 15. No compounds classified in furans, heterocyclics, and boron compounds were identified.

The number of compounds detected in almost all the categories decreased in brocaded coffee berries: 19% in benzyl compounds, and 75% among ketones, aldehydes, furans, and terpenes when compared to healthy berries. However, brocaded coffee berries observed a 35% increase in the non-aromatic cyclic compounds category. This increase may be associated with oxidation processes since a decrease in aromatic benzyl compounds was detected simultaneously. It is expected that once the insect drills the berries, the blends that were protected by the bean's skin, carbohydrates, and proteins are exposed to the environment's oxygen, heat, and moisture.

In the ripening stages of healthy berries, healthy yellow berries achieve the maximum number of compounds. As they ripen, some of the compounds decrease or disappear. This was the case with furans; compounds classified in this category doubled in the third stage of ripening YH after remaining constant in the first two stages of ripening PSH, and GH then disappeared in the last stage, RH. Furans are compounds of high volatility and low water solubility. Changes in the composition of the berry and environmental temperature can modify their distribution. The distribution of compounds present in the coffee berries could be associated with the effect of microbial fermentation metagenomics in the production of functional metabolites, volatiles, and their sensorial aspects. The bioactivity of bacteria and fungi induced complex changes in physicochemical features of the berries like pH (4.2–5.2), Brix° (9.5–3.0), and metabolic transitions in the different ripened stages. These changes determine the presence and abundance of the different volatile organic compounds (Shankar et al., 2022).

This contradicts some of the literature that suggested that green beans were more attractive to CBB (Ortiz et al., 2004). The first eleven categories into which the total compounds were classified by maturity stage, yellow fruits (YH-YHS), are when the categories reach the maximum value (Fig. 4A). At the stage of full maturity (RH-RHS), there is a 30% reduction Fig. 4A. Volatile, semivolatile, polar. Non-polar compounds differ in quantity according to the stage of maturation, being the yellow bean the stage that contains more compounds, data that agrees with Jaramillo et al. (2013). It can be linked with ripening bean transcriptomes, as reported by Chen et al. (2018). The expression of transcripts increases as berries ripen from the green to the yellow stage and decrease from the yellow to the red stage. The same tendency was found in our results, with the yellow berries pointed as the stage with higher amounts of different compounds (Cheng et al., 2018).

The trend of compounds sorted by maturity categories of brocaded berries is similar in compound variation to healthy berries. However, green berries had twice as many benzylic and nitrogenous compounds as yellow berries, while red berries' halogenated compounds disappeared, Fig. 4B. Two of the four major categories were found in CBB; benzyl and cyclic compounds Fig. 4B. Trace amounts of terpenes were detected in all healthy berries, but none in green or red brocaded berries. Terpenes are volatile signals that attract insects (Boncan et al., 2020).

3.2. Total compounds in coffee berries and CBB

Different numbers and combinations of compounds were found in each healthy ripening stage of the berries. Yellow berries had the highest number of compounds, with 35.2% of total compounds identified, followed by healthy green berries at 28.6%, healthy red berries at 25.8%, and healthy pea-size berries at 10.3%, Table 1. For healthy berry skins, the pattern changed slightly. However, yellow berry skins had the highest number of compounds, yellow berry skins with 29.0%, red berry skins with 28.0%, green berry skins with 18.3%, and pea-sized berry skins with 24.7%, Table 1.

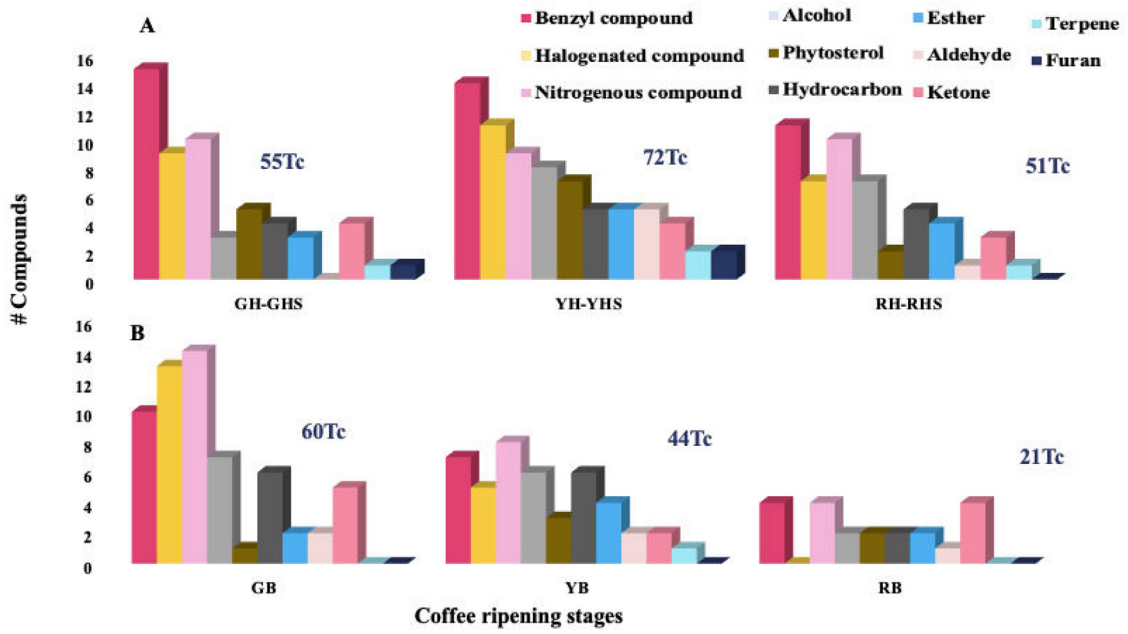

Fig. 4. Total number of compounds per category of the different coffee berries ripening stages. **A)** Healthy coffee berries; GH: healthy green, GHS: healthy green skin, YH: healthy yellow, YHS healthy yellow skin, RH: healthy red, RHS: healthy red skin. **B)** Brocaded berries; GB: green brocaded, YB: yellow brocaded and RB: red brocaded. Total compounds by ripening stage of coffee berries at the top of the bars in blue. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

In addition, each ripening stage of healthy berries presented a unique compound profile. The highest number of unique compounds again was found in healthy yellow berries, with 46 compounds, followed by healthy green berries, with 33 compounds, healthy red berries 24, and pea size with seven unique compounds, Fig. 5A. In contrast, as healthy berries ripening advances over time, the compounds in the hulls increase in exclusivity. The hulls with the least compounds were pea-sized green berries with two compounds, followed by green and yellow with 5 and 8, respectively. Moreover, the hulls from red berries with 13 compounds were the highest number of exclusive compounds, Fig. 5B.

On the other hand, green brocaded berries had the highest number of unique compounds detected, with four compounds, and red berries with nine unique compounds. Additionally, ten common compounds were found for green, yellow, and red stages in infested berries Fig. 5C.

It is important to notice that in the field, early green berries are less propensity to be attacked by CBB, while the yellow berries are found more commonly infested. Therefore, the compounds exclusively detected in the yellow berries and the change in chemical composition observed after these berries become infected could provide information to formulate improved coffee berry borer attractants with defined compositions. Many of these exclusive compounds are aromatics having a benzene ring or conjugated double bonds in their structure. Methoxylated aromatic compounds, which are important signals in many insects in a different context (e.g., pheromones), were also in the aromatic mix, thus suggesting that the berry borer is attracted to the berries at this state (Dötterl et al., 2012).

Also, compounds linked to fermentation processes, such as saccharides, are included in this list. (Cheng et al., 2018; De la Rosa-Cancino et al., 2021). The volatile and semivolatile compounds emitted by CBB were studied to determine if they are like those detected in the different stages of CBB-preferring coffee berries and if they varied according to sex. Indole derivatives were detected in the chemical signaling emitted by the CBB and healthy berries at all stages. Many indole derivatives are known to be intracellular signal molecules; indole regulates various aspects of microorganism physiology, including spore formation, plasmid stability, drug resistance, biofilm formation, and virulence (Naim et al., 2016). The compound 2 methyl seven phenylindole was detected in brocaded berries and the signaling of the male CBB. Another significant finding is that compound 4-(Nonafluoro-tert-butyl) nitrobenzene was detected in the male CBB signaling and the healthy yellow berry. CBB males spend most of their life cycle inside the coffee berry. At the same time, females fly outside it Fig. 5D. This may explain why females are more attracted to yellow berries since their aroma is related to male chemical signaling.

Throughout the year, the coffee-growing area of Puerto Rico can experience maximum temperatures between 29.3 to 33.9 °C (Ruiz-Diaz & Verle Rodrigues, 2021). For this reason, the impact of temperature on CBB signaling was studied (Landolt & Phillips, 1997; Oudenhove et al., 2011; Martín & López, 2015; Groot & Zizzari, 2019). Males at room temperature had 18% fewer components secreted than females. With increasing temperature, females reduced the total number of compounds by 64% and males by 33%, Fig. 4D. Only two compounds were detected both in males and females; 6-Chloro-2,3-dimethyl-4-phenylquinoline and 1-Indole-2-carboxylic acid, 6-(4-methoxyphenyl)−3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester. It is reported that Coffee borers emitted lower chemical signals at higher temperatures, consistent with our results (Oudenhove et al., 2011; Groot & Zizzari, 2019, Landolt & Phillips, 1997).

3.3. Phenology and CBB infestation

The results showed a significant difference between the levels of berry borer infestation on the plant and the percentage of yellow berries for green and red or ripe berries on the coffee branches ($F = 18.58$, $p\text{-value}=0.002$). In contrast, for red berries was no significant difference between infestation and red berries in the branches ($F = 1.87$, $p\text{-value} = 0.204$). These results suggest that the periods of highest infestation coincide with the highest number of yellow berries on the branch. On the other hand, the results also showed significance between the number of volatile and semivolatile compounds between brocaded and healthy berries ($X^2 = 3.8571$, $p\text{-value} = 0.045$), suggesting the impact of CBB on the decrease of compounds in brocaded coffee berries. Our results could be because YH has unique compounds from the other ripening stages (PH, GH, and RH), Fig. 5. According to the results, YH

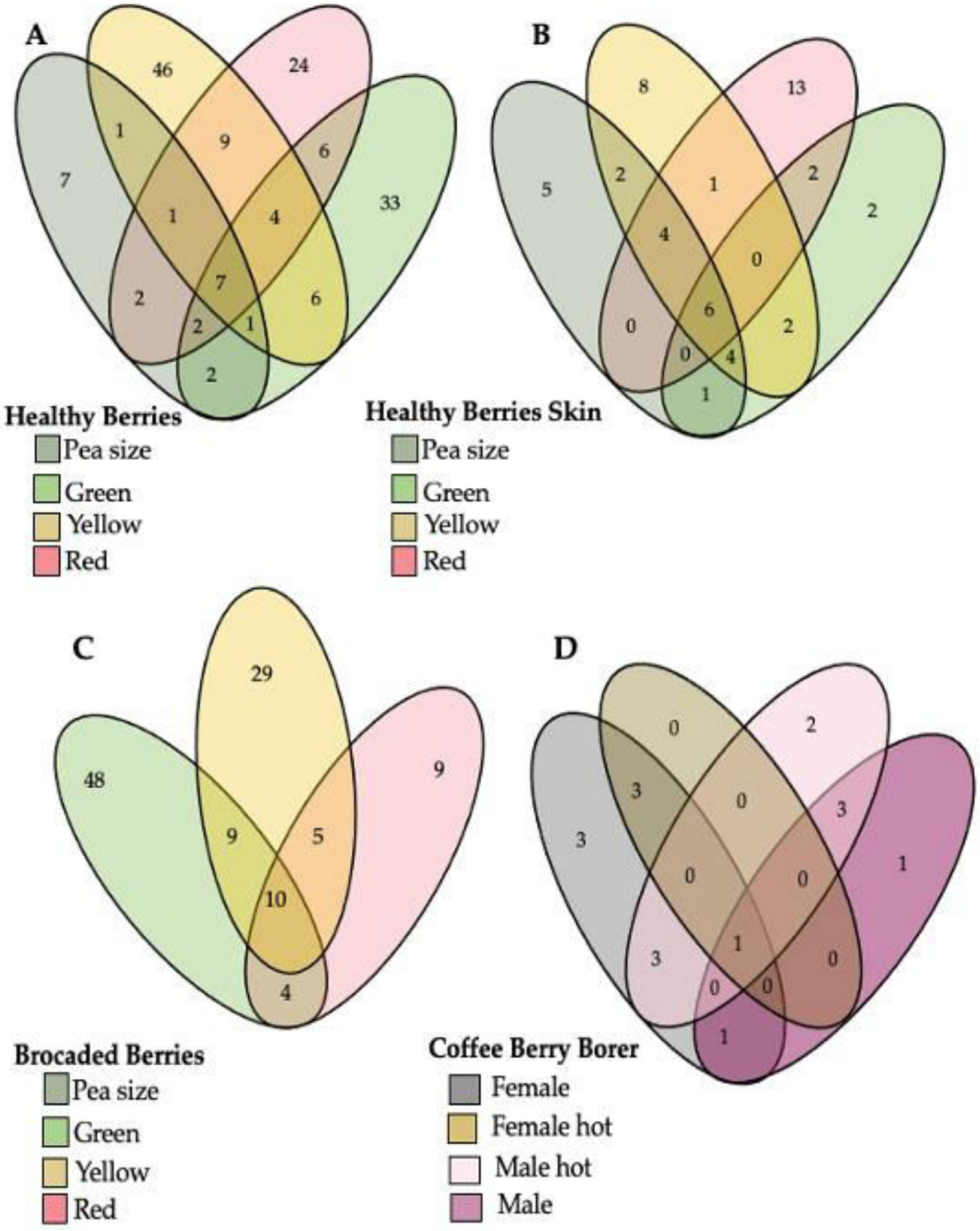

Fig. 5. Ven diagram with total aromatic compounds. A) samples of pea size, green healthy, yellow and red healthy coffee berries, B) Skin pea size, green healthy, yellow and red healthy, C) green, yellow, and red berries brocades, D) aromatics compound from coffee berry borer or CBB. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

are more attractive to CBBs because they have more compounds that attract them, such as halogenated and phytosterol, which they can use to achieve mating and have better food (Cheng et al., 2018; Jaramillo et al., 2013). In addition, the results show that the highest CBB infestation coincides with the time when there are more yellow berries on the coffee trees.

4. Conclusions

In this study, eight extraction methods were used to complete the aromatic profile of coffee beans during the ripening process (pea size, green, yellow, and red) and of the coffee berry (green, yellow, and red), as well as of female and male coffee berry borers. Polar, non-polar, volatile, and semivolatile compounds were found. A total of 228 compounds were identified and classified into 20 categories according to their chemical functionality. Our results suggest that coffee berries emit chemical signals that olfactory receptor neurons can easily detect in the CBB, such as aldehyde, alcohol, and benzyl. Therefore, CBB is likely to infest coffee berries according to the number of compounds that attract them (i.e., if berries have fewer compounds that attract them, they will be less infested). Also, our results suggested that yellow berries attracted the most CBB and contained the most volatile and semi-volatile

compounds. In addition, our study shows that the highest CBB infestation coincides with the time when there are more yellow berries on the coffee trees than the other ripening stages, PSH, GH, and RH. Thus, the profile of volatile and semivolatile compounds generated by our work can help develop effective bait traps, considering berry ripening stages and unique compounds for effective CBB control. Therefore, yellow coffee berry compounds could be used in place of the standard methanol-ethanol mixture (3:1 or 1:1), which has been used as bait for CBB traps through time to control the significant coffee pest.

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Conflicts of interest

The authors declare no conflict of interest.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Claudia Patricia Ruiz-Diaz: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **José C. Verle Rodrigues:** Writing – review & editing, Visualization, Supervision, Funding acquisition. **Erick Miro-Rivera:** Data curation, Visualization, Methodology. **Liz M. Diaz-Vazquez:** Methodology, Investigation, Formal analysis, Validation, Writing – review & editing.

Data availability

No data was used for the research described in the article.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.focha.2022.100154.

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