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Influence of our Special Treatment on Green Coffee Beans
By Dr.Hu, Middlebury CT

Introduction

The green coffee chemical composition consists primarily of two fractions: non-volatile and volatile compounds.
In general, the compounds of the non-volatile fraction form the basis of the green coffee and are divided into the several groups according to their characteristics. These groups are given as: (1) Carbohydrates/Fiber, which include Sucrose, Polysaccharides, Lignin and Pectin; (2) Nitrogenous Compounds, which include Protein/Peptides, Free Amino Acids, Caffeine and Trigonelline; (3) Lipids, which include Coffee Oil (Triglycerides with unsaponifiables, Sterols/Tocopherols), Diterpenes and Minerals; (4) Acids and Esters, which include Chlorogenic Acids, Aliphatic Acids and Quinic Acids. Many such compounds and their derivatives that are related to these four groups were identified and published in the literatures during past years.
The volatile fraction of the green coffee seeds gives them a weak but characteristic aroma. Approximately more than 100 different volatile compounds have been identified in green coffee beans. The most abundant classes of volatile compounds are categorized as: Alcohols, Esters, Hydrocarbons and Aldehydes. In addition, Ketones, Pyrazines, Furan and Sulfur compounds have also been identified. It was interested in observing that the volatile composition of coffee berries is dominated by high level of Alcohols, mainly Ethanol, in all stage of ripeness. Overripe coffee berries, however, exhibit high concentration of volatile compounds by Esters, followed by Alcohols, Ketones and Aldehydes.
Depending on genetic aspects, especially species, and physiologic aspects such as degree of maturation, as well as extrinsic factors such as soil composition, climate, agricultural practices and storage conditions, the different green coffee beans differ in many ways. However, the basic chemical composition of the green coffee is quite similar, and the most organic compounds described above are present in different coffee species, with their portions relatively varied.
On the basis of the general coffee constitution, the current report, in a preliminary R&D stage, represents an attempt in identifying chemical compounds for the coffee selected by using gas chromatography with mass spectrometry, and analyzing how green coffee beans are influenced by “our special treatment”

Identification Procedures

Various chromatographic analytic instruments have been used to separate and identify all kinds of organic compounds in coffee. Gas chromatography with mass spectrometry

(GC-MS) is one of these instruments, and has recently come into wide use. Through its mass spectrometry service, PerkinElmer, the company that specializes in producing various mass spectrum equipment, helped us to perform the mass spectrum scanning for two kind of coffee we selected by using several GC-MS techniques, and also provided us with the NIST GC-MS Spectra and Library Search, a comprehensive software that collects more than 300,000 organic compounds detected capably by the GC-MS technique, for our compound identification.

Two coffee employed in our study were Uganda kapkwai Sipi Falls Green Beans and Horqueta La Berlina Green Beans "geisha" Panama. For each coffee, there were three different status prepared. For Uganda kapkwai Sipi Falls Green Beans, three status were given as: 1) Green originally; 2) the beans exposed to Domaine Dupont's Pommeau along with Meyer Lemons, & Bouquet de Fleurs from our citrus greenhouse 3) Roasted after our special treatment. Similarly, three status prepared for Horqueta La Berlina Green Beans "geisha" Panama were: 1) Original green beans; 2) the beans exposed to "OEC" Grandis 2014 along with Meyer Lemons from our citrus greenhouse. 3) Roasted after special treatment.

In processing GC-MS examination, three sampling methods and their correspondent scanning operations, Extraction, Headspace and Headspace Trap, were all applied for our each coffee with different status. The purpose to use these three modes together for our coffee was trying to detect the compounds as much as possible, covering a breadth of compound spectrums from non-volatile to volatile in terms of the intrinsic compound molecular weights. Generally in GC-MS, Extraction is mainly used for detecting non-volatile compounds and partially for some volatile compounds, and the most volatile compounds are normally detected by Headspace. The difference between Headspace (Non-Trap) and Headspace Trap, however, is that the former detects the compounds in ppm level and the latter can get into ppb level. In other words, the Headspace Trap scanning is much more sensitive to the volatile compounds and can see the most.

Combining the coffee types, the bean status prepared and sampling, a total of eighteen GC-MS scanning were thus carried out in analyzing the intrinsic changes or variations of constitutional compounds in our selected coffee.

Various estimates in literatures suggested that coffee contains 2000 - 3000 compounds, and most occur after roasting. Although the GC-MS technique has been widely accept to detect the compounds in coffee, it is worth, however, mentioning that subject to the compound molecular weight, compound stability, sample preparation and response to the gases used for compound separation, and as well as many other factors, complexity in inspection for coffee results in very limited compounds detected capably by this technique. In order to detect and analyze a broad spectrum of compounds occurred in coffee, many other types of instruments such as High Performance Liquid Chromatography MS, Ion Chromatography MS, Thin Layer Chromatography MS, Pyrolysis Gas Chromatography MS and etc. should be employed.

This report currently focuses on compound identification and comparison between the original green and special treatment status for each coffee. Many more compounds did present in GC-MS after roast, but it was not clear whether or not occurrence of aromatic (volatile) compounds in this stage was due to special treatment or a well-known Maillard reaction, an important chemical reaction usually occurred in roast and cook. Therefore, the GC-MS results obtained for our roasted beans were excluded in this report for the time being, and those compounds appeared during roast will be analyzed later. While the compounds detected using the GC-MS technique were limited as described above, our identification results were still interested and promising.

Results

(A) The compounds detected by GC-MS for original green beans

To identify non-volatile and volatile compounds in our green coffee eans, specially treated and roasted, PerkinElmer used a specific Clarus SQ8 GC-MS instrument with TurboMatrix Sampler by which three modes, Extraction, Headspace and Headspace Trap, can be operated, respectively. For each green coffee bean examined under each operation mode, the scan revealed that with the peak heights relatively varied, the GC-MS compound spectrums presented were almost the same for both Uganda kapkwai Sipi Falls Green Beans and Horqueta La Berlina Green Beans “geisha” Panama, basically indicating the same chemical constitution or compounds existed in both green beans.

As inspection was limited due to the GC-MS technique itself, the non-volatile compounds detected in coffee green were only related two major categories compared with those listed in Introduction: Nitrogenous Compounds and Lipids.

Caffeine - the most common Nitrogenous Compound - was clearly identified. Caffeine is a Methylxanthine with bitter characteristics, simulating the central nervous system as an adenosine-receptor antagonist. Caffeine, also as a kind of Alkaloid, is the most widely consumed and studied psychoactive substance in history. Caffeine is heat stable, and its concentration in C. Robusta is approximately twice that found in C. Arabica. In addition to Caffeine, another type of Nitrogenous Compounds – Amino Acids – was also detected, such as Alanine, Arginine and Taurine.

Lipids are major compositions of coffee and are originated from the coffee oil. In coffee, the Lipid fraction is composed mainly of Glycerols (approximately 75%), Fatty acids (included esterified), Sterols (unesterified and esterified with Fatty acids), and so on. The Lipid fraction also contains Diterpenes in proportions of up to 20% of the total Lipid fraction.

The Lipid compounds detected specifically in our green beans were: the Palmitic acid and its Methyl, Ethyl and Glycerol palmitates (esterified), as well as its derivatives such as Palmitoyl chloride; the Linoleic acid and its Methyl, Ethyl and Butyl Linoleates (esterified); the Oleic acid; the Stearic acid and its Methyl and Ethyl Stearates (esterified); and the Eicosanoic acid and its Methyl and Ethyl Eicosanoates (esterified). In addition to these saturated and unsaturated fatty acids and their derivatives, a group of the acetates esterified from the Prenols (Pregnenolone or Pregnane) with the Acetic acid were also detected. The Prenol is a natural alcohol, and it is one of the simplest Terpenoids. It is a clear colorless oil that is reasonably soluble in water and miscible with most common organic solvents. Prenol occurs naturally in citrus fruits such as cranberry, currants, grapes, raspberry, blackberry, tomato, hop oil and coffee etc., and it has a fruity odor.

Several major volatile compounds were detected and categorized as: Alcohols such as Ethanol, Methanol, Propanol, Butanediol and Isopentyl alcohol (Fusel alcohol); Aldehydes such as Acetaldehyde, Isovaleraldehyde (3-methylbutanal), Crotoaldehyde (Butenal) and Stearyl aldehyde; Aliphatic compounds and acids such as Ethylene, Squalene and Acetic acid, Esters such as Ethyl acetate and Ethyl isovalerate (Ethyl 3-methylbutanoate); and Ketone such as Butanone.

Green coffee can contain minor constituents that may be undesirable both for flavor and bioactivity of the brew. Most of these compounds are microbial byproducts that occur due to inappropriate harvesting, weather conditions during primary processing, or improper storage.

Examples of such incidental compounds are ochratoxin A (OTA) and specific biogenic amides.
In both Uganda kapkwai Sipi Falls Green Beans and Horqueta La Berlina Green Beans "geisha" Panama, biogenic amides were observed regardless to their green and special treatment conditions, such as Aziridineethanamine, Benzeneethanamine, Ethanediamide (Oxalic acid diamide), Isopropanolamine (Amino propanol) and Propanamide (lactic amide) etc. Biogenic amides are aliphatic, alicyclic or heterocyclic organic base of low molecular weight and synthesized by the regular metabolic processes of plants and microorganisms. They are formed by the enzymes of raw materials or produced by decarboxylation of specific amino acids. In coffee, biogenic amines originate from the action of microbial decarboxylases on amino acids during fermentative processes (wet or natural fermentation), inappropriate storage, or low quality defective fermented seeds.

(B) Influence of Special Treatment on Compound Spectrum

(B1) Extraction Analyses

The GC-MS compound spectrums with the Extract operation before and after the green beans specially treated are shown in following Figs. 1 and 2, respectively, for Horqueta La Berlina Green Beans "geisha" Panama, the influence of the special treatment on variation of the compounds in coffee can be analyzed by comparing these two graphs.
In these graphs, the peaks represent the intensity occurred for the compounds. The numbers associated with these peaks are called the retention time, a time that elapses for a compound to be detected through GC-MS scanning. When the MS spectrum equipment and test method (i.e. extract or headspace) are selected, the retention time for a compound to be detected in a substance is approximately ascertained.
The Y axis in these graphs is called Relative Abundance of Intensity since it is accounted by the number of the molecular ions detected and received by equipment. The most intense peak presented in the compound spectrum is defined the base peak, and intensities of other peaks are expressed relatively as a percentage of the base peak intensity.
From Figs. 1 and 2, it can be seen that the retention times associated with the peaks are almost the same before and after the green beans specially treated. By examining the peaks in these two figures, it was noted that the corresponding compounds detected are also basically the same before and after the green beans specially treated, indicating that special treatment did not change the non-volatile compound constitution of coffee green (the Extract analyses are mainly used for identification of this type of compounds).
However, it was interested to observe from Figs. 1 and 2 that the peak intensities varied considerably before and after the green beans specially treated.
In Fig. 1, the base peak and the secondary intense peak locate at 22.40 and 22.04, respectively. In the range from 21.83 to 22.60, the correspondent compounds were identified as a group of the acetates esterified from the Prenols with the acetic acid, as described previously. Meanwhile, the correspondent compound at 16.30 is Caffeine, and the rest peaks detected are mainly Lipids and Amino Acids.
But, the peak intensities in Fig. 2 showed quite differently. When compared with Fig.1, the peak intensities at 22.40 and 22.04 reduce considerably, while the Caffeine's peak is the most intense and becomes a base peak. The rest of peaks show more or less reduction.
Quite similar variation of the peak intensity patterns was also observed in the GC-MS compound spectrums with the Extract operation for Uganda kapkwai Sipi Falls Green Beans

before and after special treatment. The results and analyses for this coffee bean were not, therefore, included here.
Reduction of the peak intensity implies that the amount of the compound in the substance reduces. Accordingly, in our tested coffee, the acetates which have the higher intensities detected in the original green must be reacted during special treatment, leading to the amount of this group of acetates reduced from coffee green.
Following the literatures, the acetate has tendency to dissolve and form acetate anion (CH_3COO^- or $C_2H_3O_2^-$), which is one of the carboxylate family and the conjugate base of acetic acid. This reaction is simply written as below:

$$CH_3COOH = CH_3COO^- + H^+$$

The acetate anion can also undergo a despropotionation reaction to produce carbon dioxide and methane:

$$CH_3COO^- + H^+ = CO_2 + CH_4$$

leading to reduction of the acetate amount. This reaction is catalyzed by yeasts or microorganisms in their fermentative metabolism. One electron is transformed from the carbonyl function of the carboxylic group to the methyl group of acetic acid to produce CO_2 and methane gas, respectively.
Combined the information that the peak intensities of acetates varied in the GC-MS compound spectrums with the simple chemical reaction given above, it is indicative that fermentation did take place in green beans during special treatment, and it could also be confirmative with an observation why the vacuumed bag used for our special treatment expanded during this special processing. However, it should be mentioned that the real fermentation process occurred during the special treatment could be much more complex than these simple reactions proposed above.

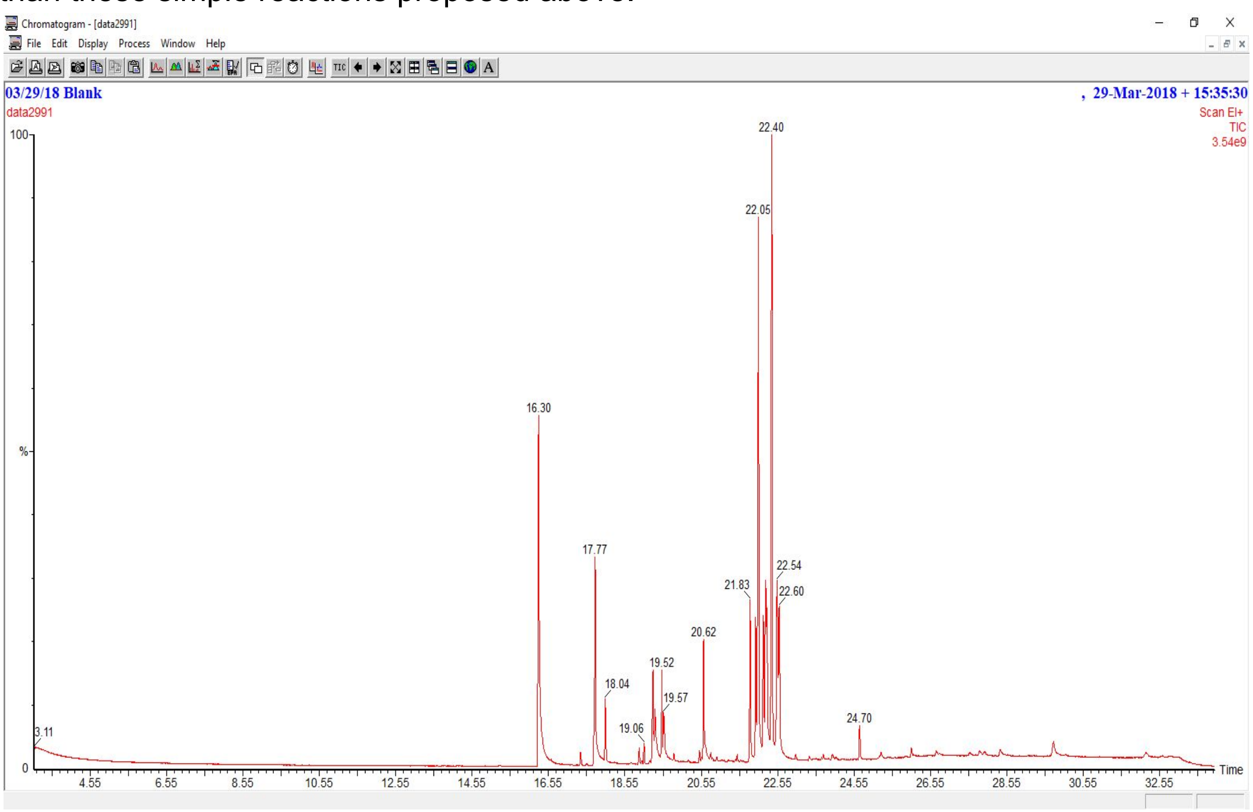

Fig. 1 Extraction Analysis – Horqueta La Berlina Green Beans “geisha”, Panama

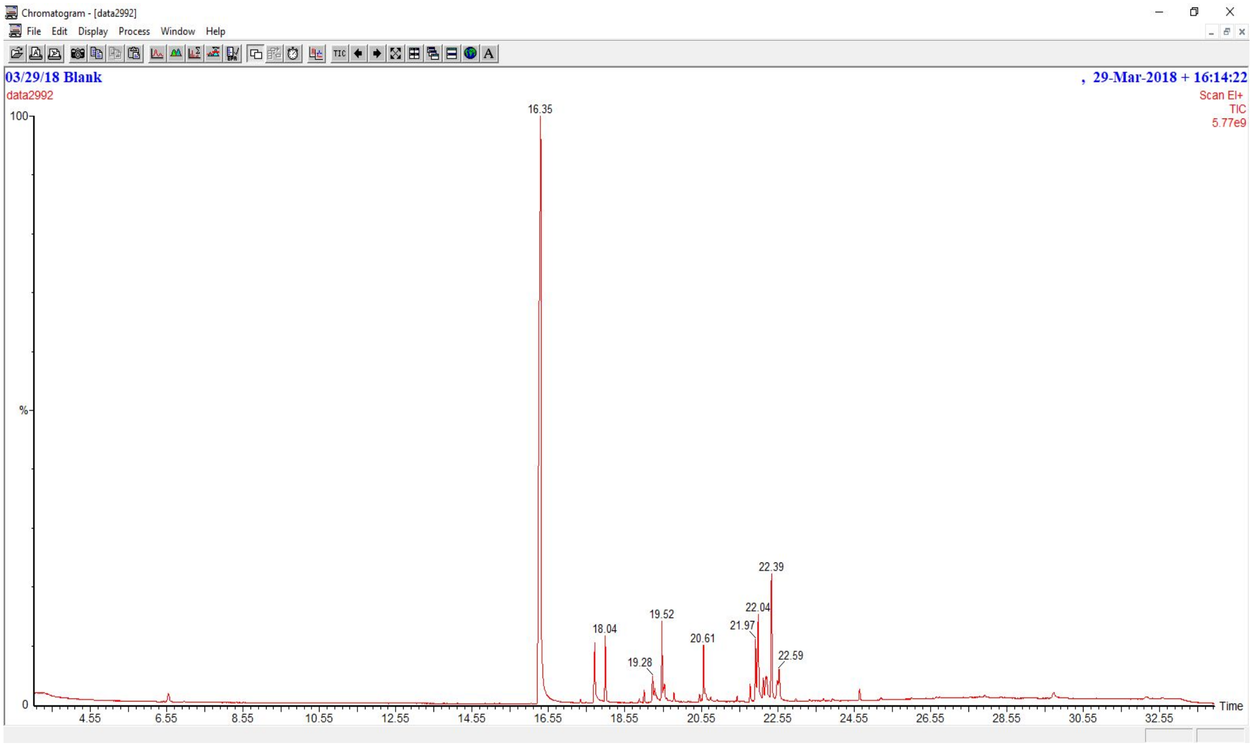

Fig. 2 Extraction Analysis – Horqueta La Berlina Specially Treated Beans “geisha”, Panama

As described previously, it was noted from Figs.1 and 2 that the peak intensity of Caffeine enhanced after the beans specially treated, indicative that the amount of Caffeine in coffee promoted by our special treatment. So far, no one reported such evidence that the Caffeine amount was affected by so-called fermentation in coffee. However, *the fact that Caffeine was influenced by fermentation in tea was indeed reported and studied in l*iterature. An explanation why Caffeine amount changes during fermentation remains to be seen, and it might be important to further confirm and study this phenomenon.

Interestingly, when the intensity levels presented in Fig. 2 were compared with those obtained after roast, it was noted that roast didn’t change the peak intensity of Caffeine very much and still keep the most intense with a little variation of relative abundance, but the intensities of acetates recover back in a certain of levels. Since the analyses of GC-MS compound spectrums obtained from coffee roasted are excluded in this report, the question how roast affects the coffee behavior contributed by special treatment (fermentation) remains to be further studied.

(B2) Headspace and Headspace Trap Analyses

The GC-MS compound spectrums with the Headspace and Headspace Trap operations for Horqueta La Berlina Green Beans “geisha” Panama before and after the green beans specially treated are shown in following Figs. 3, 4, 5 and 6, respectively. Since the Headspace and Headspace Trap GC-MS scanning are mostly employed to detect the volatile compounds, which give the green coffee characteristic aroma, accordingly, while variations of the peak intensities were examined in these figures, identification was also focused on whether or not new volatile compounds appear after special treatment.

The Headspace analyses for Horqueta La Berlina Green Beans “geisha”, Panama before and after special treatment are presented in Figs. 3 and 4. It was noted that in these figures the base peak intensity (the most intense) changes from one corresponding to biogenic amides (around 1.28) in original green to one related to ethanol (around 3.88) after special treatment, and the peak intensities of acetaldehyde (around 1.72) and ethyl acetate (around 3.24) are enhanced greatly from the negligible levels after the beans treated specially. In addition, the new compound Limonene (around 8.32) was detected thereafter.

The Headspace Trap analyses for Horqueta La Berlina Green Beans "geisha", Panama before and after special treatment are shown in Figs. 5 and 6. It was observed in these figures that Alcohol, i.e. Ethanol (around a range from 4.00 – 4.35), is the most abundant of volatile compounds regardless whether or not the beans treated specially. When Figs. 5 and 6 are compared each other, it was also noted that similar to the Headspace analyses, except that the peak intensity of biogenic amides (around 1.29) reduced, the other peak intensities correspondent to those compounds existed in the original green are also enhanced greatly from either the negligible or low levels after the beans specially treated, such as around 1.81, 3.30, 5.61, 6.07, 6.58, 8.48, 9.42, 9.75 and so on. In addition, the new compounds Limonene (around 7.47) and Terpinene (around 8.16 and 8.98) were detected thereafter.

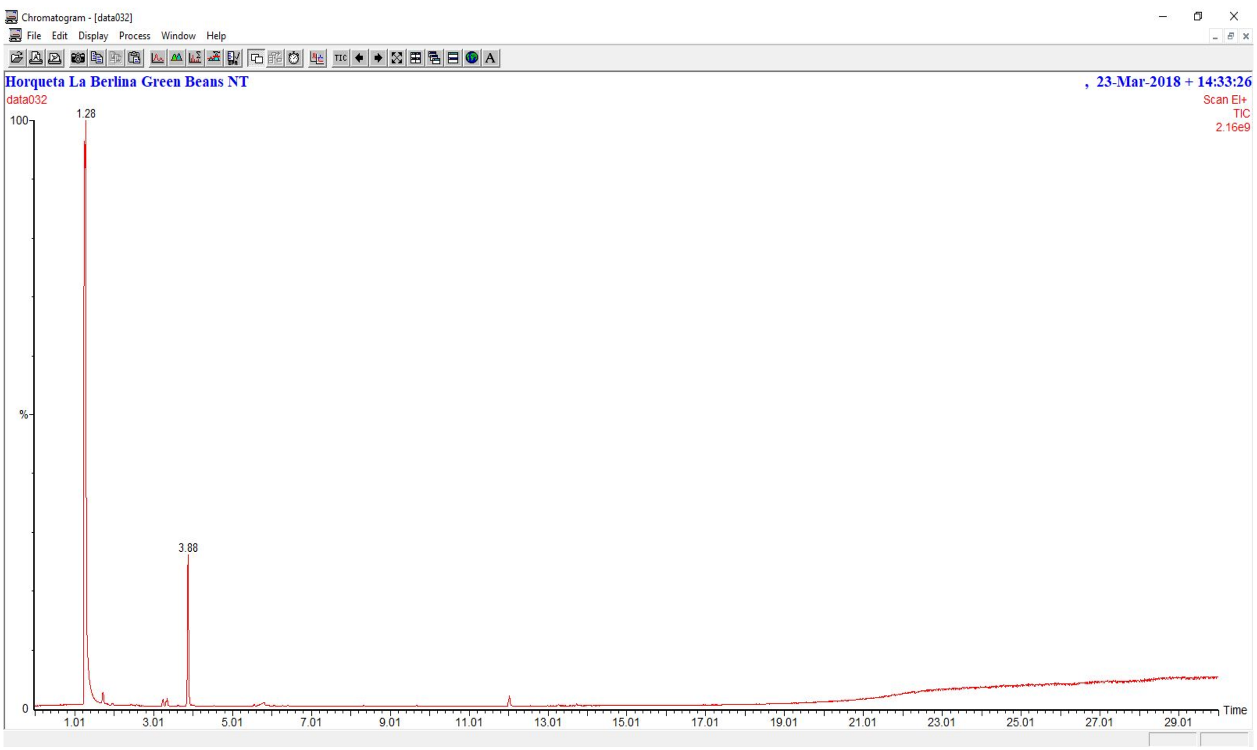

Fig. 3 Headspace Analysis – Horqueta La Berlina Green Beans "geisha", Panama

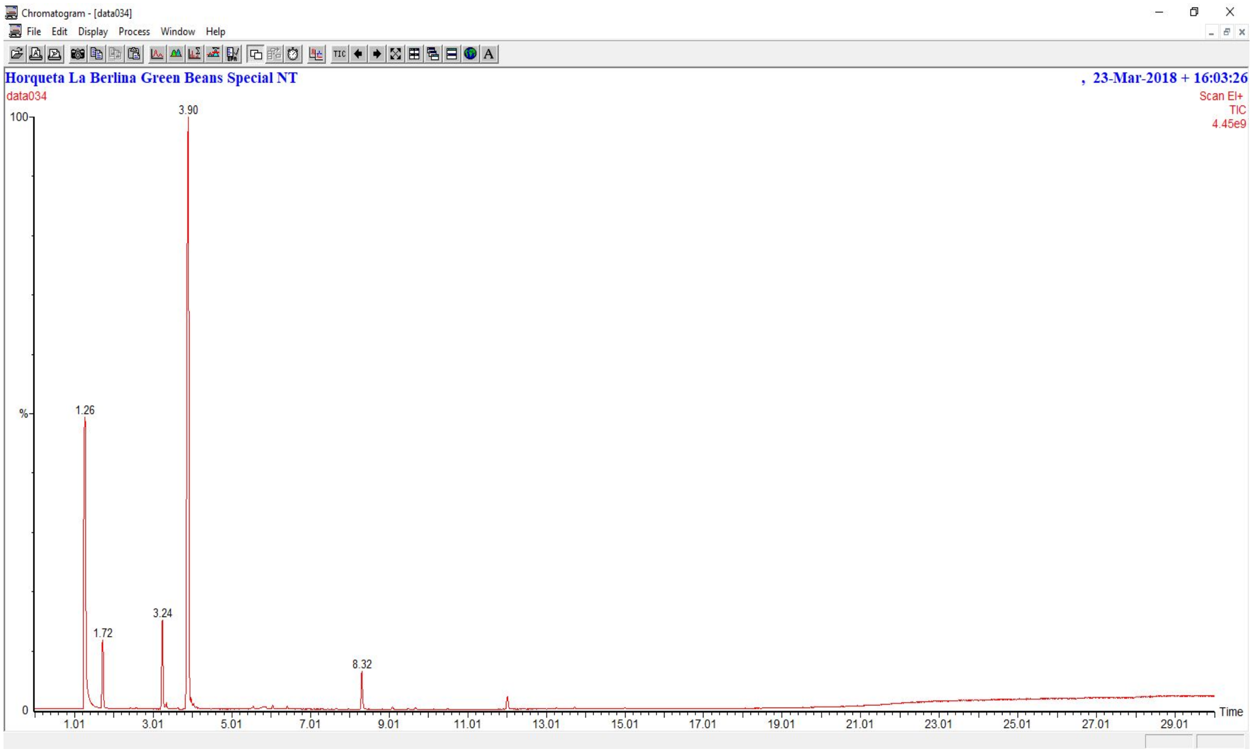

Fig. 4 Headspace Analysis – Horqueta La Berlina Specially Treated Beans "geisha", Panama

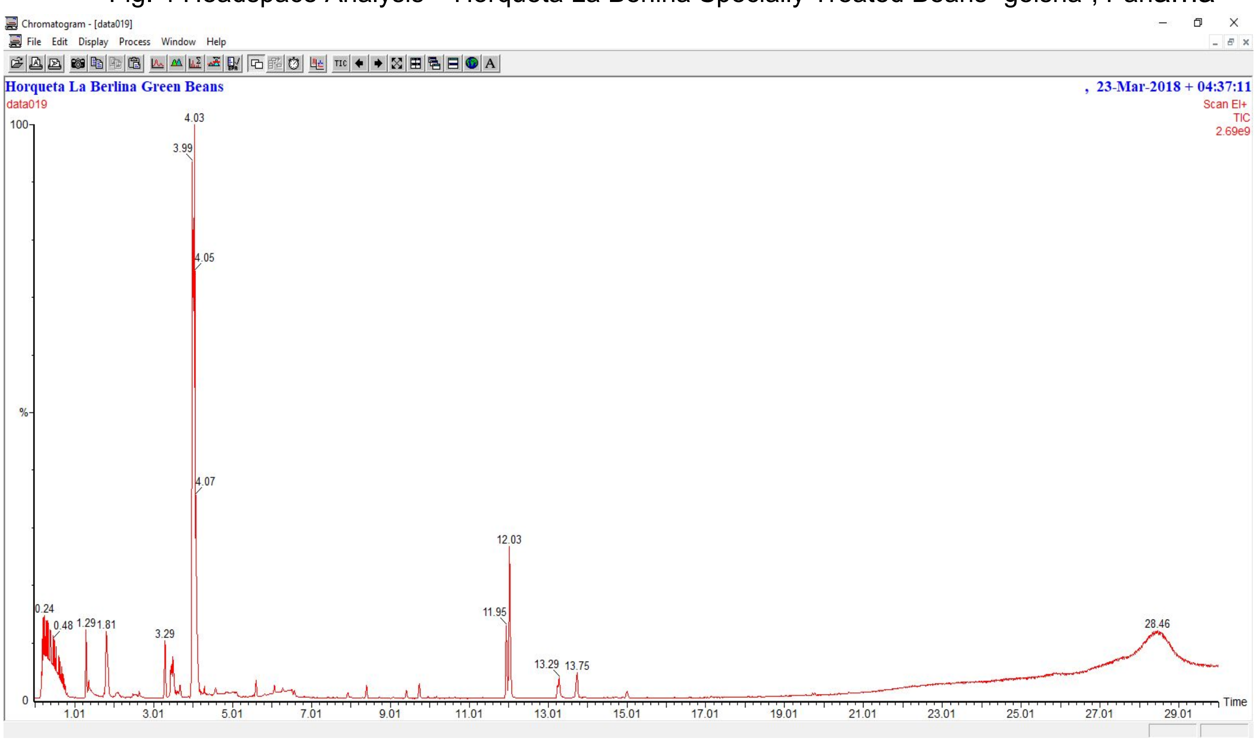

Fig. 5 Headspace Trap Analysis – Horqueta La Berlina Green Beans "geisha", Panama

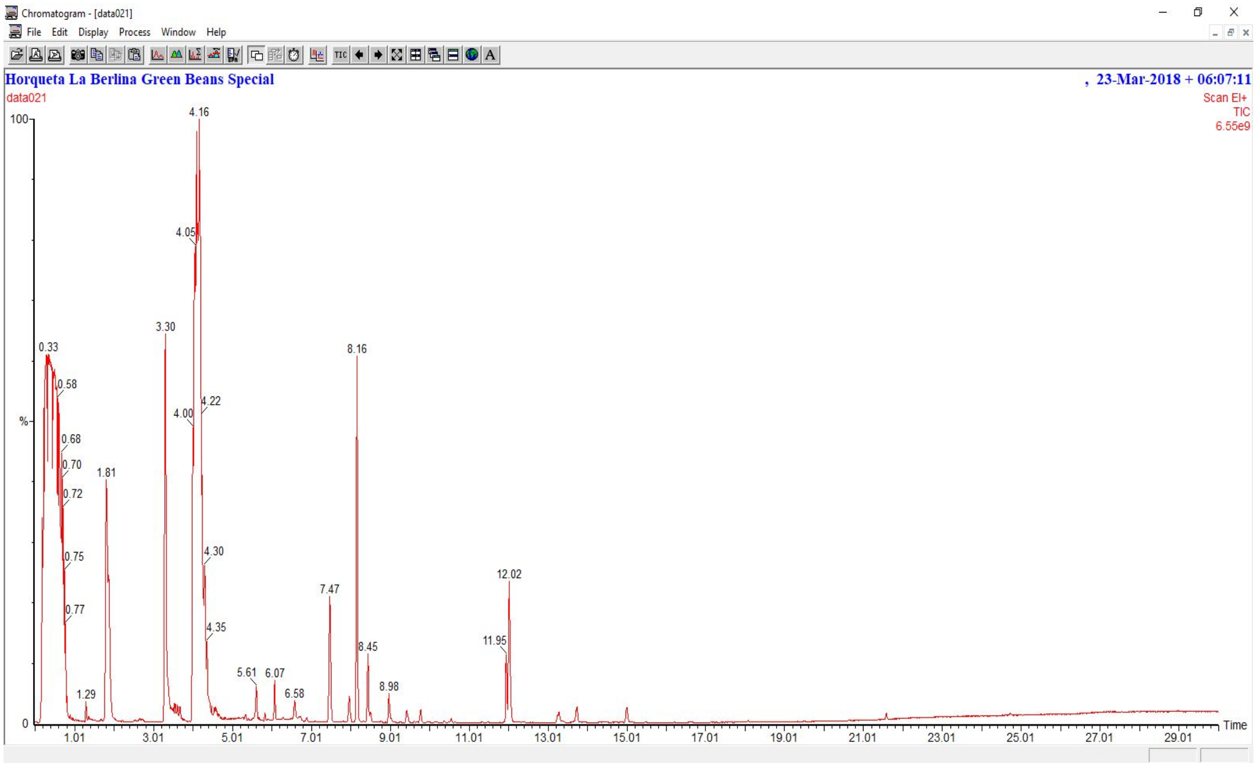

Fig. 6 Headspace Trap Analysis – Horqueta La Berlina Specially Treated Beans “geisha”, Panama

Figs. 3 – 6 showed clearly a fact that that both the peak intensity of the most compounds existed in original green enhanced and the new compounds occurred after the beans were treated specially. Since these volatile compounds are categorized as Alcohol, Aldehydes, Aliphatic Compounds and Acids, Esters and Ketone and they all characterize the coffee’s aroma, one would accordingly expect <u>that coffee flavor would be improved by using this special treatment</u>.

It is well known that the aromatic (volatile) compounds could be induced through the chemical reactions catalyzed by fermentation, however, it was unclear that the changes or variations in compound spectrums shown in Figs.3 – 6 were solely due to fermentation taken place by our special treatment. As acknowledged, those aromatic compounds detected in coffee green also occur in the beer, enhancement in the peak intensity of the existed compounds and appearance of the new ones, therefore, might be caused at the same time by a process so-called chemical diffusion from the beers used for our special treatment. It is interesting and also might be very difficult to distinguish fermentation and diffusion during special treatment in future studies.

Similar variations of the peak intensities in the Headspace and Headspace Trap GC-MS compound spectrums were also observed for Uganda kapkwai Sipi Falls Green Beans before and after special treatment. Again, the results and analyses for this coffee bean were not included here.

Conclusions and Remarks

Identification of chemical compounds for the coffee selected was carried out by using gas chromatography with mass spectrometry (GC-MS) with three ways, Extraction, Headspace and Headspace Trap, and the influence of our special treatment on green coffee beans was analyzed. The spectrum results revealed that the peak intensities of the detected non-volatile and volatile compounds varied greatly. After special treatment, green coffee beans showed that Lipids reduced, Caffeine increased, the peak intensities enhanced for the most aromatic compounds but not for the biogenic amides. In addition, the new aromatic compounds appeared thereafter. The fact that the peak intensities of the Prenol-typed acetates were reduced considerably might indicate that fermentation did take place during our special treatment, and the observation that the aromatic compounds were enriched after the beans treated specially might bring an expectation that the coffee flavor could be improved. However, it is unclear whether or not enhancement of the peak intensities for the aromatic compounds and appearance of the new ones were only related to fermentation and whether or not a chemical diffusion process involved during special treatment.

Since the results shown in this report were very preliminary for the study of the influence of special treatment on green coffee beans, it might be necessary for them to be confirmed again. It is suggestive that further compound identification in coffee might need to extend into a broader range of constitutions by combining GC-MS with other types of mass spectrometry instruments because of limitation of compound detection capable by gas chromatography. In addition, it would be also proposed that a quantitative compound identification might need to be incorporated for better understanding.

The focus of the current report was on green coffee beans only, as a result, the question arises whether or not the compound spectrums obtained after the special treatment will be changed by following roast. Compound identification after roast needs to be continuous immediately, and it is very interesting in studying how the peak intensity patterns change from the status treated specially to one roasted.

The most difficult understanding in this report was why the peak intensity of Caffeine increased after the beans treated specially. For coffee, there was no information published or reported in literatures about the relationship between Caffeine and fermentation or due to what else the other effect was, it would be necessary, therefore, for this phenomenon to be confirmed and studied further. If confirmed and understood, it could be probable that Caffeine can be applied as a specification to inspect the specialty coffee we will produce in future.