Characterization and analysis of the volatile organic compounds in green coffee affected by potato taste

Eric Marshall & Dr. Susan Jackels

Abstract

Some East African coffees have been affected by a defect known as potato taste (PTD) which imparts a disagreeable potato-like flavor, rendering the coffee unsellable and harming farmer's livelihoods. Prior studies have linked the defect to an insect native to the region, commonly referred to as the antestia bug, which feeds upon coffee cherries. In order to evaluate the surface volatile compounds associated with PTD, green coffee was sampled by headspace analysis using solid-phase microextraction, and the resultant volatiles were detected and identified by GC-MS. Data indicates that the PTD and unaffected coffees have distinct differences, and statistical analysis has been used to identify the associated volatiles.

Introduction

Rwandan coffee, as well as that of other East African nations, has been impacted with a flavor defect referred to as the potato taste defect (PTD), which imparts a taste and aroma of potato skins to roasted coffee. As these East African economies have focused on the production of specialty coffees, quality is paramount; any instance of PTD detected by a buyer is cause for immediate rejection of the entire lot of coffee. Consequently this defect stands to cause direct and immediate harm to these countries' economies and the livelihoods of their farmers. In 2008 alone, approximately a quarter of the entire Rwandan crop was lost to PTD – putting a significant strain upon the country.[1]

The exact mechanism by which PTD develops remains unclear. Prior studies have examined coffee beans with a "peasy" flavor defect, another way in which to describe PTD flavor, identifying the aroma with isopropyl-3-methoxypyrazine (IMP) and finding upwards of a thirty-fold increase in affected coffees.[2] Occurrence of the defect has also been associated with a local variegated stink bug, of the genus *antestiopsis*, which feeds upon coffee cherries.[3] The prevalent hypothesis is that this insect deposits a bacterium after feeding, which in turn produces IMP as a metabolite, adhering to the surface of the beans and causing the potato/peasy flavor. Other relevant studies of coffee defects unrelated to PTD have demonstrated the ability to distinguish between defective and non-defective coffee by their overall aroma profile, using solid-phase microextraction (SPME) and GC-MS.[4] However, no such analysis has been performed on PTD coffee.

The object of this study has been primarily to identify compounds that distinguish PTD coffee from unaffected coffee by examining the overall aroma profile, as well as to evaluate whether IMP is present on the surface of the bean. The analytical method is derived from proven methods in the literature,[5] however with a novel approach in examining compounds located on the surface of the beans via sampling whole green coffee. No other studies have been conducted on the surface volatiles of green coffee beans; from the proposed mechanism of *antestia* action, it is hypothesized that IMP should be detectable on the surface of the coffee bean and other potential marker compounds of PTD may be as well.

Materials & Methods

Sample Preparation and Analysis:

Samples of green coffee were sourced from Rogers Family Coffee for study. Each sample was assigned a lot number and possessed an associated cupping score, indicating the quality of the coffee and whether or not potato taste had been detected. For sampling, 70 grams of whole green coffee or 3.5 grams of ground green coffee were measured into a headspace vial, sealed with a septum, and then transferred to a water bath heated to 60°C. A Supelco SPME (50/30 µm triple phase fiber, divinylbenzne/carboxen/polydimethylsiloxane) was inserted into the headspace for extraction over 60 minutes. After sampling, the SPME device was transferred into the GC injection port for desorption at 250 °C over 5 minutes. Analysis was performed in triplicate to assess reproducibility.

GC/MS analysis**:**

Compound separation and analysis was performed via an HP 5890/5972 GC/MS. A Restek Stabilwax 60m column with 0.25 mm i.d. and 0.5 µm phase coating was used. The following chromatographic conditions were used: injection - splitless injection (5 minutes); injection temperature – 250 °C; temperature program – 40 °C (11 minutes) followed by a temperature ramp of 3°C per minute to 190 °C (10 minutes), followed by an additional ramp of 15 °C per minute to 250 °C (0 minutes); detector – quadrupole, temperature – 250 °C; carrier gas – helium; flow rate – 18 ml/min. The mass acquisition range was 50 to 350 amu. Compound identities were determined from the mass spectrums by comparison against the NIST05 and FFNSC databases for high quality (>75%) matches.

Statistical analysis:

Total ion count and retention time data were extracted from each generated chromatogram and transformed using principal component analysis. The distribution from the first two principal components was used to identify statistically significant differences between PTD and non PTD coffees.

Data & Results:

A total of 74 samples of green coffee were obtained for analysis, which were used to generate 128 GC/MS runs. The initial focus of these runs was in generating and analyzing chromatograms, before later submitting the data for statistical analysis. After completing many successive runs of both PTD and non PTD coffees, a general trend was observable from the chromatograms alone. Figure 1, a chromatogram taken to be representative of the non PTD profile, is observed to have a background of peaks of approximately the same scale around 32, 34, 40, 43, 56 and 63 minutes. Figure 2, in contrast, is representative of the general PTD profile. Predominant peaks are observed at retention times of approximately 33 and 37 minutes; furthermore, these peaks represent a tenfold increase in scale relative to those in the non PTD coffee. Table 1 lists all peaks by retention time that were identifiable by analysis of the mass spectrum.

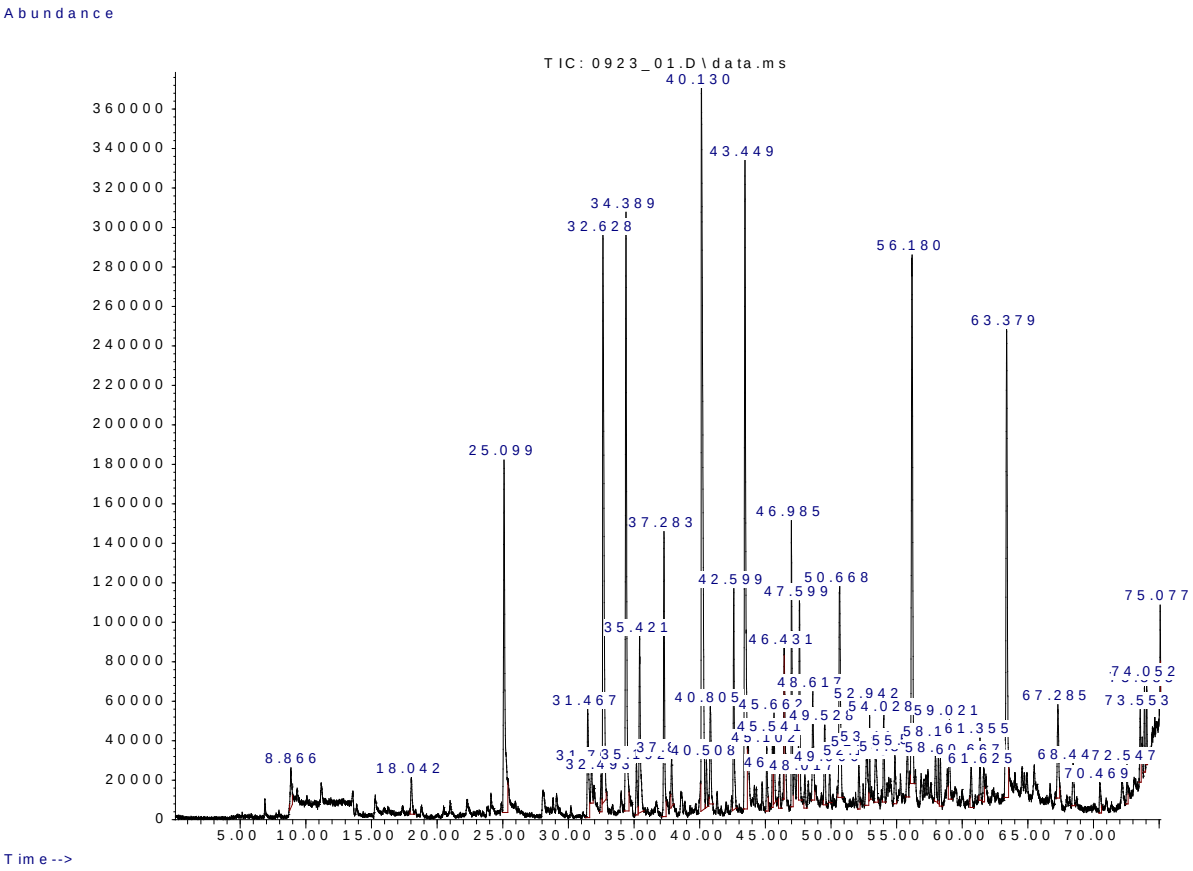

Figure 1: Representative chromatogram of non-potato taste coffee, sample SG-1407.

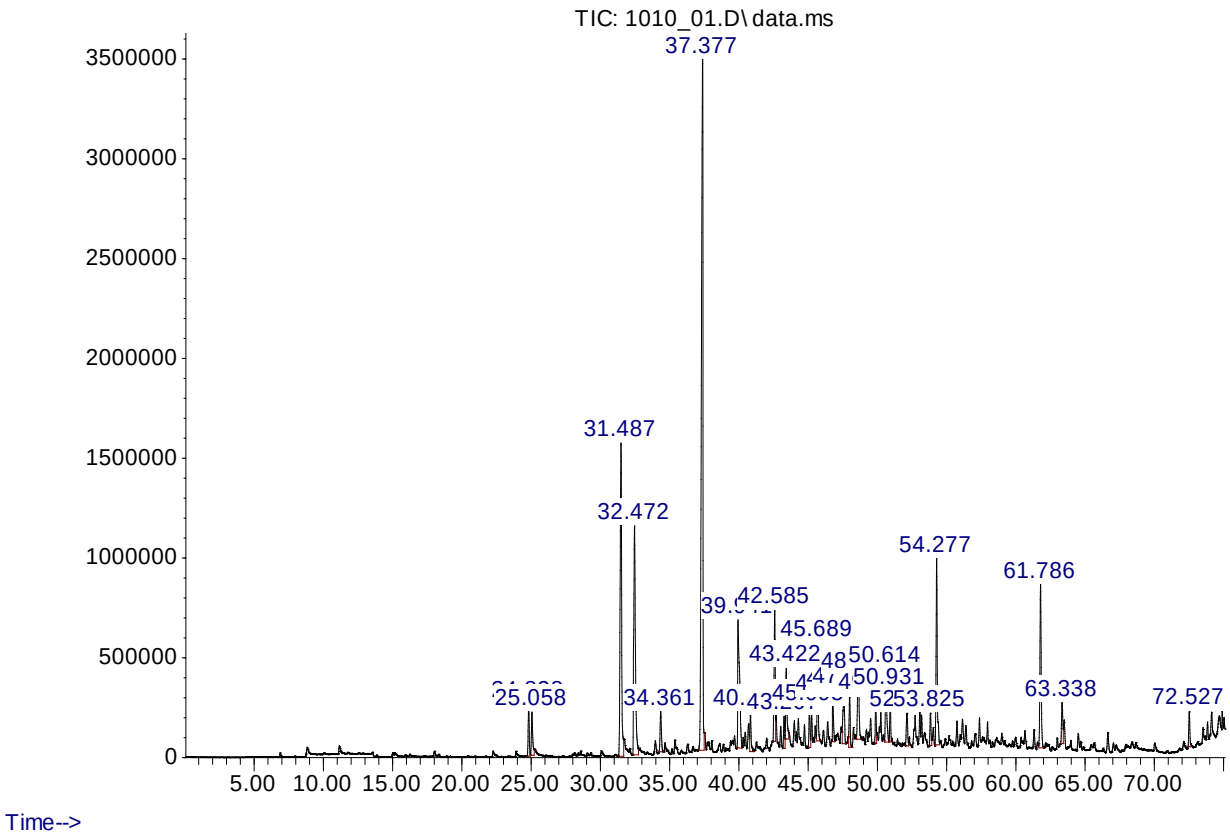

Figure 2: Representative chromatogram of potato taste coffee, sample SG-3387.

Table 1: Candidate compounds identifiable[a] from mass spectrums of chromatographic peaks

Sample SG-1407 (representative non PTD)		Sample 3387 (representative PTD)	
RT(min)	Compound Name	RT(min)	Compound Name
Not detected	Not detected	24.8	Undecane
25.1	Hexanal	25.1	Hexanal
31.5	Dodecane	31.5	Dodecane
32.5	D-Limonene	32.5	D-Limonene
34.4	1-Dodecene	34.4	1-Dodecene
35.4	2,6-Dimethylpyridine	Not detected	Not detected
37.3	Tridecane	37.4	Tridecane
42.6	Tetradecane	42.6	Tetradecane
43.4	Nonanal	43.4	Nonanal
45.1	1-Tetradecene	45.1	1-Tetradecene
45.7	1-Octen-3-ol	Not detected	Not detected
47.6	2-Ethyl-1-hexanol	47.6	2-Ethyl-1-hexanol
48.5	Decanal	Not detected	Not detected
50.7	Benzaldehyde	Not detected	Not detected
Not detected	Not detected	54.3	1-Methoxybenzene
56.1	3-Methoxybutanoic acid	Trace	
Not detected	Not detected	61.8	Methylsalicylate
63.4	Hexanoic acid	63.3	Hexanoic acid
67.3	Phenylethylalcohol	Not detected	Not detected

[a]Identification criteria was a >75% match to either of the two mass spectral databases

The total ion count and retention time from each chromatogram were then extracted and submitted for principal component analysis (PCA). The first principal component, PC1, was found to account for 26.6% of sample variability alone. Figure 3 depicts PC1 plotted against an abstraction of the retention time; peaks with large positive values, corresponding to chromatographic peaks of higher abundance in PTD versus non PTD coffee, were those at 31.5, 37.3, 42.6 and 47.5 minutes. Peaks at 32.7, 43.4 and 56.1 minutes possessed negative values, signifying that they were less represented in PTD coffee. Figure 4 is the loading for the second principal component PC2 (19.4% of sample variability), with positive peaks at 32.7, 37, 40.3, and 47.2 minutes and negative peaks at 32.5, 40.7 and 56.1 minutes.

Plotting the principal components against one another allows for statistical separation of the samples. In figure 5, PC2 is plotting vertically while PC1 is plotted horizontally. A blue circle, indicating the 95% confidence level, is drawn around the majority of the black circles, representing non PTD samples. Each of the five PTD samples (represented by red triangles) are both clustered with one another and outside the blue circle meaning that, to within 95% confidence, they are statistically distinct from the non PTD samples. Furthermore, the first principal component accounts for enough sample variability that it alone is necessary to distinguish the PTD coffee. The peaks in the PC1 loading plot are thus sufficient in establishing a profile of the surface volatiles that separate these sample types. Summarizing the corresponding compounds that were identifiable in Table 2, three long chain alkanes and one fatty alcohol are present in higher quantities and 3-methylbutanoic acid (a poor smelling compound itself) is present in lower quantities in PTD coffees.

A small subset of samples were ground to compare the profile of interior volatiles to that of those on the surface. Figure 6 is ground sample SG-1407, the same as represented in Figure 1; the ground non PTD coffee appears to have a background characterized by relatively equivalent peaks at about 25, 34, 40, 43, 50, 55 and 63 minutes. Figure 8, a ground sample of SG-3387, appears markedly different from Figure 2, with prominent peaks at 37, 39, 46, 56 and 63 minutes. Figures 7 and 9 represent a selected region (44 to 50 minutes) from Figures 6 and 8, respectively. Each chromatogram possessed peaks at approximately 45.2 and 49.7 minutes, corresponding to IMP and a related compound IBMP (isobutyl methoxypyrazine).

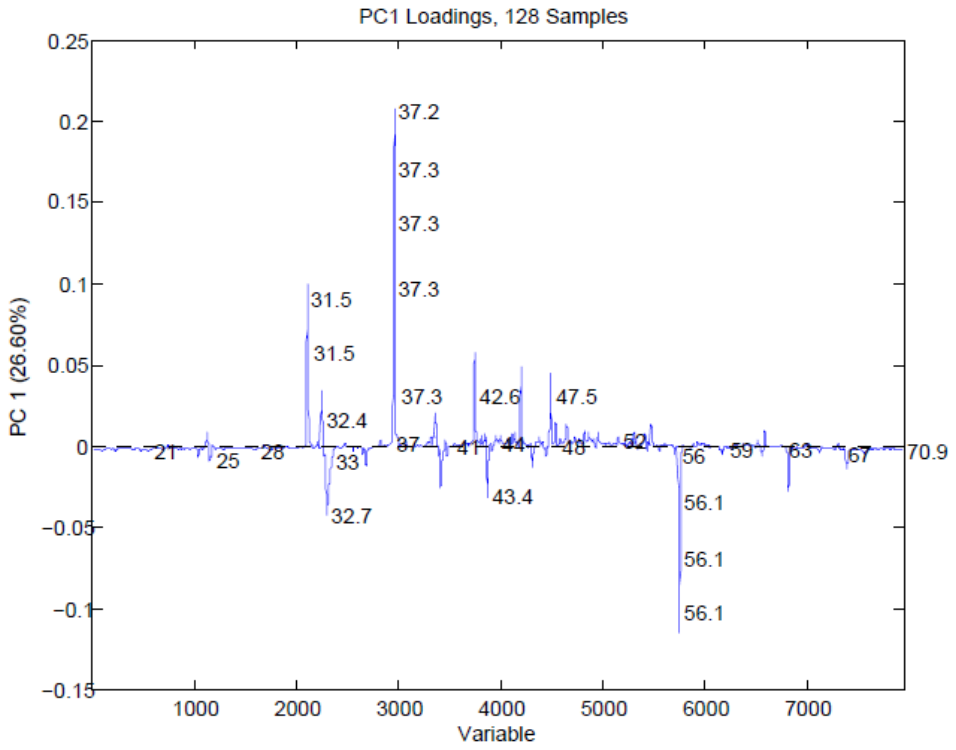

Figure 3: PC1 loading plot, accounting for 26.6% of the data variability.

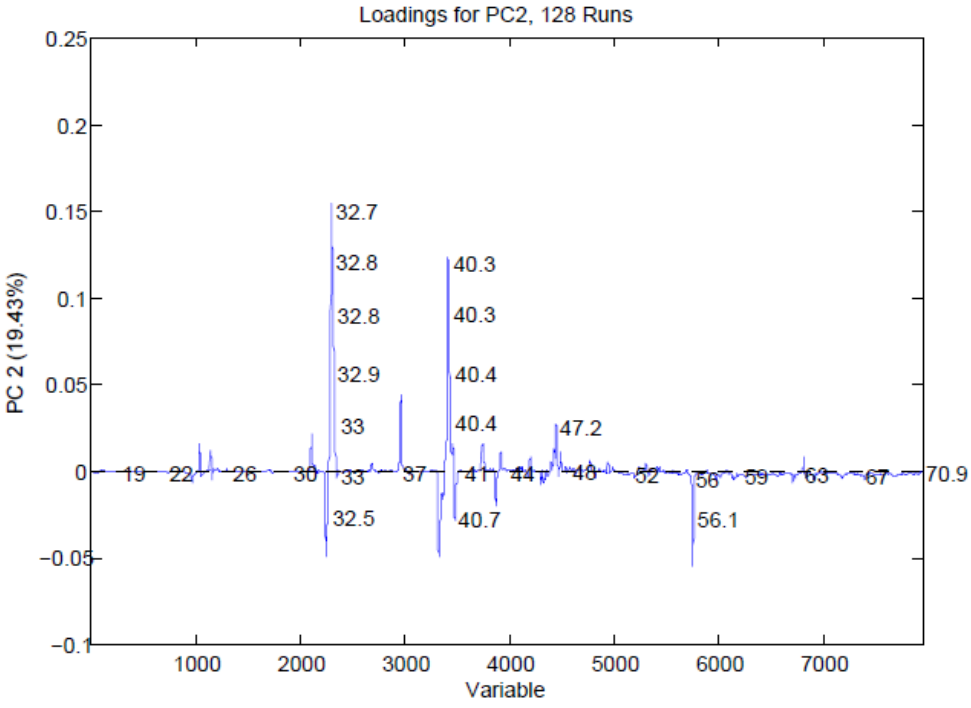

Figure 4: PC2 loading plot, accounting for 19.4% of the data variability.

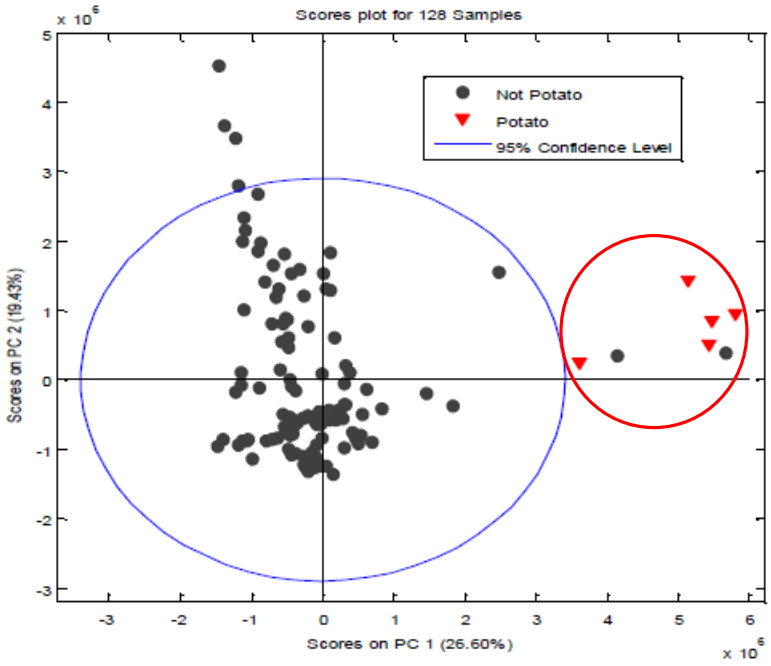

Figure 5: PC2 vs PC1 scores plot showing sample separation

Table 2: Identifiable Compounds and Retention Times Distinguishing PTD and non PTD coffees

Compound name	Retention Time
Dodecane	31.5
Tridecane	37.3
Tetradecane	42.5
2-Ethyl-1-hexanol	47.8
3-Methylbutanoic acid[b]	56.1

[b]Compound negatively correlated with PTD

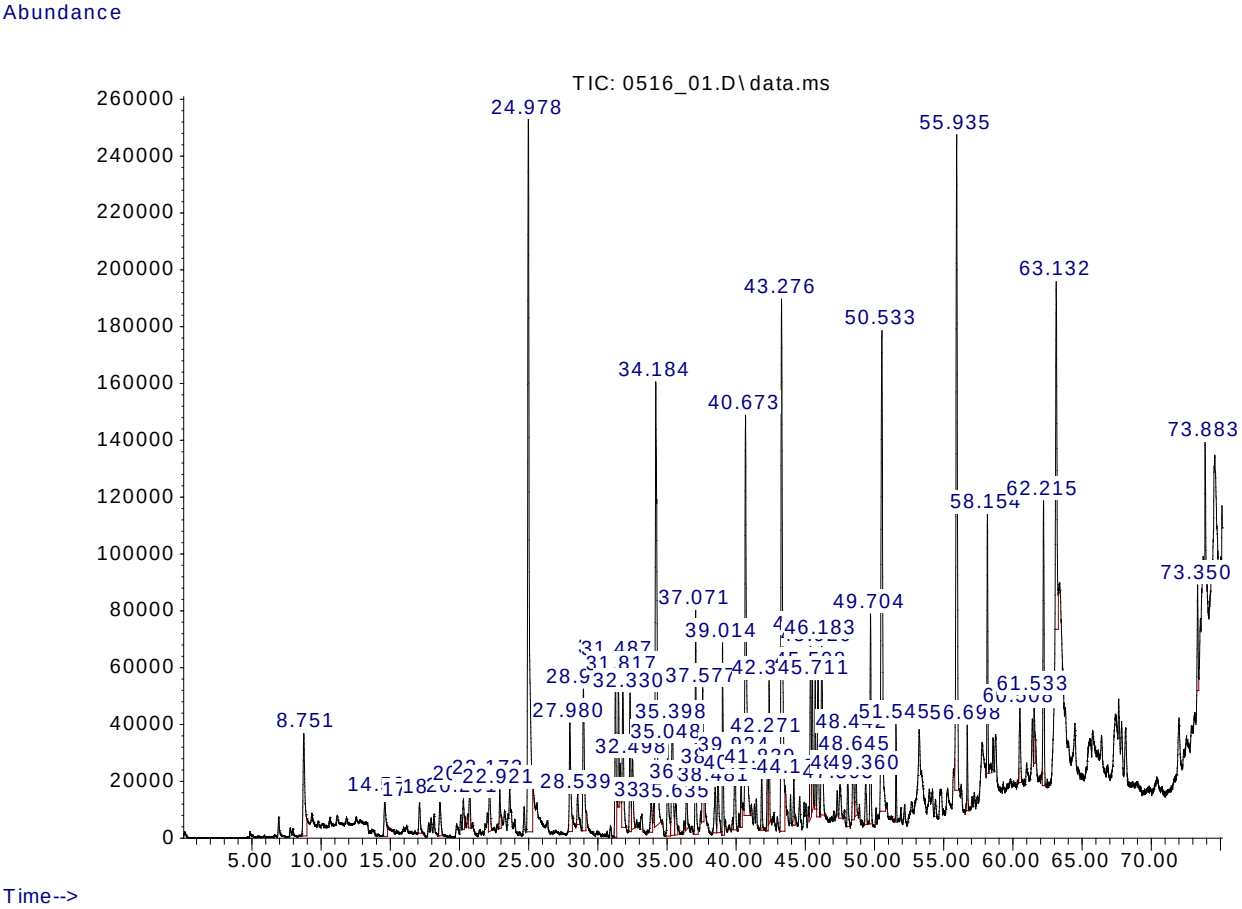

Figure 6: Chromatogram of ground non PTD sample SG-1407

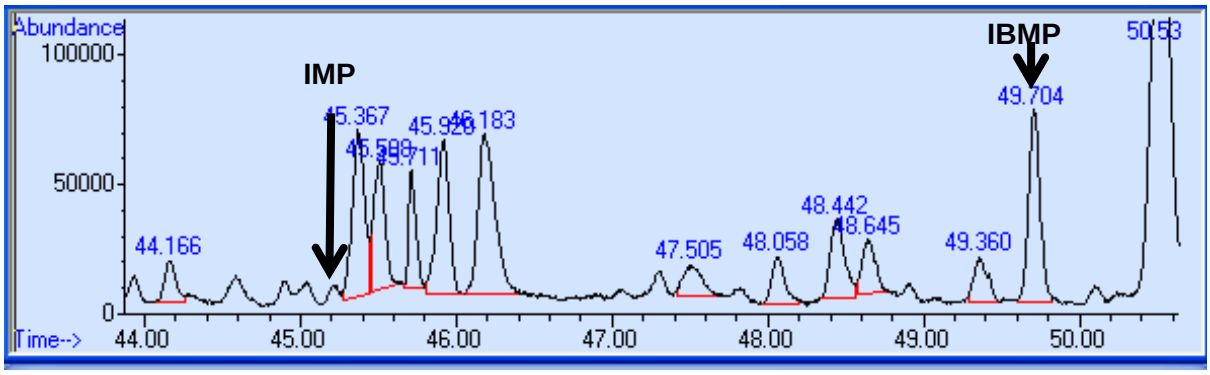

Figure 7: Selected region of ground SG-1407 chromatogram

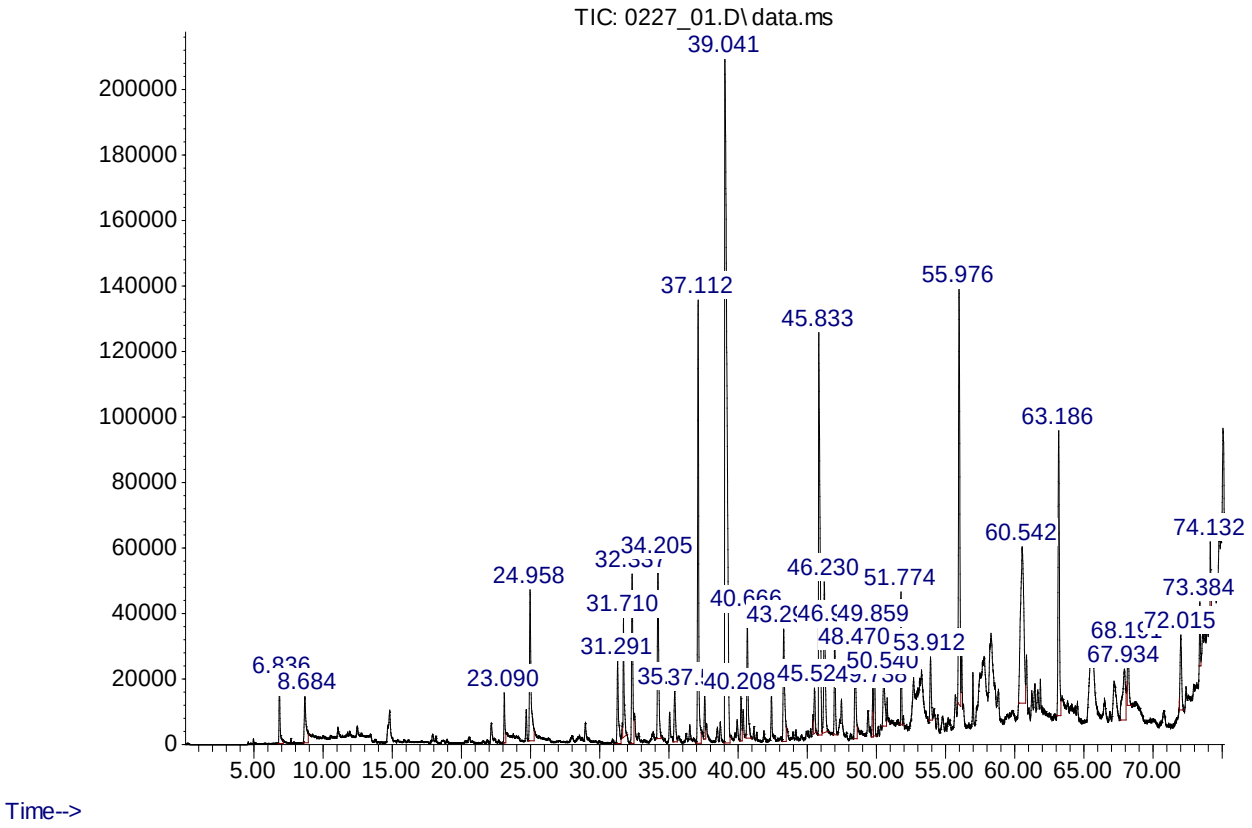

Figure 8: Chromatogram of ground PTD sample SG-3387

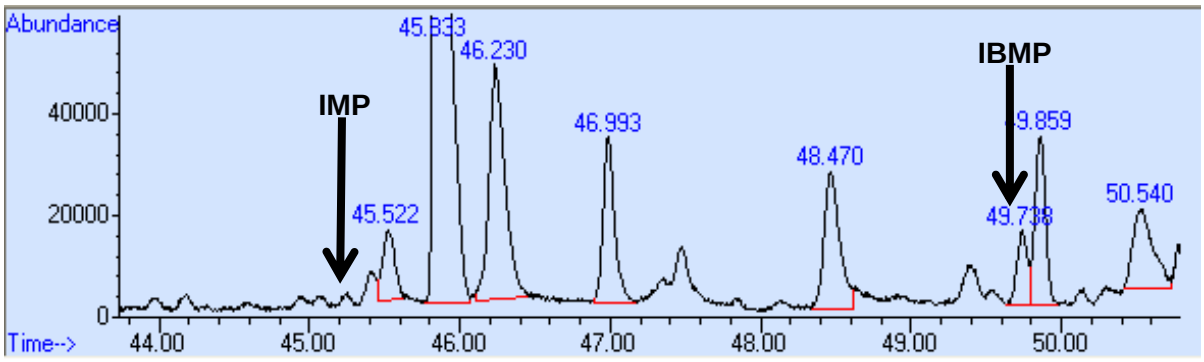

Figure 9: Selected region of ground SG-3387 chromatogram

Discussion

The results of the analysis of surface volatile compounds indicates a clear pattern of distinction between PTD and non PTD coffees, within 95% confidence. The four prominent identifiable compounds found in higher levels in PTD coffee were dodecane, tridecane, tetradecane and 2-ethyl-1-hexanol; 3-methylbutanoic acid was determined to be negatively correlated with PTD. Long-chain alkanes have not previously been associated with the aroma profile of green coffee, while the other two listed compounds have.[6] However, these alkanes were absent in the profile of ground coffee, indicating their presence to be limited to the bean surface. Long-chain organic compounds are often constituents of waxy substances, such as present on the exterior of the coffee bean, though these are generally fatty acids and lipids. Several studies include long-chain alkanes on the list of defensive pheromones secreted by stink bugs, including undecane, dodecane, tridecane and tetradecane.[7,8] Defensive pheromones are those produced in response to disturbance or aggression, primarily to ward off predators. While no study has been explicitly conducted upon the *antestia* bug, it appears plausible that heightened levels of these compounds on the coffee bean surface could have their origin in *antestia* activity.

Also notable among the results is that IMP, the compound linked to the peasy flavor itself, was not detectable on the surface of any of the PTD and non PTD coffees evaluated. This appears contradictory to the proposed PTD mechanism: a metabolite adhering to the surface of the bean at high enough levels to affect taste should be therefore be detectable on the surface. Furthermore, though only a small number of samples were analyzed by the ground coffee method, IMP and IBMP were observed among the profile of both PTD and non PTD coffees, with generally increased levels among affected samples. The implication of this is that IMP is actually produced by the coffee plants, rather than introduced by an external factor. This opens the possibility that increased levels of IMP in PTD coffee are a result of a response to stress, of which the *antestia* bug remains the likely cause.

Conclusions

A novel approach to the study of green coffee, the analysis of its surface volatile compounds, was conducted in order to develop a profile differentiating PTD and non PTD coffees. PCA of the chromatographic data revealed five compounds as the key distinguishing factors; three of the compounds were long-chain alkanes, not previously associated with any coffee profile. Alkanes such as this may be linked with defensive pheromones produced by stink bugs like the *antestia* bug,with heightened levels caused by stresses on the insect. The peasy smelling compound from previous studies was not detected on the surface of the beans,

suggesting that the PTD mechanism is still not well understood; IMP was however present in trace amounts during ground coffee analysis, suggesting production by the coffee plant itself. Further analyses of *antestia* pheromones and the profile of ground coffee are underway to better develop these connections.

References

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